Volume 2

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General Notices

General Statements

The General Notices provide the basic guidelines for the interpretation and application of the standards, tests, assays, and other specifications of the Indian Pharmacopoeia (IP), as well as to the statements made in the monographs and other texts of the Pharmacopoeia.

A monograph is to be constructed in accordance with any general monograph or notice or any appendix, note or other explanatory material that is contained in this Pharmacopoeia and that is applicable to that monograph. All statements contained in the monograph, except where a specific general notice indicates otherwise and with the exceptions given hereafter, constitute standards for the official articles. An article is not of pharmacopoeial quality unless it complies with all of the requirements stated.

Exceptions to the General Notices do exist, and where they do, the wording in the individual monograph or an appendix takes precedence and specifically indicates directions or the intent. Thus, the specific wording of standards, tests, assays and other specifications is binding wherever deviations from the General Notices exist. Likewise, where there is no specific mention to the contrary, the General Notices apply.

Name. The full name or title of this book, including addenda thereto, is Indian Pharmacopoeia 2007, abbreviated to IP 2007. In the texts, the term ‘Pharmacopoeia’ or ‘IP’ without qualification means the Indian Pharmacopoeia 2007 and any addenda thereto.

Official and Official Articles. The word ‘official’ wherever used in this Pharmacopoeia or with reference thereto, is synonymous with ‘pharmacopoeial’, with ‘IP’ and with ‘compendial’. The designation IP in conjunction with the official title on the label of an article is an indication that the article purports to comply with IP standards.

The following terms are used where the articles for which monographs are provided are to be distinguished.

An official substance is a single drug or a drug entity or a pharmaceutical aid for which the monograph title includes no indication of the nature of a dosage form.

An official preparation is a drug product (dosage form) and is the finished or partially finished preparation or product of one or more official substances formulated for use on the patient.

An article is an item for which a monograph is provided, whether an official substance or an official preparation.

Official Standards. The requirements stated in the monographs apply to articles that are intended for medicinal use but not necessarily to articles that may be sold under the same name for other purposes.

The active pharmaceutical ingredients (drug substances), excipients (pharmaceutical aids), pharmaceutical preparations (dosage forms) and other articles described in the monographs are intended for human and veterinary use (unless explicitly restricted to one of these uses).

The requirements given in the monographs are not framed to provide against all possible impurities, contaminants or adulterants; they provide appropriate limitation of potential impurities only.

A preparation must comply throughout the shelf-life assigned to it by the manufacturer; for opened or broached containers the maximum period of validity for use may sometimes be stated in the individual monograph. Nevertheless, the responsibility for assigning the period of validity shall be with the manufacturer.

Added Substances. An official substance, as distinguished from an official preparation, contains no added substances except when specifically permitted in the individual monograph. Unless otherwise specified in the individual monograph, or elsewhere in the General Notices, suitable substances may be added to an official preparation to enhance its stability, usefulness or elegance, or to facilitate its preparation. Such auxiliary substances shall be harmless in the amounts used, shall not exceed the minimum quantity required to provide their intended effect, shall not impair the therapeutic efficacy or the bioavailability or safety of the preparation and shall not interfere with the tests and assays prescribed for determining compliance with the official standards. Particular care should be taken to ensure that such substances are free from harmful organisms. The freedom to the manufacturers to add auxiliary substances imposes on them the responsibility of satisfying the licensing authorities on the purpose of the addition and the innocuity of such substances.

Alternative Methods. The tests and assays described are the official methods upon which the standards of the Pharmacopoeia are based. Alternative methods of analysis may be used for control purposes, provided that the methods used are shown to give results of equivalent accuracy and enable an unequivocal decision to be made as to whether compliance with the standards of the monographs would be achieved if the official methods were used. Automated procedures utilising the same basic chemistry as the test procedures given in the monograph may also be used to determine compliance. Such alternative or automated procedures must be validated.

In the event of doubt or dispute, the methods of analysis of the Pharmacopoeia are alone authoritative and only the result obtained by the procedure given in this Pharmacopoeia is conclusive.
Meanings of Terms

Alcohol. The term “alcohol” without qualification means ethanol (95 per cent). Other dilutions of ethanol are indicated by the term “alcohol” or “alcohol” followed by a statement of the percentage by volume of ethanol (C₂H₆O) required.

Desiccator. A tightly-closed container of suitable size and design that maintains an atmosphere of low moisture content by means of silica gel or phosphorus pentoxide or other suitable desiccant.

Drying and ignition to constant weight. Two consecutive weighings after the drying or igniting operations do not differ by more than 0.5 mg, the second weighing following an additional period of drying or of ignition respectively appropriate to the nature and quantity of the residue.

Ethanol. The term “ethanol” without qualification means anhydrous ethanol or absolute alcohol.

Filtration. Unless otherwise stated, filtration is the passing of a liquid through a suitable filter paper or equivalent device until the filtrate is clear.

Freshly prepared. Made not more than 24 hours before it is issued for use.

Label. Any printed packing material, including package inserts that provide information on the article.

Negligible. A quantity not exceeding 0.50 mg.

Solution. Where the name of the solvent is not stated, “solution” implies a solution in water. The water used complies with the requirements of the monograph on Purified Water. The term ‘distilled water’ indicates Purified Water prepared by distillation.

Temperature. The symbol ° used without qualification indicates the use of the Celsius thermometric scale.

Water. If the term is used without qualification it means Purified Water of the Pharmacopoeia. The term ‘distilled water’ indicates Purified Water prepared by distillation.

Water-bath. A bath of boiling water unless water at another temperature is indicated. Other methods of heating may be used provided the required temperature is approximately maintained but not exceeded.

Provisions Applicable To Monographs and Test Methods

Expression of Content. Where the content of a substance is defined, the expression “per cent” is used according to circumstances with one of two meanings:

— per cent w/w (percentage, weight in weight) expressing the number of grams of substance in 100 grams of final product,

— per cent v/v (percentage, volume in volume) expressing the number of millilitres of substance in 100 millilitres of final product.

The expression “parts per million” refers to the weight in weight, unless otherwise stated.

Where the content of a substance is expressed in terms of the chemical formula for that substance an upper limit exceeding 100 per cent may be stated. Such an upper limit applies to the result of the assay calculated in terms of the equivalent content of the specified chemical formula. For example, the statement ‘contains not less than 99.0 per cent and not more than 101.0 per cent of C₇H₆O₂ implies that the result of the assay is not less than 99.0 per cent and not more than 101.0 per cent, calculated in terms of the equivalent content of C₇H₆O₂.

Where the result of an assay or test is required to be calculated with reference to the dried, anhydrous, ignited substance, or the substance free from solvent, the determination of loss on drying, water content, loss on ignition, content of the specified solvent, respectively is carried out by the method prescribed in the relevant test in the monograph.

Expression of Concentrations. The following expressions in addition to the ones given under Expression of Content are also used:

— per cent w/v (percentage, weight in volume) expressing the number of grams of substance in 100 millilitres of product

— per cent v/w (percentage, volume in weight) expressing the number of millilitres of substance in 100 grams of product.

Usually, the strength of solutions of solids in liquids is expressed as percentage weight in volume, of liquids in liquids as percentage volume in volume, of solids in semi-solid bases (e.g. creams) and of gases in liquids as percentage weight in weight.

When the concentration of a solution is expressed as parts of dissolved substance in parts of solution, it means parts by weight (g) of a solid in parts by volume (ml) of the final solution; as parts by weight (g) of a gas in parts by weight (g) of the final solution.

When the concentration of a solution is expressed in molarity designated by the symbol M preceded by a number, it denotes the number of moles of the stated solute contained in sufficient Purified Water (unless otherwise stated) to produce 1 litre of solution.

Abbreviated Statements. Incomplete sentences are employed in parts of the monographs for directness and brevity (for example, Iodine Value. Not more than ……; Relative Density. ……to……….) Where the tests are abbreviated, it is to be understood that the test method referred to in brackets
provides the method to be followed and that the values specified are the applicable limits.

**Weights and Measures.** The metric system of weights and measures is employed in the Pharmacopoeia. All measures are required to be graduated at 25º and all measurements in tests and assays, unless otherwise stated, are to be made at that temperature. Graduated glass apparatus used in analytical operations shall comply with the requirements stated in Chapter 2.1.6

**Monographs**

**General Monographs**

General monographs on dosage forms include requirements of general application and apply to all preparations within the scope of the Introduction section of the general monograph, except where a preamble limits the application. The requirements are not necessarily comprehensive for a given specific preparation; additional requirements may sometimes be given in the individual monograph for it.

**Production.** Statements given under the heading Production relate to particular aspects of the manufacturing process and are not necessarily comprehensive. However, they are mandatory instructions to manufacturers. They may relate, for example, to source materials, to the manufacturing process and its validation and control, to any in-process testing that is to be carried out by the manufacturer on the final product either on selected batches or on each batch prior to release. All this cannot be verified on a sample of the final product by an independent analyst. It is for the licensing authority to verify that the instructions have been followed.

The absence of a section on Production does not imply that attention to features such as those given above is not required. An article described in a monograph of the Pharmacopoeia is to be manufactured in accordance with the principles of good manufacturing practice and in accordance with the requirements of the Drugs and Cosmetics Rules, 1945. The general principles applicable to the manufacture and quality assurance of drugs and preparations meant for human use apply equally to veterinary products as well.

**Manufacture of Drug Products.** The opening definitive statement in certain monographs for drug products is given in terms of the active ingredient(s) only. Any ingredient(s) other than those included in the statement, must comply with the general notice on Excipients and the product must conform to the Pharmacopoeial requirements.

Official preparations are prepared only from ingredients that comply with the requirements of the pharmacopoeial monographs for those individual ingredients for which monographs are provided.

**Excipients.** Any substance added in preparing an official preparation shall be innocuous, shall have no adverse influence in the therapeutic efficacy of the active ingredients and shall not interfere with the tests and assays of the Pharmacopoeia. Care should be taken to ensure that such substances are free from harmful organisms.

**Individual Monographs**

Drug products that are the subject of an individual monograph are also required to comply with the tests given in the general monographs.

**Titles.** The main title for a drug substance is the International Non-proprietary Name (INN) approved by the World Health Organization. Subsidiary names and synonyms have also been given in some cases; where included, they have the same significance as the main title.

The main titles of drug products are the ones commonly recognised in practice. Synonyms drawn from the full non-proprietary name of the active ingredient or ingredients have also been given. Where, however, a product contains one or the other of different salts of an active molecule, the main title is based on the full name of the active ingredient. For example, Chloroquine Phosphate Tablets and Chloroquine Sulphate Tablets.

**Chemical Formulae.** When the chemical structure of an official substance is known or generally accepted, the graphic and molecular formulae are normally given at the beginning of the monograph for information. This information refers to the chemically pure substance and is not to be regarded as an indication of the purity of the official material. Elsewhere, in statement of purity and strength and in descriptions of processes of assay, it will be evident from the context that the formulae denote the chemically pure substances.

Where the absolute stereochemical configuration is specified, the International Union of Pure and Applied Chemistry (IUPAC) $R/S$ and $E/Z$ systems of designation have been used. If the substance is an enantiomer of unknown absolute stereochemistry, the sign of the optical rotation, as determined in the solvent and under the conditions specified in the monograph, has been attached to the systematic name. An indication of sign of rotation has also been given where this is incorporated in a trivial name that appears on an IUPAC preferred list.

**Atomic and Molecular Weights.** The atomic weight or molecular weight is shown, as and when appropriate at the top right hand corner of the monograph. The atomic and molecular weights and graphic formulae do not constitute analytical standards for the substances described.

**Definition.** The opening statement of a monograph is one that constitutes an official definition of the substance,
preparation or other article that is the subject of the monograph. In certain monographs for pharmaceutical preparations the statement is given in terms of the principal ingredient(s).

In monographs on vegetable drugs, the definition indicates whether the subject of the monograph is, for example, the whole drug or the drug in powdered form.

Certain pharmaceutical substances and other articles are defined by reference to a particular method of manufacture. A statement that a substance or article is prepared or obtained by a certain method constitutes part of the official definition. A statement that a substance may be prepared or obtained by a certain method, however, indicates that this is one possible method and does not imply that other methods are not permissible.

**Statement of content.** The limits of content stated are those determined by the method described under Assay.

**Description.** The statements under the heading Description are not to be interpreted in a strict sense and are not to be regarded as official requirements.

**Solubility.** Statements on solubility are given in Chapter 2.4.26 and are intended as information on the approximate solubility at a temperature between 15º and 30º, unless otherwise stated, and are not to be considered as official requirements. However, a test for solubility stated in a monograph constitutes part of the standards for the substance that is the subject of that monograph.

**Test Methods**

References to general methods of testing are indicated by test method numbers in brackets immediately after the heading of the test or at the end of the text.

**Identification.** The tests given under the heading Identification are not necessarily sufficient to establish absolute proof of identity. They provide a means of verifying that the identity of the material under examination is in accordance with the label on the container.

In certain monographs alternative series of identification tests are given; compliance with either one or the other set of tests is adequate to verify the identity of the article.

When tests for infrared absorption are applied to material extracted from formulated preparations, strict concordance with the specified reference spectrum may not always be possible, but nevertheless a close resemblance between the spectrum of the extracted material and the specified reference spectrum should be achieved.

**Tests and Assays**

The tests and assays are the official methods upon which the standards of the Pharmacopoeia depend. The requirements are not framed to take into account all possible impurities. It is not to be presumed, for example, that an impurity that is not detectable by means of the prescribed tests is tolerated. Material found to contain such an impurity is not of pharmacopoeial quality if the nature or amount of the impurity found is incompatible with good pharmaceutical practice.

Pharmacopoeial methods and limits should be used merely as compliance requirements and not as requirements to guarantee total quality assurance. Tests and assays are prescribed for the minimum sample available on which the attributes of the article should be measured. Assurance of quality must be ensured by the manufacturer by the use of statistically valid sampling and testing programmes.

**Tests.** Unless otherwise stated, the assays and tests are carried out at a temperature between 20º and 30º.

Where it is directed that an analytical operation is to be carried out ‘in subdued light’, precautions should be taken to avoid exposure to direct sunlight or other strong light. Where a procedure is directed to be performed ‘protected from light’ precautions should be taken to exclude actinic light by the use of low-actinic glassware, working in a dark room or similar procedures.

For preparations other than those of fixed strength, the quantity to be taken for a test or an assay is usually expressed in terms of the active ingredient. This means that the quantity of the active ingredient expected to be present and the quantity of the preparation to be taken are calculated from the strength stated on the label.

**Other Tests.** In the monographs on dosage forms and certain preparations, under the sub-heading ‘Other tests’ it is stated that the article complies with the tests stated under the general monograph of the relevant dosage form or preparation. Details of such tests are provided in the general monographs.

**Limits.** The limits given are based on data obtained in normal analytical practice. They take into account normal analytical errors, of acceptable variations in manufacture and of deterioration to an extent that is acceptable. No further tolerances are to be applied to the limits for determining whether or not the article under examination complies with the requirements of the monograph.

**Quantities.** Unless otherwise stated, the quantities to be taken for assays, limit tests and other tests are of the substance under examination.

In tests with numerical limits and assays, the quantity stated on the label.

In tests with numerical limits and assays, the quantity stated on the label.
conditions, the stated quantity is taken for testing. Reagents are used in the prescribed amounts.

Quantities are weighed or measured with an accuracy commensurate with the indicated degree of precision. For weighings, the precision is plus or minus 5 units after the last figure stated. For example, 0.25 g is to be interpreted as 0.245 g to 0.255 g. For the measurement of volumes, if the figure after the decimal point is a zero or ends in a zero, e.g. 10.0 ml or 0.50 ml, the volume is measured using a pipette, a volumetric flask or a burette, as appropriate; in other cases, a graduated measuring cylinder or a graduated pipette may be used. Volumes stated in microlitres are measured using a micropipette or microsyringe.

The term ‘transfer’ is used generally to indicate a quantitative operation.

**Apparatus.** Measuring and weighing devices and other apparatus are described in the chapter entitled ‘Apparatus for Tests and Assays’. A specification for a definite size or type of container or apparatus in a test or assay is given merely as a recommendation.

Unless otherwise stated, comparative tests are carried out using identical tubes of colourless, transparent, neutral glass with a flat base, commonly known as Nessler cylinders.

**Reagents and Solutions.** The reagents required for the tests and assays of the Pharmacopoeia are defined in the various chapters showing their nature, degree of purity and the strengths of the solutions to be made from them. The requirements set out are not intended to imply that the materials are suitable for use in medicine; regents not covered by monographs in the pharmacopoeia shall not be claimed to be of IP quality.

The term ‘analytical reagent grade of commerce’ implies that the chemical is of a high degree of purity wherein the limits of various impurities are known. Where it is directed to use a ‘general laboratory reagent grade of commerce’ it is intended that a chemically pure grade material, not necessarily required to be tested for limiting or absence of certain impurities, is to be used.

**Indicators.** Where the use of an indicator solution is mentioned in an assay or test, approximately 0.1 ml of the solution shall be added, unless otherwise directed.

**Reference Substances.** Certain monographs require the use of a chemical reference substance or a biological reference preparation or a reference spectrum. These are authentic specimens chosen and verified on the basis of their suitability for intended use as prescribed in the Pharmacopoeia and are not necessarily suitable in other circumstances.

IP Reference Substances, abbreviated to IPRS (and referred to as RS in the individual monographs) are issued by the Indian Pharmacopoeia Commission (IPC). They are the official standards to be used in cases of arbitration. Secondary Standards (Working Standards) may be used for routine analysis, provided they are standardized at regular intervals against the Reference Substances.

Biological Reference Substances, also abbreviated to IPRS and Standard Preparations of antibiotics are issued by agencies authorised by the IPC. They are standardized against the International Standards and Reference Preparations established by the World Health Organization (WHO). The potency of these preparations is expressed in International Units.

Reference spectra are published by the IPC and they are accompanied by information concerning the conditions used for sample preparation and recording of the spectra.

**Test animals.** Unless otherwise directed, animals used in a test or an assay shall be healthy and are drawn from a uniform stock, and have not previously been treated with any material that will interfere with the test or the assay.

**Calculation of results.** In determining compliance with a numerical limit in assay or test, the result should be calculated to one decimal place more than the significant figures stated and then rounded up or down as follows: if the last figure calculated is 5 to 9, the preceding figure is increased by 1; if it is 4 or less, the preceding figure is left unchanged.

**Storage.** Statements under the side-heading Storage constitute non-mandatory advice. The articles of the Pharmacopoeia are to be stored under conditions that prevent contamination and, as far as possible, deterioration. Precautions that should be taken in relation to the effects of the atmosphere, moisture, heat and light are indicated, where appropriate, in the individual monograph.

Specific directions are given in some monographs with respect to the temperatures at which Pharmacopoeial articles should be stored, where it is considered that usage at a lower or higher temperature may produce undesirable results. The storage conditions are defined by the following terms:

- Store in a dry, well-ventilated place at a temperature not exceeding 30º
- Store in a refrigerator (2º to 8º). Do not freeze
- Store in a freezer (-2º to -18º)
- Store in a deep freezer (Below -18º)

Storage conditions not related to temperature are indicated in the following terms:

- Store protected from light
- Store protected from light and moisture

Where no specific storage directions or limitations are given in the monograph or by the manufacturer, it is to be understood
that the storage conditions include protection from moisture, freezing and excessive heat (any temperature above 40º).

Storage Containers. The requirements, guidance and information on containers for pharmaceutical use are given in the chapter entitled Containers (6.1)

In general, an article should be packed in a well-closed container i.e. one that protects the contents from contamination by extraneous solids, liquids or vapours and from loss of the article under normal conditions of handling and storage.

Where, additionally, loss or deterioration of the article from effervescence, deliquescence or evaporation under normal conditions of storage is likely, the container must be capable of being tightly closed, and re-closed after use.

In certain cases, special requirements of pack have been indicated in some monographs under Storage, using expressions that have been defined in chapter 6.1.

Labelling. The labelling of drugs and pharmaceuticals is governed by the Drugs and Cosmetics Rules, 1945. The statements that are given in the monographs under the side-heading ‘Labelling’ are not comprehensive. Only those that are necessary to demonstrate compliance or otherwise with the monograph have been given and they are mandatory. For example, in the monograph on Betamethasone Sodium Tablets the labelling statement is “The label states the strength in terms of the equivalent amount of betamethasone”. Any other statements are included as recommendations.
## DOSAGE FORMS

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General requirements

The Pharmacopoeia provides monographs of dosage forms for most of the pharmacopoeial drug substances. Additionally, the general requirements including the processes for the preparation of many of them and the tests of a general nature applicable to each type of dosage form are given in the following pages. In addition to defining the dosage forms, this section presents the general principles involved in the production of some of them.

The requirement for compliance with the tests given under each dosage form is indicated in each monograph of a drug product under the heading ‘Other tests’. These tests are mandatory and are additional to the tests given in the individual monograph.

Capsules

Capsules are solid dosage forms in which the drug or a mixture of drugs is enclosed in Hard Gelatin Capsule Shells, in soft, soluble shells of gelatin, or in hard or soft shells of any other suitable material, of various shapes and capacities. They usually contain a single dose of active ingredient(s) and are intended for oral administration. The consistency of soft shells may be adjusted by the addition of substances such as Glycerin and Sorbitol. Excipients such as opaque fillers, antimicrobial preservatives, sweetening agents, flavouring agents and one or more colouring agents permitted under the Drugs and Cosmetic Rules, 1945 may be added. Capsules may bear surface markings.

The contents of capsules may be of solid, liquid or paste-like consistency. They consist of the medicament(s) with or without excipients such as vehicles, solvents, diluents, lubricants, fillers, wetting agents and disintegrating agents. The contents do not cause deterioration of the shell, but the capsules are attacked by the digestive fluids thereby releasing the contents.

The contents of capsules other than Modified-release (Sustained-release) Capsules do not contain any added colouring agent.

Hard Gelatin Capsules. Hard gelatin capsules contain the medicament(s) in the solid form. Where two mutually incompatible drugs are present in the mixture, one of the drugs can be put as a tablet or pellet or in small capsule and then enclosed with the other drug in a large capsule.

Production

Hard gelatin capsules are made by a process that involves dipping shaped pins into gelatin solutions, after which the gelatin films are dried, trimmed, and removed from the pins, and the body and cap pieces are joined.

Soft Gelatin Capsules. Soft gelatin capsules made from gelatin (sometimes called softgels) or other suitable material require large-scale production methods. The soft gelatin shell is somewhat thicker than that of hard-shell capsules and may be plasticized by the addition of a polyol such as sorbitol or glycerin. The ratio of dry plasticizer to dry gelatin determines the “hardness” of the shell and may be varied to accommodate environmental conditions as well as the nature of the contents. Like hard shells, the shell composition may include approved dyes and pigments, opaquing agents such as titanium dioxide, and preservatives. Flavors may be added and up to 5 per cent sucrose may be included for its sweetness and to produce a chewable shell. Soft gelatin shells normally contain 6 per cent to 13 per cent of water.

Soft gelatin capsules shells are usually formed, filled with medicament and sealed in a combined operation on machines. In some cases, shells for extemporaneous use may be performed. The shells which are thicker than those of hard capsules are formed to produce capsules which are spherical, oval or cylindrical with hemispherical ends.

Soft gelatin capsules also may be manufactured in a bubble process that forms seamless spherical capsules. The shells may sometimes contain a medicament. They may contain a preservative to prevent growth of fungi.

The contents of soft capsules usually consist of liquids or solids dissolved or dispersed in suitable excipients to give a paste-like consistency. With suitable equipment, powders, granules and other dry solids also may be filled into soft-shell capsules. As soft gelatin shells contain appreciable amounts of water, migration of capsule contents, particularly of watersoluble ingredients, may occur.

Modified-release Capsules. Modified-release (Sustained-release) Capsules are hard or soft capsules in which the contents or the shell, or both, contain auxiliary substances or are prepared by a special process designed to modify the rate at which the active ingredients are released.

Enteric Capsules (Gastro-resistant Capsules). Enteric Capsules are hard or soft capsules prepared in such a manner that the shell resists the action of the gastric fluid but is attacked by the intestinal fluid to release the contents.

During manufacture, packaging, storage and distribution of capsules, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 5.9.

Tests

Content of active ingredients. Determine the amount of active ingredient(s) by the method described in the Assay and calculate the amount of active ingredient(s) in each capsule. The result lies within the range for the content of active
ingredient(s) stated in the monograph. This range is based on the requirement that 20 capsules, or such other number as may be indicated in the monograph, are used in the Assay. Where 20 capsules cannot be obtained, a smaller number, which must not be less than 5, may be used, but to allow for sampling errors the tolerances are widened in accordance with Table 1. The requirements of Table 1 apply when the stated limits are between 90 and 110 per cent. For limits other than 90 to 110 per cent, proportionately smaller or larger allowances should be made.

### Table 1

<table>
<thead>
<tr>
<th>Weigh of Active ingredients in each Capsules</th>
<th>Subtract from the lower limit for samples of</th>
<th>Add to the upper limit for samples of</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.12 g or less</td>
<td>0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>More than 0.12 g and less than 0.3 g</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>0.3 g or more</td>
<td>0.1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

**Uniformity of weight.** This test is not applicable to capsules that are required to comply with the test for Uniformity of content for all active ingredients.

Weigh an intact capsule. Open the capsule without losing any part of the shell and remove the contents as completely as possible. To remove the contents of a soft capsule the shell may be washed with *ether* or other suitable solvent and the shell allowed to stand until the odour of the solvent is no longer detectable. Weigh the shell. The weight of the contents is the difference between the weighings. Repeat the procedure with a further 19 capsules. Determine the average weight. Not more than two of the individual weights deviate from the average weight by more than the percentage deviation shown in Table 2 and none deviates by more than twice that percentage.

### Table 2

<table>
<thead>
<tr>
<th>Average weight of capsule contents</th>
<th>Percentage deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 300 mg</td>
<td>10</td>
</tr>
<tr>
<td>300 mg or more</td>
<td>7.5</td>
</tr>
</tbody>
</table>

**Uniformity of content.** This test is applicable to capsules that contain less than 10 mg or less than 10 per cent w/w of active ingredient. For capsules containing more than one active ingredient carry out the test for each active ingredient that corresponds to the afore-mentioned conditions.

The test should be carried out only after the content of active ingredient(s) in a pooled sample of the capsules has been shown to be within accepted limits of the stated content.

**NOTE —** The test is not applicable for capsules containing multivitamins and trace elements.

Determine the content of active ingredient in each of 10 capsules taken at random using the method given in the monograph or by any other suitable analytical method of equivalent accuracy and precision. The capsules comply with the test if not more than one of the individual values thus obtained is outside the limits 85 to 115 per cent of the average value and none is outside the limits 75 to 125 per cent. If two or three individual values are outside the limits 85 to 115 per cent of the average value repeat the determination using another 20 capsules. The capsules comply with the test if in the total sample of 30 capsules not more than three individual values are outside the limits 85 to 115 per cent and none is outside the limits 75 to 125 per cent of the average value.

**Disintegration.** The disintegration test is not applicable to Modified-release Capsules. For those Hard Capsules and Soft Capsules for which the dissolution test (2.5.2) is included in the individual monograph, the test for Disintegration is not required.

**Hard Capsules.** Comply with the disintegration test (2.5.1). Unless otherwise directed in the individual monograph use *water* as the medium. If the capsules float on the surface of the medium, a disc may be added. If the capsules adhere to the discs, attach a removable piece of stainless steel woven gauze with mesh aperture of 2.00 mm to the upper plate of the basket rack assembly and carry out the test omitting the discs. Operate the apparatus for 30 minutes unless otherwise directed.

**Soft Capsules.** Comply with the disintegration test (2.5.1). Unless otherwise directed in the individual monograph use *water* as the medium and add a disc to each tube. Operate the apparatus for 60 minutes unless otherwise directed.

**Enteric Capsules.** Use the apparatus described under disintegration test (2.5.1), using one capsule in each tube. Operate the apparatus for 2 hours without the discs in *0.1 M hydrochloric acid*. No capsule shows signs of disintegration or of rupture permitting the escape of the contents. Replace the medium in the vessel with *mixed phosphate buffer pH 6.8*, add a disc to each tube and operate the apparatus for a further 60 minutes. Remove the apparatus from the medium and examine the capsules. They pass the test if no residue remains on the screen or on the underside of the discs, or, if a residue remains, it consists of fragments of shell or of a soft mass with no palpable, unmoistened core.

**Storage.** Store at a temperature not exceeding 30º.

**Labelling.** The label states the name of any added antimicrobial preservative.
**Creams**

Creams are homogeneous, semi-solid or viscous preparations that possess a relatively fluid consistency and are intended for external application to the skin or certain mucous membranes for protective, therapeutic or prophylactic purposes especially where an occlusive effect is not necessary. They are semisolids usually consisting of solutions or dispersions of one or more medicaments in suitable bases*. They are formulated using hydrophilic or hydrophobic bases to provide preparations that are essentially miscible with the skin secretion.

In recent times the term cream has been restricted to products consisting of oil-in-water emulsions or aqueous microcrystalline dispersions of long-chain fatty acids or alcohols that are water-washable and more cosmetically and aesthetically acceptable. Creams can be used for administering drugs via the vaginal route.

The base should not produce irritation or sensitisation of the skin, nor should it retard wound healing; it should be smooth, inert, odourless or almost odourless, physically and chemically stable and compatible with the skin and with incorporated medicaments.

Creams may contain suitable antimicrobial preservatives unless the active ingredients or the bases themselves have sufficient bactericidal or fungicidal activity. They may contain other suitable auxiliary substances such as antioxidants, stabilisers, thickeners and emulsifiers.

If a cream is specifically intended for use on large open wounds or on severely injured skin it should be sterile.

Creams should not normally be diluted; should dilution be necessary care should be taken to prevent instability and, in particular, microbial contamination.

**Production**

Creams should be packed in well-closed containers fitted with closures that minimise contamination with micro-organisms. When practicable, creams should be packed in collapsible tubes of suitable metal or plastic.

During manufacture, packaging, storage and distribution of creams, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 5.9.

**Tests**

Creams comply with the requirements of tests stated under the individual monographs and with the following requirements.

**Uniformity of weight.** Comply with the test for contents of packaged dosage forms (2.5.6).

**Sterility.** When the cream is labelled as sterile, it complies with the test for sterility (2.2.11).

**Storage.** Store at temperatures below 25°C unless otherwise directed. Do not freeze.

**Labelling.** The label states (1) that the cream is sterile, where necessary; (2) the name and concentration of any added antimicrobial preservative; (3) the storage conditions.

* The term basis as a synonym for base in some of the monographs means a carrier, composed of one or more excipients, for the active pharmaceutical ingredient(s) in semi-solid and solid preparations.

**Ear Drops**

Otic Drops; Otic Solutions

Ear Drops are aqueous or oily solutions or suspensions of one or more medicaments intended for instillation into the outer ear. They may contain suitable auxiliary substances such as buffers, stabilising agents, dispersing agents, solubilising agents and agents to adjust the tonicity or viscosity of the preparation. However, if buffering agents are used in preparations intended for use in surgical procedures, care should be taken to ensure that the nature and concentration of the selected agents are suitable. Where the active ingredients are susceptible to oxidative degradation, a suitable antioxidant may be added but care should be taken to ensure compatibility between the antioxidant and the other ingredients of the preparations. Any additive in the preparation should not adversely affect the intended medicinal action nor, at the concentrations used, cause undue local irritation. Certain Ear Drops may be supplied in dry, sterile form to be constituted in an appropriate sterile liquid immediately before use.

Aqueous preparations supplied in multiple application containers contain suitable antimicrobial preservatives at appropriate concentrations except when the product itself has adequate antimicrobial properties. The antimicrobial preservatives should be compatible with the other ingredients of the preparation and should be effective throughout the period of use of the Ear Drops. Containers for multiple application preparations should permit the withdrawal of successive doses of the preparation. Such containers should normally hold not more than 10 ml.

During development of a formulation of ear drops containing an antimicrobial preservative, the need for and the efficacy of the chosen preservative shall be demonstrated by the test for efficacy of antimicrobial preservation (2.2.2).

During manufacture, packaging, storage and distribution of ear drops, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 5.9.
Ear Drops intended for use in surgical procedures or for application to injured ear, are sterile. Such preparations should not contain antimicrobial preservatives and should be packed in single dose containers.

**Production**

Sterile Ear Drops are prepared using methods designed to ensure their sterility and to avoid the introduction of contaminants and growth of micro-organisms. Methods of sterilisation that may be used in the manufacture of Ear Drops are described in Chapter 5.3.

**Description.** Ear Drops that are solutions are practically clear and practically free from particles when examined under suitable conditions of visibility. Ear Drops that are suspensions may show a sediment that readily disperses when shaken. The suspension remains sufficiently dispersed to enable the correct dose to be removed from the container.

**Tests**

**Uniformity of volume.** Comply with the test for contents of packaged dosage forms (2.5.6).

**Particle size.** This test is applicable only to Ear Drops that are suspensions. Introduce a suitable volume of the Ear Drops into a counting cell or onto a microscope slide, as appropriate. Scan under a microscope an area corresponding to 10 µg of the solid phase. Scan at least 50 representative fields. Not more than 20 particles have a maximum dimension greater than 50 µm, not more than 10 particles have a maximum dimension greater than 20 µm and none has a maximum dimension greater than 100 µm.

**Sterility.** Where the label indicates that the Ear Drops are sterile, it complies with the test for sterility (2.2.11). Droppers supplied separately also comply with these tests. Remove the dropper out of the package using aseptic precautions and transfer it to a tube containing suitable culture medium so that it is completely immersed. Incubate and carry out the tests for sterility on the medium.

**Storage.** Ear Drops should be packed in well-closed containers. If the preparation is sterile, store in sterile, tightly-closed, tamper-evident containers. Containers should be made from materials that do not cause deterioration of the preparation as a result of diffusion into or across the material of the container or by yielding foreign substances to the preparation.

The container and package of a single application preparation should be such as to maintain sterility of the contents and the applicator up to the time of use. Containers for multiple application preparations should be fitted with an integral dropper or with a screw cap made of suitable material incorporating a dropper and plastic or rubber teat. Alternatively, such a cap assembly may be packed separately.

**Labelling.** The label states (1) the names and concentrations in percentages, or weight or volume per ml, of the active ingredient(s); (2) the names and concentrations of any added antioxidant, stabilising agent or antimicrobial preservative; (3) that, for multiple application containers, the contents should not be used for more than 1 month after opening the container; (4) that, for multiple application containers, care should be taken to avoid contamination of the contents during use; (5) that the preparation is NOT FOR INJECTION; (6) that, where applicable, the preparation is sterile; (7) the storage conditions.

**Eye Drops**

**Ophthalmic Drops**

Eye Drops are sterile, aqueous or oily solutions or suspensions of one or more medicaments intended for instillation into the conjunctival sac. They may contain suitable auxiliary substances such as buffers, stabilising agents, solubilising agents and agents to adjust the tonicity or viscosity of the preparation. However, if buffering agents are used in preparations intended for use in surgical procedures care should be taken to ensure that the nature and concentration of the selected agents are suitable. Where the active ingredient is susceptible to oxidative degradation, a suitable antioxidant may be added but care should be taken to ensure compatibility between the antioxidant and the other ingredients of the preparation. Any additive in the preparation should not adversely affect the intended medicinal action nor, at the concentrations used, cause undue local irritation. Certain Eye Drops may be supplied in dry, sterile form to be constituted in an appropriate sterile liquid immediately before use.

Aqueous preparations supplied in multiple application containers contain suitable antimicrobial preservatives at appropriate concentrations except when the product itself has adequate antimicrobial properties. The antimicrobial preservatives should be compatible with the other ingredients of the preparation and should be effective throughout the period of use of the Eye Drops.

If the preparation does not contain an antimicrobial preservative it should be packed in single application containers. Eye Drops intended for use in surgical procedures should not contain antimicrobial preservatives and should be packed in single application containers.

Eye Drops are prepared using methods designed to ensure their sterility and to avoid the introduction of contaminants and growth of micro-organisms. Methods of sterilisation that may be used in the manufacture of Eye Drops are described in chapter 5.3.

**Containers.** Eye Drops should be packed in tamper-evident containers. Containers should be made from materials that do
not cause deterioration of the preparation as a result of diffusion into or across the material of the container or by yielding foreign substances to the preparation.

The container and package of a single dose preparation should be such as to maintain sterility of the contents and the applicator up to the time of use. Containers for multiple application preparations should be fitted with an integral dropper or with a sterile screw cap of suitable materials incorporating a dropper and plastic or rubber teat. Alternatively, such a cap assembly may be packed separately after it is sterilised. Containers of multiple application preparations should permit the withdrawal of successive doses of the preparation. Such containers should normally hold not more than 10 ml.

**Description**. Eye Drops that are solutions are practically clear and practically free from particles when examined under suitable conditions of visibility. Eye Drops that are suspensions may show a sediment that readily disperses when shaken. The suspension remains sufficiently dispersed to enable the correct dose to be removed from the container.

**Tests**

**Uniformity of volume**. Comply with the test for contents of packaged dosage forms (2.5.6).

**Particle size**. This test is applicable only to Eye Drops that are suspensions. Introduce a suitable volume of the Eye Drops into a counting cell or onto a microscope slide, as appropriate. Scan under a microscope an area corresponding to 10 µg of the solid phase. Scan at least 50 representative fields. Not more than 20 particles have a maximum dimension greater than 25 µm, not more than 10 particles have a maximum dimension greater than 50 µm and none has a maximum dimension greater than 100 µm.

**Sterility**. Comply with the test for sterility (2.2.11). Droppers supplied separately also comply with these tests. Remove the dropper out of the package using aseptic precautions and transfer it to a tube containing suitable culture medium so that it is completely immersed. Incubate and carry out the test.

**Storage**. Store in sterile containers sealed so as to protect from micro-organisms.

**Labelling**. The label states (1) the names and concentrations in percentages, or weight or volume per ml, of the active ingredients; (2) the names and concentrations of any added antimicrobial preservative; (3) that, for multiple application containers, the contents should not be used for more than 1 month after opening the container; (4) that, for multiple application containers, care should be taken to avoid contamination of the contents during use; (5) that the preparation is NOT FOR INJECTION; (6) the conditions under which the preparation should be stored.

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**Eye Ointments**

**Ophthalmic Ointments**

Eye Ointments are sterile, semi-solid preparations of homogenous appearance intended for application to the eye. They may contain one or more medicaments dissolved or dispersed in a suitable basis. Bases, which are usually non-aqueous, may contain suitable auxiliary substances such as stabilising agents, antimicrobial preservatives and antioxidants. The base selected must be non-irritant to the conjunctiva, allow the drug to diffuse throughout the secretions of the eye and retain the activity of the medicaments for a reasonable period of time under the stated conditions of storage.

Eye Ointments are prepared using methods designed to ensure their sterility and to avoid the introduction of contaminants and growth of micro-organisms. Methods of sterilisation that may be used in the manufacture of Eye Ointments are described in Chapter 5.3.

**Containers**. Eye Ointments should be packed in small, sterilised collapsible tubes of metal or of suitable plastic fitted or provided with a nozzle of suitable shape to facilitate the application of the product without contamination and with a cap. The content of such containers is not more that 5 g of the preparation. Eye Ointments may also be packed in single application containers of such a shape as to facilitate administration without contamination; such containers may be individually wrapped. Other requirements concerning containers are given in Chapter 6.2.

**Tests**

**Uniformity of weight**. Comply with the test for contents of packaged dosage forms (2.5.6).

**Particle size**. Gently spread a small quantity of the Eye Ointment as a thin layer on a microscope slide. Scan under a microscope an area corresponding to 10 µg of the solid phase. Scan at least 50 representative fields. Not more than 20 particles have a maximum dimension greater than 25 µm, not more than 10 particles have a maximum dimension greater than 50 µm and none has a maximum dimension greater than 100 µm.

**Sterility** (2.2.11). Comply with the test for sterility.

**Storage**. Store at temperatures below 30 º unless otherwise directed. Do not freeze.

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**Gels**

Gels are homogeneous, semi-solid preparations usually consisting of solutions or dispersions of one or more medicaments in suitable hydrophilic or hydrophobic bases.
They are normally prepared with the aid of suitable gelling agents. They are intended to be applied to the skin or certain mucous membranes for protective, prophylactic or therapeutic purposes. Gels may contain suitable added substances such as antioxidants, stabilisers and antimicrobial preservatives.

During manufacture, packaging, storage and distribution of gels, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 5.9.

Gels specifically intended for use on large open wounds or on severely injured skin should be sterile.

Containers. Gels should be packed in suitable well-closed or, if the preparation contains water or other volatile ingredients, suitable tightly-closed containers. The containers should be fitted with closures that minimise contamination with microorganisms. To the extent possible, collapsible tubes of suitable metal or plastic should be used.

Storage. Store at temperatures below 30° unless otherwise directed. Do not freeze.

Labelling. The label states (1) that the gel is sterile, where necessary; (2) the storage conditions.

Tests

Uniformity of weight. Comply with the test for contents of packaged dosage forms (2.5.6).

Sterility. Gels labelled as sterile comply with the test for sterility (2.2.11).

**Inhalation Preparations**

Inhalation Preparations are liquid or solid dosage forms intended for administration as vapours or aerosols to the lung in order to obtain a local or systemic effect. They contain solutions or dispersions of one or more active ingredients which may be dissolved or dispersed in a suitable vehicle.

Inhalation Preparations contain propellants, diluents, antimicrobial agents, solubilising and stabilising agents etc. depending on the type of preparation. They are available in single-dose or multidose containers.

Inhalation Preparations intended to be administered as aerosols (dispersions of solid or liquid particles of active ingredient(s) in a gas) are administered by pressurized metered-dose inhalers or by powder inhalers.

**Production**

Inhalation preparations should be manufactured in conditions designed to minimise microbial and particulate contamination.

During the development of a preparation that contains an antimicrobial preservative, the effectiveness of the preservative selected, shall be determined as described in chapter 2.2.2 (Efficacy of antimicrobial preservation).

The size of aerosol particles shall be controlled so that a significant fraction is deposited in the lung.

The most commonly used method of preparation involves filling under pressure and sometimes by filling after refrigeration to temperatures below 0°. In filling under pressure, the requisite volume of the concentrate of the active ingredient(s) is filled in the container and either the propellant is forced under pressure through the valve orifice after the valve is sealed, or the propellant is allowed to flow under the valve cap and the valve assembly is sealed. In either case, the air in the container must be evacuated by means of vacuum or displacement with a small amount of the propellant.

During production, strict control should be exercised by process controls that include propellant and medicament fill weights, pressure test and leak test of the finished product.

For preparations adversely affected by water present in quantities beyond certain limits, care should be taken to protect the products from moisture.

**Storage.** Avoid storage under extremes of temperature and in an environment with undue fluctuations in temperature.

Labelling. The label states (1) the name(s) of the active ingredient(s); (2) the total amount of the active ingredient(s) in the container except in the case of metered-dose preparation for inhalation); (3) that the container should be shaken before use; (4) the other instructions for use; (5) the date after which the preparation is not intended to be used; (6) the conditions under which it should be stored; (7) a warning that the container is under pressure and that it must not be punctured, broken or incinerated even when apparently empty; (8) the statement “Warning. Keep away from children”.

In the case of metered-dose aerosols and pressurized metered dose inhalers, the label states in addition (1) the total number of deliveries available from the container; (2) the amount of active ingredient(s) released each time the valve is actuated.

In the case of dry powder inhalers the label on the container states (1) the date after which the dry powder inhaler is not intended to be used; (2) the conditions under which the powder for Inhalation should be stored. Where the powder for Inhalation is supplied in a capsule, the label also states (3) the quantity of the active ingredient contained in each capsule; (4) that the capsules are intended for use in an inhaler and are not to be swallowed.

Information on use of the preparation provided in the pack shall include (1) the direction for correct use of the aerosol; (2) a warning that the container may explode if punctured, exposed to excessive heat or direct sunlight; (3) the directions for the disposal of the used or partly-used container.
Pressurised metered-dose preparations are solutions, suspensions or emulsions supplied in containers equipped with a metering valve and which are held under pressure with suitable propellants or mixtures of liquefied propellants.

Pressurised Metered Dose Inhalers are dosage forms containing therapeutically active ingredients that are packaged under pressure in a sealed container and are released as a fine mist of spray upon actuation of a suitable valve system.

The basic components of an aerosol system are the container, the propellant, the concentrate containing the active ingredient(s), the valve and the actuator.

Pressurised metered dose preparations are of two types, the two-phase system consisting of gas and liquid or the three-phase system consisting of gas, liquid and solid or liquid. The two-phase preparation comprises a solution of active ingredient(s) in liquefied propellant and the vapourised propellant. The solvent is usually the propellant or a mixture of the propellant and co-solvents such as ethanol, propylene glycol and polyethylene glycols. The three-phase preparation consists of a suspension or emulsion of the active ingredient(s) and the vapourised propellants. In the suspension the ingredient(s) may be dispersed in the propellant system with the aid of suitable pharmaceutical aids such as wetting agents, solubilising agents, emulsifying agents, suspending agents and lubricating agents to prevent clogging of valves.

Active ingredients. For satisfactory bioavailability the active ingredient(s) should have the majority of particles under 10 µm in size in the case of inhalation aerosols and not more than 100 µm for other types of aerosols.

Propellants. For pressurised metered dose inhalations propellants perform the essential function of expelling the material from the container by supplying the necessary pressure within the aerosol system. They are liquefied or compounded gases having vapour pressures exceeding atmospheric pressure. The commonly used propellants in aerosol systems are hydrocarbons, especially the fluorochloro-derivatives of methane and ethane, the butanes and pentanes and compressed gases such as nitrogen and carbon dioxide. Mixtures of propellants are often employed to obtain the necessary delivery and spray characteristics of the aerosol.

Valves. The valve regulates the flow of the active ingredient(s) and propellant from the container and determines the spray characteristics of the aerosol. It must be manufactured from materials which are inert to the contents of the aerosol. The commonly used materials are rubber, plastic, aluminium and stainless steel.

Products for oral or nasal inhalation require metered-dose valves which ensure delivery of a uniform quantity of spray and an accurate dose of the active ingredient(s), both within specified tolerances, with each activation of the valve.

Metered valves may need priming before use if the aerosol packages have not been stored properly or have not been used for long periods of time.

Actuators. The actuator or adaptor which is fitted to the aerosol valve stem is a device which on depression or any other required movement opens the valve and directs the spray to the desired area. The design of the actuator which incorporates an orifice of varying size and shape and expansion chamber is very important in influencing the physical characteristics of the spray or foam, particularly in the case of inhalation aerosols, where the active ingredient(s) must be delivered in the proper particle size range. A proportion of the active ingredient(s) is usually deposited on the inner surface of the actuator; the amount available is therefore less than the amount released by actuation of the valve.

Containers. Aerosol containers are made of metal (stainless steel, aluminum or tin-plated steel), glass or plastic or a combination of these materials. The containers must be so designed that they provide the maximum in pressure safety and impact resistance.

Tests

Pressurised Metered-dose Preparations

Content of active ingredient delivered per actuation.

Apparatus

A small sample vessel suitable for shaking. The size of the vessel is such that when the aerosol is discharged into the specified volume of solvent under the conditions described in the Method below, the discharge takes place not less than 25 mm below the surface of the solvent. A stainless steel base plate with 3 legs and a central circular indentation with a hole about 1.5 mm in diameter is placed in the sample vessel. The arrangement should prevent particle entrapment and side-of-stem leakage during the delivery of the sample.

Procedure

Remove the pressurised container from the actuator and remove all labels and markings which may be present on the container with a suitable solvent. Dry the container, replace in its actuator, shake for about 30 seconds and holding it in an inverted position actuate the valve by discharging about 5 sprays to waste. Remove the pressurised container from its actuator, clean the valve stem (internally and externally) and valve ferrule by washing with a suitable solvent. Dry the complete valve assembly using an air-supply line fitted with an appropriate narrow jet to ensure that all solvent is removed from the inside of the valve stem. Wash the actuator after the initial discharge of 5 sprays to waste, with a suitable solvent and allow it to dry.
For test solution add to the sample vessel a volume of solvent or solvent mixture specified in the monograph so that the final concentration of the active ingredient in the test solution corresponds to the reference solution. Shake the pressurised container for about 30 seconds and place it inverted in the vessel. Discharge 10 deliveries below the surface of the solvent actuating the valve at intervals of not less than 5 seconds, maintaining the pressurised container in the vertical plane and discharging the aerosol through the hole in the centre of the base plate. With some preparations it may be necessary to shake the pressurised container between each actuation of the valve; in such cases shaking should be carried out without removing the pressurised container from its inverted position in the vessel. Remove the pressurised container, wash it with the specified solvent and dilute the combined solution and washings to the volume specified in the monograph. Determine the amount of active ingredient by the method described under Assay in the individual monograph. This amount of active ingredient is referred to as metered dose assay (A) for metered dose inhalers.

Fit the washed and dried actuator to the pressurised container and actuate the valve 10 times at intervals of not less than 5 seconds. Remove the actuator carefully from the pressurised container and wash it with small quantities of the specified solvent or solvent mixture. Dilute the combined washings suitably and on the resulting solution determine the amount of active ingredient as per the method given in the individual monograph under the test for ‘Content of active ingredient delivered per actuation’ and calculate the amount of active ingredient per actuation of the valve. This amount of active ingredient is referred to as actuator retention (B) for metered dose inhalers.

Calculate the content of active ingredient delivered per actuation from the expression A – B.

**Uniformity of delivered dose**

The delivered dose is the dose delivered from the inhaler to the patient. For some preparations, the dose has been established as a metered dose. The metered dose is determined by adding the amount deposited on the inhaler device to the delivered dose. It may also be determined directly.

The test is applicable to inhalation preparations containing the drug formulation (e.g., solution, suspension, or powder) either in reservoirs or in premetered dosage units, and for drug formulations packaged in reservoirs or in premetered dosage units where these containers are labeled for use with a named inhalation device.

**Apparatus**

Most of the containers usually operate in a valve-down position. For those containers that operate in a valve-up position, an equivalent test is applied using methods that ensure the complete collection of the delivered dose.

For all the cases, prepare the inhaler as directed in the instructions to the patient and connect to a dose collection apparatus, which must be capable of quantitatively capturing the delivered dose (see Fig. 1).

The apparatus consists of a filter-support base with an open-mesh filter-support, such as a stainless steel screen, a sample collection tube that is clamped or screwed to the filter-support base, and a mouthpiece adapter to ensure an airtight seal between the sample collection tube and the mouthpiece. Use a mouthpiece adapter which ensures that the front face of the inhaler mouthpiece fits with the front face or the 2.5 mm indented shoulder of the sample collection tube, as appropriate. The vacuum connector is connected to a system comprising a vacuum source and a flow regulator. The source should be adjusted to draw air through the complete assembly, including the filter and the inhaler to be tested, at 28.3 litres per minutes (± 5 per cent). Air should be drawn continuously through the apparatus to avoid loss of the active substance into the atmosphere. The filter-support base is designed to accommodate 25 mm diameter filter disks.

The filter disk and other materials used in the construction of the apparatus must be compatible with the active substance and solvents that are used to extract the active substance from the filter.

One end of the collection tube is designed to hold the filter disk tightly against the filter-support base. When assembled, the joints between the components of the apparatus are airtight so that when a vacuum is applied to the base of the filter, all of the air drawn through the collection tube passes through the inhaler.

**Procedure**

Unless otherwise prescribed in the instructions to the patient, shake the inhaler for 5 seconds and discharge one delivery to waste. Attach the inverted inhaler to the apparatus, depressing the valve for a sufficient time to ensure complete discharge. Repeat the procedure until the number of deliveries that constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance.

Repeat the procedure for a further 2 doses.

Discharge the device to waste, waiting not less than 5 seconds between actuations until \((n/2) + 1\) deliveries remain, where \(n\) is the number of deliveries stated on the label. Collect 4 doses using the procedure described above.

Discharge the device to waste, waiting not less than 5 seconds between actuations until 3 doses remain. Collect these 3 doses using the procedure described above.
For preparations containing more than one active substance, carry out the test for uniformity of delivered dose for each active substance.

**Acceptance criteria**

Unless otherwise justified and authorised, the preparation complies with the test if 9 out of 10 results lie between 75 per
cent and 125 per cent of the average value and all lie between 65 per cent and 135 per cent. If 2 or 3 values lie outside the limits of 75 per cent to 125 per cent, repeat the test for 2 more inhalers. Not more than 3 of the 30 values lie outside the limits of 75 per cent to 125 per cent and no value lies outside the limits of 65 per cent to 135 per cent.

**Particle size**

*NOTE — Carry out the test in a laminar flow cabinet. Filter all solvents through an appropriately sized filter before use.*

Assemble a suitable membrane filtration apparatus. Use a filter holder fitted with an input chamber designed to prevent any loss of material when the actuator mouthpiece of the aerosol is inserted and the valve actuated. Before assembly wash all parts of the membrane filter holder with water and methanol and dry in a stream of nitrogen or allow to dry in a laminar flow cabinet. Use a membrane filter with a nominal pore size not greater than 5 µm and with the filtering surface free from foreign particles when examined microscopically using a magnification of not less than ×40.

Discharge 50 deliveries from the pressurised container into the orifice of the input chamber, actuating the valve at intervals of not less than 5 seconds and washing down the particles deposited in the input chamber with successive 10-ml quantities of light petroleum (40° to 60°), ethanol (95 per cent) and water after 20, 40 and 50 actuations of the valve. Remove the pressurised container and dry the membrane filter. Examine its entire filtering surface microscopically using a magnification of not less than ×40. Record the number and size of all individual particles (not agglomerates) more than 10 µm in length measured along the longest axis. The number of particles longer than 20 µm does not exceed 50 and no particle exceeds 100 µm in length.

**Number of deliveries per container.** Take the pressurised container used in the test for Particle size and discharge the remaining contents to waste, actuating the valve at intervals of not less than 5 seconds. Record the number of deliveries discharged. The total number of deliveries so discharged in the test for Particle size is not less than the number stated on the label.

**Leak test.** Select 12 pressurised containers at random, and record the date and time to the nearest half-hour. Weigh each container to the nearest mg, and record the weight, in mg, of each as \( W_i \). Allow the container to stand in an upright position at room temperature for not less than 3 days, and again weigh each container, recording the weight, in mg, of each as \( W_i \) and recording the date and time to the nearest half-hour. Determine the time, \( T \), in hours, during which the containers were under test. Calculate the leakage rate, in mg per year, of each container from the expression 365 x 24/\( T \times (W_i - W_i) \).

Empty the contents of each container tested by chilling to reduce the internal pressure, removing the valve and pouring. Remove any residual contents by rinsing with suitable solvents, then rinse with a few portions of methanol. Retain as a unit the container, the valve, and all associated parts, and heat them at 100° for 5 minutes. Cool, weigh and record the weight as \( W_j \), and determine the net fill weight (\( W_i - W_j \)) for each container tested.

The requirements are met if the average leakage rate of the 12 containers is not more than 3.5 per cent of the net fill weight per year and none of the containers leaks more than 5.0 per cent of the net fill weight per year. If 1 container leaks more than 5.0 per cent per year, and if none of the containers leaks more than 7.0 per cent per year, determine the leakage rate of an additional 24 containers as directed herein. Not more than 2 of the 36 containers leak more than 7.0 per cent of the net fill weight per year.

Where the net fill weight is less than 15 g the requirements are met if the average leakage rate of the 12 containers is not more than 525 mg per year and none of the containers leaks more than 750 mg per year. If 1 container leaks more than 750 mg per year but not more than 1.1 g per year, determine the leakage rate of an additional 24 containers as directed herein. Not more than 2 of the 36 containers leak more than 750 mg per year and none of the 36 containers leaks more than 1.1 g per year.

**Deposition of the emitted dose**

The deposition of the emitted dose is a measure of the drug deposition during inhalation. This test is used to determine the fine particle characteristics of the aerosol clouds generated by preparations for inhalation and may be expected to correlate with the drug dose or that fraction of the drug dose that penetrates the lung during inhalation. Individual monographs may also define the emitted fractions of the delivered dose in more than one particle size range.

**Stage Mensuration.** Manufacturers of cascade impaction devices provide a definitive calibration for the separation characteristics of each impaction stage in terms of the relationship between the stage collection efficiency and the aerodynamic diameter of particles and droplets passing through it as an aerosol. Calibration is a property of the jet dimensions, the spatial arrangement of the jet and its collection surface, and the airflow rate passing through it. Because jets can corrode and wear over time, the critical dimensions of each stage, which define that impaction stage’s calibration, must be measured on a regular basis. This process, known as stage mensuration, replaces the need for repetitive calibration (using standard aerosols) and ensures that only devices that conform to specifications are used for testing inhaler output. The process involves the measurement and adjustment of the critical dimensions of the instrument.

**Re-entrainment (for apparatus B).** To ensure efficient particle...
capture, coat each plate with glycerol, silicone oil or similar high viscosity liquid, typically deposited from a volatile solvent. Plate coating must be part of method validation and may be omitted where justified and authorised.

Mass balance. The total mass of the active substance is not less than 75 per cent and not more than 125 per cent of the average delivered dose determined during testing for uniformity of delivered dose. This is not a test of the inhaler but it serves to ensure that the results are valid.

Unless otherwise specified, one of the following apparatus and test procedures is used.

Apparatus A. Glass impinger

The apparatus is shown in Fig. 2 and the dimensions are given in Table 1.

Procedure

Place the actuator adapter in position at the end of the throat so that the mouthpiece end of the actuator, when inserted to a depth of about 10 mm, lines up along the horizontal axis of the throat and the open end of the actuator, which accepts the pressurised container, is uppermost and in the same vertical plane as the rest of the apparatus.

Introduce 7 ml and 30 ml of a suitable solvent into the upper and lower impingement chambers, respectively.

Connect all the component parts. Ensure that the assembly is vertical and adequately supported and that the lower jet-spacer peg of the lower jet assembly just touches the bottom of the lower impingement chamber. Connect a suitable pump to the outlet of the apparatus. Adjust the air flow through the apparatus, as measured at the inlet to the throat, to 60 ± 5 litres per minute.

Prime the metering valve by shaking for 5 seconds and discharging once to waste; after not less than 5 seconds, shake and discharge again to waste. Repeat for further 3 times.

Shake for about 5 seconds, switch on the pump to the apparatus and locate the mouthpiece end of the actuator in

Dimensions in millimeters (tolerances ± 1 mm, unless otherwise specified)

Fig. 2: Apparatus A. Glass impinger
Table 1

<table>
<thead>
<tr>
<th>Code</th>
<th>Item</th>
<th>Description</th>
<th>Dimensions</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Mouthpiece adaptor</td>
<td>Moulded rubber adapter for actuator mouthpiece.</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Throat</td>
<td>Modified round-bottomed flask: ground-glass inlet socket 29/32</td>
<td>50ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ground-glass outlet cone 24/29</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Neck</td>
<td>Modified glass adapter: ground-glass inlet socket 24/29</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ground-glass outlet cone 24/29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lower outlet section of precision-bore</td>
<td>bore diameter 14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>glass tubing:</td>
<td>Selected bore light-wall glass tubing: external diameter 17</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Upper impingement chamber</td>
<td>Modified round-bottomed flask</td>
<td>100ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ground-glass inlet socket 24/29</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ground-glass outlet cone 24/29</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>Coupling tube</td>
<td>Medium-wall glass tubing: ground-glass cone 14/23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bent section and upper vertical section:</td>
<td>external diameter 13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lower vertical section:</td>
<td>external diameter 8</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>Screw thread, side-arm adaptor</td>
<td>Plastic screw cap 28/13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Silicone rubber ring 28/11</td>
<td>PTFE washer 28/11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glass screw thread:</td>
<td>thread size 28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Side-arm outlet to vacuum pump:</td>
<td>minimum bore diameter 5</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>Lower jet assembly</td>
<td>Modified polypropylene filter holder connected to lower vertical section</td>
<td>See Figure1</td>
</tr>
<tr>
<td></td>
<td>of coupling tube by PTFE tubing</td>
<td>Acetal circular disc with the centres of four jets 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>arranged on a projected circle of diameter 5.3 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>with an integral jet spacer peg: peg diameter 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>peg protrusion 2</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>Lower impingement chamber</td>
<td>Conical flask 250ml</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ground-glass inlet socket 24/29</td>
<td></td>
</tr>
</tbody>
</table>

the adapter, discharge once immediately. Remove the assembled inhaler from the adapter, shake for not less than 5 seconds, relocate the mouthpiece end of the actuator in the adapter and discharge again. Repeat the discharge sequence. The number of discharges should be minimised and typically would not be greater than 10. After the final discharge wait for not less than 5 seconds and then switch off the pump. Dismantle the apparatus.
Wash the inner surface of the inlet tube to the lower impingement chamber and its outer surface that projects into the chamber with a suitable solvent, collecting the washings in the lower impingement chamber. Determine the content of active substance in this solution. Calculate the amount of active substance collected in the lower impingement chamber per discharge and express the results as a percentage of the dose stated on the label.

**Apparatus B. Andersen Cascade impactor**

The Andersen 1 ACFM non-viable cascade impactor consists of 8 stages together with a final filter. Material of construction may be aluminium, stainless steel or other suitable material. The stages are clamped together and sealed with O-rings. Critical dimensions applied by the manufacturer of apparatus B are provided in Table 2. In use, some occlusion and wear of holes will occur. In-use mensuration tolerances need to be justified. In the configuration used for pressurised inhalers (Fig. 3) the entry cone of the impactor is connected to an induction port (see Fig. 4). A suitable mouthpiece adapter is used to provide an airtight seal between the inhaler and the induction port. The front face of the inhaler mouthpiece must be flush with the front face of the induction port.

<table>
<thead>
<tr>
<th>Description</th>
<th>Number</th>
<th>Dimension (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 0 nozzle diameter</td>
<td>96</td>
<td>2.55 ± 0.025</td>
</tr>
<tr>
<td>Stage 1 nozzle diameter</td>
<td>96</td>
<td>1.89 ± 0.025</td>
</tr>
<tr>
<td>Stage 2 nozzle diameter</td>
<td>400</td>
<td>0.914 ± 0.0127</td>
</tr>
<tr>
<td>Stage 3 nozzle diameter</td>
<td>400</td>
<td>0.711 ± 0.0127</td>
</tr>
<tr>
<td>Stage 4 nozzle diameter</td>
<td>400</td>
<td>0.533 ± 0.0127</td>
</tr>
<tr>
<td>Stage 5 nozzle diameter</td>
<td>400</td>
<td>0.343 ± 0.0127</td>
</tr>
<tr>
<td>Stage 6 nozzle diameter</td>
<td>400</td>
<td>0.254 ± 0.0127</td>
</tr>
<tr>
<td>Stage 7 nozzle diameter</td>
<td>201</td>
<td>0.254 ± 0.0127</td>
</tr>
</tbody>
</table>

In the configuration for powder inhalers, a pre-separator is placed above the top stage to collect large masses of non-
respirable powder. It is connected to the induction port as shown in Fig. 5. To accommodate high flow rates through the impactor, the outlet nipple, used to connect the impactor to the vacuum system is enlarged to have an internal diameter of greater than or equal to 8 mm.

**Procedure**

Assemble the Andersen impactor with a suitable filter in place. Ensure that the system is airtight. In that respect, follow the manufacturer’s instructions. Place a suitable mouthpiece

---

**Note:**

1. Material may be aluminium, stainless steel or other suitable material.
2. Machine from 38 mm bar stock.
3. Bore 19 mm hole through bar.
4. Cut tube to exact 45° as shown.
5. The inner bores and tapers should be smooth – surface roughness Ra approx. 0.4 µm.
6. Mill joining cads of stock to provide a liquid tight leak-free seal.
7. Set up a holding fixture for aligning the inner 19 mm bore and for drilling and tapping M4 x 0.7 threads. There must be virtually no mismatch of the inner bores in the miter joint.

*Dimensions in millimeters unless otherwise stated*

Fig. 4: Induction port
adapter in position at the end of the induction port so that the mouthpiece end of the actuator, when inserted, lines up along the horizontal axis of the induction port and the inhaler unit is positioned in the same orientation as the intended use. Connect a suitable pump to the outlet of the apparatus and adjust the air flow through the apparatus, as measured at the inlet to the induction port, to 28.3 litres per minute (± 5 per cent). Switch off the pump.

Unless otherwise prescribed in the patient instructions, shake the inhaler for 5 seconds and discharge one delivery to waste. Switch on the pump to the apparatus, locate the mouthpiece end of the actuator in the adapter and discharge the inverted inhaler into the apparatus, depressing the valve for a sufficient time to ensure complete discharge. Wait for 5 seconds before removing the assembled inhaler from the adapter. Repeat the procedure. The number of discharges should be minimised and typically would not be greater than 10. The number of discharges is sufficient to ensure an accurate and precise determination of the fine particle dose. After the final discharge, wait for 5 seconds and then switch off the pump.

Dismantle the apparatus. Carefully remove the filter and extract the active substance into an aliquot of the solvent. Remove

---

*(Dimensions are in millimeters unless otherwise stated)*

Fig. 5: Connection of the induction port to the preseparator of the Andersen cascade impactor
the induction port and mouthpiece adapter from the apparatus
and extract the active substance into an aliquot of the solvent.
Extract the active substance from the inner walls and the
collection plate of each of the stages of the apparatus into
aliquots of solvent.
Using a suitable method of analysis, determine the quantity
of active substance contained in each of the aliquots of
solvent.
Calculate the fine particle dose as described below.

Calculations

From the analysis of the solutions, calculate the mass of active
substance deposited on each stage per discharge and the
mass of active substance per discharge deposited in the
induction port, mouthpiece adapter and when used, the pre-
separator.

Starting at the final collection site (filter or MOC), derive a
table of cumulative mass versus cut-off diameter of the
respective stage (see Table 3). Calculate by interpolation
the mass of the active substance less than 5 µm. This is the
Fine Particle Dose (FPD).

If necessary, and where appropriate (e.g., where there is a log-
normal distribution), plot the cumulative fraction of active
substance versus cut-off diameter (see Table 4) on log
probability paper, and use this plot to determine values for
the Mass Median Aerodynamic Diameter (MMAD) and
Geometric Standard Deviation (GSD) as appropriate.
Appropriate computational methods may also be used.

Powders for Inhalation

Powders for inhalation are presented as single-dose powders
or multidose powders. To facilitate their use, active substances
may be combined with a suitable carrier. They are generally
administered by powder inhalers. For pre-metered inhalers,
the inhaler is loaded with powders pre-dispensed in capsules
or other suitable pharmaceutical forms. For inhalers using a
powder reservoir, the dose is created by a metering mechanism
within the inhaler.

They are intended either for inhalation for local action in the
lungs or for systemic absorption through the alveoli or for
topical application to the skin or various body orifices.
Inhalation aerosols are metered dose preparations which
provide controlled amounts of the active ingredient(s).

Tests

Uniformity of delivered dose

Procedure

Prepare the inhaler as directed in the instructions to the patient.
The dose collection apparatus must be capable of
quantitatively capturing the delivered dose. A dose collection
apparatus similar to that described for the evaluation
of pressurised metered-dose inhalers may be used provided
that the dimensions of the tube and the filter can accommodate
the measured flow rate. A suitable tube is defined in Table 4.
Connect the tube to a flow system according to the
scheme specified in Fig. 6 and Table 4.

Unless otherwise stated, determine the test flow rate and
duration using the dose collection tube, the associated flow
system, a suitable differential pressure meter and a suitable
volumetric flowmeter, calibrated for the flow leaving the
meter, according to the following procedure.

Prepare the inhaler for use and connect it to the inlet of the
apparatus using a mouthpiece adapter to ensure an airtight
seal. Use a mouthpiece adapter which ensures that the front
face of the inhaler mouthpiece fits with the front face of
the sample collection tube. Connect one port of a differential
pressure meter to the pressure reading point, P1, in Figure 6

<table>
<thead>
<tr>
<th>Cut-off diameter (µm)</th>
<th>Mass of active substance deposited per discharge</th>
<th>Cumulative mass of active substance deposited per discharge</th>
<th>Cumulative fraction of active substance (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d0 = 0.4</td>
<td>mass from stage 8, m8</td>
<td>c7 = m8</td>
<td>f7 = (c7/c) × 100</td>
</tr>
<tr>
<td>d5 = 0.7</td>
<td>mass from stage 7, m7</td>
<td>c6 = c7 + m7</td>
<td>f6 = (c6/c) × 100</td>
</tr>
<tr>
<td>d6 = 1.1</td>
<td>mass from stage 6, m6</td>
<td>c5 = c6 + m6</td>
<td>f5 = (c5/c) × 100</td>
</tr>
<tr>
<td>d6 = 2.1</td>
<td>mass from stage 5, m5</td>
<td>c4 = c5 + m5</td>
<td>f4 = (c4/c) × 100</td>
</tr>
<tr>
<td>d5 = 3.3</td>
<td>mass from stage 4, m4</td>
<td>c3 = c4 + m4</td>
<td>f3 = (c3/c) × 100</td>
</tr>
<tr>
<td>d6 = 4.7</td>
<td>mass from stage 3, m3</td>
<td>c2 = c4 + m3</td>
<td>f2 = (c2/c) × 100</td>
</tr>
<tr>
<td>d6 = 5.8</td>
<td>mass from stage 2, m2</td>
<td>c1 = c4 + m2</td>
<td>f1 = (c1/c) × 100</td>
</tr>
<tr>
<td>d6 = 9.0</td>
<td>mass from stage 1, m1</td>
<td>c0 = c1 + m1</td>
<td>f0 = (c0/c) × 100</td>
</tr>
<tr>
<td>d0 = 9.0</td>
<td>mass from stage 0, m0</td>
<td>c = c0 + m0</td>
<td>100</td>
</tr>
</tbody>
</table>

INHALATION PREPARATIONS
Table 4 – Specifications of the apparatus shown in Fig. 6

<table>
<thead>
<tr>
<th>Code</th>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Sample collection tube</td>
<td>Capable of quantitatively capturing the delivered dose, e.g. dose collection tube similar to that described in Figure A with dimensions of 34.85 mm ID x 12 cm length (e.g. product number XX40 047 00, Millipore Corporation, Bedford, MA 01732 with modified exit tube, ID ≥ 8 mm, fitted with Gelman product number 61631), or equivalent.</td>
</tr>
<tr>
<td>B</td>
<td>Filter</td>
<td>47 mm filter, e.g. A/E glass fibre filter (Gelman Sciences, Ann Arbor, MI 48106), or equivalent.</td>
</tr>
<tr>
<td>C</td>
<td>Connector</td>
<td>ID ≥ 8 mm, e.g. short metal coupling, with low-diameter branch to P3</td>
</tr>
<tr>
<td>D</td>
<td>Vacuum tubing</td>
<td>A length of suitable tubing having an ID ≥ 8 mm and an internal volume of 25 ± 5 ml</td>
</tr>
<tr>
<td>E</td>
<td>2-way solenoid valve</td>
<td>A 2-way, 2-port solenoid valve having a minimum airflow resistance orifice with ID ≥ 8 mm and an opening time ≤ 100 ms (e.g. type 256-A08, Burkert GmbH, D-74653 Ingelfingen), or equivalent.</td>
</tr>
<tr>
<td>F</td>
<td>Vacuum pump</td>
<td>Pump must be capable of drawing the required flow rate through the assembled apparatus with the powder inhaler in the mouthpiece adapter (e.g. product type 1023, 1423 or 2565, GAST Manufacturing Inc., Benton Harbor, MI 49022), or equivalent. Connect the pump to the 2-way solenoid valve using short and/or wide (&gt; 10 mm ID) vacuum tubing and connectors to minimize pump capacity requirements.</td>
</tr>
<tr>
<td>G</td>
<td>Timer</td>
<td>Timer capable of driving the 2-way solenoid valve for the required time period (e.g. type G814, RS Components International, Corby, NN17 9 RS, UK), or equivalent.</td>
</tr>
<tr>
<td>P1</td>
<td>Pressure tap</td>
<td>2.2 mm ID, 3.1 mm OD, flush with internal surface of the sample collection tube, centred and burr-free, 59 mm from its inlet. The pressure tap P1 must never be open to the atmosphere.</td>
</tr>
<tr>
<td>P1</td>
<td>Pressure measurements</td>
<td>Differential pressure to atmosphere (P1) or absolute pressure (P2 and P3)</td>
</tr>
<tr>
<td>P2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>Flow control valve</td>
<td>Adjustable regulating valve with maximum Cv ≥ 1, (e.g. type 8FV12LNSS, Parker Hannifin plc., Barnstable, EX31 1NP, UK), or equivalent.</td>
</tr>
</tbody>
</table>

Fig. 6: Apparatus for measuring the uniformity of delivered dose for powders for inhalation
and let the other be open to the atmosphere. Switch on the pump, open the 2-way solenoid valve and adjust the flow control valve until the pressure drop across the inhaler is 4.0 kPa (40.8 cm H₂O) as indicated by the differential pressure meter. Remove the inhaler from the mouthpiece adapter and without touching the flow control valve, connect a flowmeter to the inlet of the sampling apparatus. Use a flowmeter calibrated for the volumetric flow leaving the meter, or calculate the volumetric flow leaving the meter (Q_out) using the ideal gas law. For a meter calibrated for the entering volumetric flow (Q_in), use the following expression:

\[ Q_{\text{out}} = Q_{\text{in}} \times \frac{P_0}{P_0 \times \Delta P} \]

P₀ = Atmospheric pressure.

\( \Delta P = \) Pressure drop over the meter.

If the flow rate is above 100 litres per minutes adjust the flow control valve to obtain a flow rate of 100 litres per minute (± 5 per cent). Note the volumetric airflow rate exiting the meter and define this as the test flow rate, Q_out, in litres per minute. Define the test flow duration, T, in seconds so that a volume of 4 litres of air is drawn from the mouthpiece of the inhaler at the test flow rate, Q_out.

Ensure that critical flow occurs in the flow control valve by the following procedure; with the inhaler in place and the test flow rate Q_out, measure the absolute pressure on both sides of the control valve (pressure reading points P₂ and P₃ in Figure 6). A ratio P₃/P₂ of less than or equal to 0.5 indicates critical flow. Switch to a more powerful pump and re-measure the test flow rate if critical flow is not indicated.

**Predispensed systems:** Prepare the inhaler as directed in the instructions to the patient and connect it to the apparatus using an adapter which ensures a good seal. Draw air through the inhaler using the predetermined conditions. Repeat the procedure until the number of deliveries which constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance.

**Reservoir systems:** Prepare the inhaler as directed in the instructions to the patient and connect it to the apparatus using an adapter which ensures a good seal. Draw air through the inhaler under the predetermined conditions. Repeat the procedure until the number of deliveries which constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance.
Repeat the procedure for a further 2 doses.
Discharge the device to waste until \((n/2)+1\) deliveries remain, where \(n\) is the number of deliveries stated on the label. If necessary, store the inhaler to discharge electrostatic charges. Collect 4 doses using the procedure described above.

Discharge the device to waste until 3 doses remain. If necessary, store the inhaler to discharge electrostatic charges. Collect 3 doses using the procedure described above.

For preparations containing more than one active substance, carry out the test for uniformity of delivered dose for each active substance.

**Acceptance criteria**

The preparation complies with the test if 9 out of 10 results lie between 75 per cent and 125 per cent of the average value and all lie between 65 per cent and 135 per cent. If 2 or 3 values lie outside the limits of 75 per cent to 125 per cent, repeat the test for 2 more inhalers. Not more than 3 of the 30 values lie outside the limits of 75 per cent to 125 per cent and no value lies outside the limits of 65 per cent to 135 per cent.

In justified and authorised cases, these ranges may be extended but no value should be greater than 150 per cent or less than 50 per cent of the average value.

Deposition of emitted dose and fine particle dose

**Apparatus.** Use the apparatus described under Pressurised metered-dose Preparations.

**Procedure**

The aerodynamic cut-off diameters of the individual stages of this apparatus are currently not well-established at flow rates other than 28.3 litres per minute. Users must justify and validate the use of the impactor in the chosen conditions, when flow rates different from 28.3 litres per minute are selected.

Assemble the Andersen impactor with the pre-separator and a suitable filter in place and ensure that the system is airtight. Depending on the product characteristics, the pre-separator may be omitted, where justified and authorised. Stages 6 and 7 may also be omitted at high flow rates, if justified. The pre-separator may be coated in the same way as the plates or may contain 10 ml of a suitable solvent. Connect the apparatus to a flow system according to the scheme specified in Figure 7 and Table 5.

Unless otherwise defined, conduct the test at the flow rate, \(Q_{\text{in}}\), used in the test for uniformity of delivered dose drawing 4 litres of air from the mouthpiece of the inhaler and through the apparatus.

Connect a flowmeter to the induction port. Use a flowmeter calibrated for the volumetric flow leaving the meter, or calculate the volumetric flow leaving the meter \((Q_{\text{out}})\) using the ideal gas law. For a meter calibrated for the entering volumetric flow \((Q_{\text{in}})\), use the following expression:

\[
Q_{\text{out}} = \frac{Q_{\text{in}} \times P_0}{P_0 \times \Delta P}
\]

\(P_0 = \) atmospheric pressure,
\(\Delta P = \) pressure drop over the meter.

Adjust the flow control valve to achieve steady flow through the system at the required rate, \(Q_{\text{out}} (\pm 5 \text{ per cent})\). Switch off the pump. Ensure that critical flow occurs in the flow control valve by the following procedure.

---

Fig. 7: Experimental set-up for testing powder inhalers
With the inhaler in place and the test flow rate established, measure the absolute pressure on both sides of the control valve (pressure reading points P2 and P3 in Figure 7). A ratio P3/P2 of less than or equal to 0.5 indicates critical flow. Switch to a more powerful pump and re-measure the test flow rate if critical flow is not indicated.

Prepare the powder inhaler for use according to the patient instructions. With the pump running and the 2-way solenoid valve closed, locate the mouthpiece of the inhaler in the mouthpiece adapter. Discharge the powder into the apparatus by opening the valve for the required time, $T$ (± 5 per cent). Repeat the discharge sequence. The number of discharges should be minimised and typically would not be greater than 10. The number of discharges is sufficient to ensure an accurate and precise determination of fine particle dose.

Dismantle the apparatus. Carefully remove the filter and extract the active substance into an aliquot of the solvent. Remove the pre-separator, induction port and mouthpiece adapter from the apparatus and extract the active substance into an aliquot of the solvent. Extract the active substance from the inner walls and the collection plate of each of the stages of the apparatus into aliquots of solvent.

Using a suitable method of analysis, determine the quantity of active substance contained in each of the aliquots of solvent.

Calculate the fine particle dose as given under Calculations for Pressurised Metered-dose Preparations.

**Uniformity of Content.** For dry powder inhalers in premetered dosage units, carry out the test for uniformity of content of the contents as given in Capsules.

**Number of deliveries per container.** Discharge doses from the inhaler until empty, at the predetermined flow rate. Record the deliveries discharged. The total number of doses delivered is not less than the number stated on the label.

**Microbial contamination (2.2.9).** Total viable aerobic bacterial count. Not more than 100 cfu per g of the powder.

*E. coli.* Absent in 10 g of the powder.

Salmonella. absent in 50 g of the powder.

*Staphylococcus aureus.* Absent in 10 g of the powder.

*Pseudomonas aeruginosa.* Absent in 10 g of the powder.

**Insulin Preparations**

**Introduction**

Insulin preparations are sterile preparations of human Insulin, bovine Insulin or porcine Insulin intended for subcutaneous injection into the human or animal body. They are either solutions or suspensions or they are prepared by combining solutions and suspensions. They contain not less than 90.0 per cent and not more than the equivalent of 110.0 per cent of the amount of insulin stated on the label.

**Production**

Insulin preparations are made by methods that are designed to ensure their sterility, to avoid the introduction of foreign contaminants, bacterial endotoxins and the growth of microorganisms. The methods used should confer suitable properties with respect to the onset and duration of therapeutic action.

The use of excipients in the injections may be necessary, for example to make the preparation isotonic with respect to blood, to adjust the pH to the appropriate value, to prevent deterioration of the active substances or to provide adequate antimicrobial properties. Where appropriate, suitable substances may be added and suitable procedures carried out to confer the appropriate physical form on the insulin-containing component or components. Irrespective of the purpose for which additives are used, they should not to adversely affect the intended therapeutic action of the preparation or, at the concentration used, cause toxicity or undue local irritation.

In the course of production the strength of the insulin-containing component or components should be determined, where necessary, by adjustment so that the final preparation contains the required number of Units of insulin per ml.

Initial sterilisation of the insulin-containing component or components is done by filtration and subsequent procedures are carried out aseptically using materials that have been sterilised by suitable methods.

The final preparation is distributed aseptically into sterile glass or plastic containers or pre-filled syringes that are closed so as to exclude microbial contamination.

**Tests**

**Insulin in the supernatant - For preparations that are suspensions**

Not more than 2.5 per cent of the total insulin content, determined in the following manner.

Centrifuge 10 ml of the suspension for 10 minutes and carefully separate the supernatant liquid from the residue. Determine the insulin content of the supernatant liquid (2.3.46) and calculate as a percentage of the total insulin content determined as described under Assay in the individual monograph.

Impurities with molecular masses greater than that of insulin
Determine by size-exclusion chromatography (2.4.16).

**Test solution.** Add 4 µl of 6 M hydrochloric acid per millilitre of the preparation under examination, whether a suspension or a solution, to obtain a clear acid insulin solution. When sampling a suspension, agitate the material prior to sampling in order to obtain a homogeneous sample. If a suspension does not turn clear within 5 minutes of the initial addition of hydrochloric acid, add small aliquots of acid (less than 4 µl per millilitre) until a solution is obtained. Preparations with concentrations higher than 100 Units per ml need to be diluted with 0.01 M hydrochloric acid to avoid overloading the column with insulin monomer.

**Resolution solution.** Use a solution of insulin (approximately 4 mg per ml), containing more than 0.4 per cent of high molecular mass proteins. An injectable insulin preparation, whether a solution or a suspension, that has been clarified with a sufficient amount of 6 M hydrochloric acid, containing the indicated percentage of high molecular mass proteins, or a solution prepared from insulin, dissolved in 0.01 M hydrochloric acid, may be used. Insulin containing the indicated percentage of high molecular mass proteins may be prepared by allowing insulin powder to stand at room temperature for about ten days.

Maintain the solutions at 2° to 10° and use within 30 hours (soluble insulin injection) or 7 days (other insulin preparations). If an automatic injector is used, maintain the temperature at 2° to 10°.

**Chromatographic system**
- a stainless steel column 30 cm x 7.5 mm packed with hydrophilic silica gel (5 µm to 10 µm), of a grade suitable for the separation of insulin monomer from dimers and polymers,
- mobile phase: a filtered and degassed mixture of 15 volumes of glacial acetic acid, 20 volumes of acetonitrile and 65 volumes of a 1.0 g/l solution of arginine,
- flow rate, 0.5 ml per minute,
- spectrophotometer set at 276 nm,
- a 100 µl loop injector.

Before using a new column for chromatographic analysis, equilibrate by repeated injections of an insulin solution containing high molecular mass proteins. This can be done by at least three injections of the resolution solution. The column is equilibrated when repeatable results are obtained from two subsequent injections. If protamine-containing samples are to be analysed, the equilibration of the column is performed using a solution containing protamine.

Inject the resolution solution. When the chromatograms are recorded under the prescribed conditions, the retention times are: polymeric insulin complexes or covalent insulin-protamine complex, about 13 to 17 minutes, covalent insulin dimmer, about 17.5 minutes, insulin monomer, about 20 minutes, salts, about 22 min. If the sample solution contains preservatives, for example methyl paraben, m-cresol or phenol, these compounds elute later. The test is not valid unless the resolution, defined by the ratio of the height of the dimer peak to the height above the baseline of the valley separating the monomer and dimer peaks, is at least 2.0.

Inject the test solution. Record the chromatogram for approximately 35 min. In the chromatogram obtained, the sum of the areas of any peak with a retention time less than that of the insulin peak is not greater than 3.0 per cent (protamine-containing preparations) or 2.0 per cent (non-protamine containing preparations) of the total area of the peaks. Ignore any peak with a retention time greater than that of the insulin peak.

**Related proteins**
Determine by liquid chromatography (2.4.14) as described under Assay of Insulins (2.3.46), following the elution conditions as described in the table below:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase (a) (per cent v/v)</th>
<th>Mobile phase (b) (per cent v/v)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-30</td>
<td>42 → 58</td>
<td>58</td>
<td>isocratic</td>
</tr>
<tr>
<td>30-44</td>
<td>42 → 11</td>
<td>58 → 89</td>
<td>linear gradient</td>
</tr>
<tr>
<td>44-50</td>
<td>11 → 89</td>
<td></td>
<td>isocratic</td>
</tr>
</tbody>
</table>

Maintain the solutions at 2° to 10° and use within 24 hours. Perform a system suitability check (resolution, linearity) as described under Assay of Insulins (2.3.46). If necessary, the relative proportions of the mobile phases may be adjusted to ensure complete elution of A21 desamido porcine insulin before commencement of the gradient. The profile of the gradient may also be adjusted to ensure complete elution of all insulin related impurities.

Inject 20 µl of the test solution and 20 µl of either reference solution (a), for insulin preparations containing 100 IU/ml, or reference solution (b), for insulin preparations containing 40 IU/ml. If necessary, adjust the injection volume to a volume between 10 µl and 20 µl in accordance with the results obtained in the test for linearity as described under Assay. Record the chromatograms for approximately 50 min. If necessary, make further adjustments to the mobile phase in order to ensure that the antimicrobial preservatives present in the test solution are well separated from the insulin and show a shorter retention time. A small reduction in the concentration of acetonitrile increases the retention time of the insulin peaks relatively more than those of the preservatives. In the chromatogram obtained with either reference solution (a), or reference solution (b), as appropriate, A21 desamido insulin appears as a small peak after the principal peak and has a retention time of about...
1.3 relative to the principal peak, due to insulin. In the chromatogram obtained with the test solution the area of the peak due to A21 desamido insulin is not greater than 5.0 per cent of the total area of the peaks; the sum of the areas of any other peaks, apart from those due to insulin and A21 desamido insulin is not greater than 6.0 per cent of the total area of the peaks. Disregard the peaks due to the preservatives and protamine (early eluting peaks).

**Total zinc.** Not more than the amount stated in the individual monograph, determined by either of the following methods.

A. To an accurately measured volume of the gently shaken injection containing 200 Units add 10 ml of alkaline borate buffer pH 9.0, 0.3 ml of zincon solution and sufficient water to produce 50 ml. Allow to stand for 1 hour and measure the absorbance of the resulting solution at about 620 nm, using as the blank a solution prepared by treating 5 ml of water instead of the substance under examination in a similar manner. Calculate the content of zinc from the absorbance obtained by repeating the procedure using a suitable aliquot of a mixture of 4 volumes of zincon solution and 6 volumes of water.

B. Determine by atomic absorption spectrometry (2.4.2).

**Test solution.** Shake the preparation gently and dilute a volume containing 200 Units of insulin to 25.0 ml with 0.01 M hydrochloric acid. Dilute if necessary to a suitable concentration of zinc (for example 0.4 µg to 1.6 µg of Zn per millilitre) with 0.01 M hydrochloric acid.

**Reference solutions.** Use solutions containing 0.40 µg, 0.80 µg, 1.00 µg, 1.20 µg and 1.60 µg of Zn per millilitre, freshly prepared by diluting zinc solution AAS (5 mg/ml Zn) with 0.01 M hydrochloric acid.

Measure the absorbance at 213.9 nm using a zinc hollow-cathode lamp as source of radiation and an air-acetylene flame of suitable composition (for example 11 litres of air and 2 litres of acetylene per minute).

**Bacterial endotoxins** (2.2.3). Less than 80 Units per 100 Units of insulin.

**Sterility.** Comply with the test for sterility (2.2.11).

**Assay.** Determine as described under Assay of Insulins ((2.3.46).

**Storage.** Unless otherwise prescribed, store in sterile, airtight, tamper-proof containers, protected from light, at a temperature of 2° to 8°. Insulin preparations should not to be frozen.

**Labelling.** The label states (a) the potency in Units per millilitre; (2) the concentration in terms of the number of milligrams of insulin per ml (for preparations containing both bovine insulin and porcine insulin the concentration is stated as the combined amount of both insulins); (3) where applicable, that the substance is produced by enzymatic modification of porcine insulin; (4) where applicable, that the substance is produced by recombinant DNA technology; (5) where applicable, the animal species of origin; (6) the preparation must not be frozen; (7) where applicable, that the preparation must be re-suspended before use.

**Nasal Preparations**

Nasal Preparations are liquid, semi-solid or solid preparations containing one or more medicaments and are intended for administration to the nostrils for local or systemic effects. They should as far as possible be non-irritating and should not affect the functions of the nasal mucosa and its cilia. They are supplied in single dose or multiple dose containers of glass VD or plastic with, if necessary, a suitable device for administration. They may also be supplied in pressurised containers with a suitable adaptor and with or without a metering dose valve.

Aqueous nasal preparations are usually isotonic and, when supplied in multiple dose containers, contain a suitable antimicrobial preservative except when the product itself has adequate antimicrobial properties.

During manufacture, packaging, storage and distribution of nasal preparations, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 5.9.

**Tests**

**Uniformity of content.** Comply with the test described under Parenteral Preparations.

**Uniformity of weight.** Nasal Preparations supplied in single dose containers comply with the test for contents of packaged dosage forms (2.5.6).

**Nasal Drops, Solutions and Sprays**

These are solutions, emulsions or suspensions intended for instillation or spraying into the nostrils. Emulsions should have a uniform appearance after shaking and should not show evidence of phase separation. Suspensions should be readily re-dispersible on shaking to give a smooth and stable suspension. In suspensions, the size of the dispersed particles should be such as to localise their deposition in the nostril.

**Nasal Powders**

These are powders intended for insufflation into the nostrils by means of a suitable device. The size of the particles should be such as to localise their deposition in the nostril.

**Storage.** Store protected from light and moisture.
**Tests**

**Uniformity of content.** Comply with the test described under Parenteral Preparations.

**Uniformity of weight.** Nasal Preparations supplied in single application containers comply with the test for contents of packaged dosage forms (2.5.6).

**Ointments**

Ointments are homogeneous, semi-solid preparations intended for external application to the skin or certain mucous membranes for emollient, protective, therapeutic or prophylactic purposes where a degree of occlusion is desired. They usually consist of solutions or dispersions of one or more medicaments in suitable bases. They are formulated using hydrophobic, hydrophillic or water-emulsifying bases to provide preparations that are immiscible, miscible or emulsifiable with the skin secretion, respectively. The base should not produce irritation or sensitisation of the skin, nor should it retard wound healing; it should be smooth, inert, odourless or almost odourless, physically and chemically stable and compatible with the skin and with incorporated medicaments. The proportions of the base ingredients should be such that the ointment is not too soft or too hard for convenient use. The consistency should be such that the ointment spreads and softens when stress is applied.

Ointments may contain suitable auxiliary substances such as antioxidants, stabilisers, thickeners and emulsifiers and, when the base might support the growth of microbial contaminants, suitable antimicrobial preservatives.

During manufacture, packaging, storage and distribution of ointments, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 5.9.

If an ointment is specifically intended for use on large wounds or on severely injured skin it should be sterile.

Ointments should not normally be diluted; if dilution is necessary care should be taken to choose the right diluent to avoid risk of instability or incompatibility.

**Tests**

**Uniformity of weight.** Comply with the test for contents of packaged dosage forms (2.5.6).

**Sterility.** When the ointment is labelled as sterile, it complies with the test for sterility (2.2.11).

**Storage.** Store at a temperature not exceeding 30° unless otherwise directed. Do not freeze.

**Labelling.** The label states (1) that the ointment is sterile, where necessary; (2) the name and concentration of any added antimicrobial preservative; (3) the storage conditions.

**Oral Liquids**

Oral Liquids are homogeneous liquid preparations, usually consisting of a solution, an emulsion or a suspension of one or more medicaments in a suitable vehicle*. They are intended for oral administration either undiluted or after dilution. They may contain auxiliary substances such as suitable dispersing, emulsifying, suspending, wetting, solubilising, thickening, stabilising agents and antimicrobial preservatives. They may also contain suitable sweetening, flavouring and permitted colouring agents. If saccharin, including its sodium and potassium salts, is used as a sweetening agent, its concentration in preparations meant for paediatric use should be restricted so as to limit its intake to 5 mg per kg of body weight.

Oral Liquids other than Oral Emulsions may be supplied as liquids or prepared just before use by dissolving or dispersing granules or powder in the liquid stated on the label. The granules or powder comply with the requirements stated under Oral Powders.

During manufacture, packaging, storage and distribution of oral liquids, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 5.9.

Oral Liquids should not be diluted and stored; where, however, the individual monograph directs dilution, the diluted Oral Liquid should be freshly prepared irrespective of the nature of the diluent. Diluted Oral Liquids may be less stable physically and chemically than the corresponding undiluted preparation and should be used within the period stated on the label.

Oral Liquids are variously known as Elixirs, Linctuses Mixtures, Oral Drops, Oral Emulsions, Oral Solutions, Oral Suspensions and Syrups. These terms are defined below.

**Elixirs.** Elixirs are clear, flavoured Oral Liquids containing one or more active ingredients dissolved in a vehicle that usually contains a high proportion of Sucrose or a suitable polyhydric alcohol or alcohols and may also contain Ethanol (95 per cent) or a dilute Ethanol.

**Linctuses.** Linctuses are viscous Oral Liquids containing one or more active ingredients dissolved in a vehicle that usually contains a high proportion of sucrose, other sugars or a suitable polyhydric alcohol or alcohols. Linctuses are intended for use in the treatment or relief of cough, and are sipped and swallowed slowly without the addition of water.
**Mixtures.** Mixtures are Oral Liquids containing one or more active ingredients dissolved, suspended or dispersed in a suitable vehicle. Suspended solids may separate slowly on keeping but are easily redispersed on shaking.

**Oral Drops.** Oral Drops are Oral Liquids that are intended to be administered in small volumes with the aid of a suitable measuring device such as a dropper.

**Oral Emulsions.** Oral Emulsions are Oral Liquids containing one or more active ingredients and are stabilised oil-in-water dispersions, either or both phases of which may contain dissolved solids. Solids may also be suspended in Oral Emulsions. Emulsions may exhibit phase separation but are easily reformed on shaking. The preparation remains sufficiently stable to permit a homogeneous dose to be withdrawn.

**Oral Solutions.** Oral Solutions are Oral Liquids containing one or more active ingredients dissolved in a suitable vehicle.

**Oral Suspensions.** Oral Suspensions are Oral Liquids containing one or more active ingredients suspended in a suitable vehicle. Suspended solids may slowly separate on keeping but are easily redispersed.

In the manufacture of oral suspensions containing dispersed particles, measures shall be taken to ensure a suitable and controlled particle size with regard to the intended use of the product.

**Syrups.** Syrups are viscous Oral Liquids that may contain one or more active ingredients in solution. The vehicle usually contains large amounts of Sucrose or other sugars to which certain polyhydric alcohols may be added to inhibit crystallisation or to modify solubilisation, taste and other vehicle properties. Sugarless syrups may contain sweetening agents and thickening agents. Syrups may contain Ethanol (95%) as a preservative or as a solvent to incorporate flavouring agents. Antimicrobial agents may also be added to Syrups.

**Containers.** Oral Liquids may be supplied in multiple dose or single dose containers. Oral Emulsions and Oral Suspensions should be packed in bottles sufficiently wide-mouthed to facilitate the flow of the contents. They are administered either in volumes such as 5 ml, or multiples of 5 ml, or in small volumes (drops). Each dose of a multiple dose Oral Liquid is administered by means of a suitable measuring device which is usually provided with the container.

**Tests**

**Uniformity of content.** Unless otherwise specified, single dose liquids in suspension form or powders or granules presented in single dose containers and that contain less than 10 mg or less than 10 per cent of active ingredient comply with the following test. For Oral Liquids containing more than one active ingredient, carry out the test for each active ingredient that corresponds to the above conditions. Empty each container as completely as possible and carry out the test on the individual contents of active ingredients.

The test for Uniformity of content should be carried out only after the content of active ingredient(s) in a pooled sample of the preparation has been shown to be within the accepted limits of the stated content.

Determine the content of active ingredient(s) of each of 10 containers taken at random using the method given in the monograph or by any other suitable analytical method of equivalent accuracy and precision. The preparation complies with the test if the individual values thus obtained are all between 85 to 115 per cent of the average value. The preparation fails to comply with the test if more than one individual value is outside the limits 85 to 115 per cent of the average value or if any one individual value is outside the limits 75 to 125 per cent of the average value. If one individual value is outside the limits 85 to 115 per cent but within the limits 75 to 125 per cent of the average value, repeat the determination using another 20 containers taken at random. The preparation complies with the test if in the total sample of 30 containers not more than 3 individual values are outside the limits 85 to 115 per cent and not more than one is outside the limits 75 to 125 per cent of the average value.

**Uniformity of weight/volume.** Unless otherwise specified, Oral Liquids comply with the test for contents of packaged dosage forms (2.5.6).

**Storage.** Store Oral Liquids or powders and granules for the preparation of Oral Liquids in well-closed containers at temperatures not exceeding 30º.

**Labelling.** For Oral Liquids that are supplied as drops, the label states the number of drops per g of preparation if the dose is stated in drops or the number of drops per ml of preparation if the dose is stated in volume. For oral liquids supplied as granules or powder to be constituted before use, the label states (1) that the contents are meant for preparation of an Oral Liquid; (2) the directions for preparing the Oral liquid including the nature and quantity of the liquid to be used; (3) the conditions under which the constituted solution should be stored; (4) the period during which the constituted Oral Liquid may be expected to remain satisfactory for use when prepared and stored in accordance with the manufacturer’s recommendations; (5) the strength in terms of the active ingredient(s) in a suitable dose-volume of the constituted preparation.

* The term vehicle means a carrier, composed of one or more excipients, for the active pharmaceutical ingredient(s) in a liquid preparation.
Oral Powders

Oral Powders are finely divided powders that contain one or more medicaments with or without auxiliary substances including, where specified, flavouring and colouring agents. However, addition of saccharin or its salts is not permitted in the preparations meant for paediatric use. They are intended to be taken internally with or without the aid of water or any other suitable liquid.

Oral Powders may be single dose or multiple dose preparations. For single dose powders, each dose is enclosed in a separate container, e.g., a sachet, a paper packet or a vial. With multiple dose powders it may be necessary to provide a measuring device capable of delivering the quantity prescribed.

Effervescent Oral Powders are intended to be dissolved or dispersed in water before administration.

In the manufacture of oral powders, means are taken to ensure a suitable particle size with regard to the intended use of the product. During manufacture, packaging, storage and distribution of oral powders, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 5.9.

Storage. Store Oral Powders in containers protected from moisture.

Tests

Uniformity of content. Unless otherwise specified, Oral Powders presented in single dose containers that contain less than 10 mg of active ingredient per dose or that contain less than 10 per cent w/w of active ingredient comply with the following test. For Oral Powders containing more than one active ingredient carry out the test for each active ingredient that corresponds to the above conditions. Empty each container as completely as possible and carry out the test on the individual contents of active ingredients.

The test for Uniformity of content should be carried out only after the content of active ingredient(s) in a pooled sample of the preparation has been shown to be within the accepted limits of the stated content.

Determine the content of active ingredient(s) of each of 10 containers taken at random using the method given in the monograph or by any other suitable analytical method of equivalent accuracy and precision. The preparation complies with the test if the individual values thus obtained are all between 85 to 115 per cent of the average value. The preparation fails to comply with the test if more than one individual value is outside the limits 85 to 115 per cent of the average value or if any one individual value is outside the limits 75 to 125 per cent of the average value. If one individual value is out-side the limits 85 to 115 per cent but within the limits 75 to 125 per cent of the average value, repeat the determination using another 20 containers taken at random. The preparation complies with the test if in the total sample of 30 containers not more than 3 individual values are outside the limits 85 to 115 per cent and not more than one is outside the limits 75 to 125 per cent of the average value.

NOTE — The test for Uniformity of content is not applicable to preparations containing multivitamins and trace elements.

Uniformity of weight. Unless otherwise specified, Oral Powders presented in single dose containers comply with the test for contents of packaged dosage forms (2.5.6).

Parenteral Preparations

Injectable Preparations

NOTE — The provisions of this monograph do not necessarily apply to Blood Products or Immunological Products because of their special nature and licensing requirements.

Introduction

Parenteral Preparations are sterile products intended for administration by injection, infusion or implantation into the body. They may be preparations intended for direct parenteral administration or they may be parenteral products for constituting or diluting prior to administration. There are five main types of Parenteral Preparations, namely, Injections, Infusions, Powders for Injection, Concentrated Solutions for Injection and Implants.

Production

Parenteral Preparations should be prepared by methods designed to ensure their sterility and to avoid the introduction of foreign contaminants, the presence of pyrogens or of bacterial endotoxins and the growth of micro-organisms.

Parenteral Preparations which are solutions or suspensions require vehicles in which the medicaments are incorporated. The most commonly used vehicle is Water for Injections that complies with the requirements for water for injections in bulk stated in the monograph on Water for Injections. Any other suitable vehicles may be used provided they are safe in the volume of injections administered and also do not interfere with the therapeutic efficacy of the preparation or with its response to the prescribed tests and assays of the Pharmacopoeia. It may be necessary to include auxiliary substances to increase the stability or usefulness of the preparation, unless otherwise specified in the individual monograph. Such substances at the concentration at which they are used should not adversely affect the intended medicinal action of the preparation nor cause toxicity or local irritation and should not interfere with the responses to the
specified tests and assays. No colouring agent may be added solely for the purpose of colouring the finished preparation. Aqueous Parenteral Preparations for administration by the subcutaneous, intradermal, intramuscular, or in the case of large volumes, intravenous route, should if possible be made isotonic with blood by the addition of Sodium Chloride or other suitable substances. Buffering agents should not be used in preparations intended for intraocular or intracardiac injection, or in products that may gain access to the cerebrospinal fluid.

Parenteral Preparations that are packaged in multiple dose containers, regardless of the method of sterilisation employed, may contain suitable antimicrobial preservatives in appropriate concentration, unless otherwise directed in the individual monograph, or unless the active ingredients themselves are bacteriostatic. The effectiveness of the chosen preservative shall have been demonstrated during the development of a parenteral preparation.

Precautions to be taken for administration and for storage between successive withdrawals from such multiple dose preparations should be indicated. Preservatives should not be added when the volume to be injected as a single dose exceeds 15 ml, unless otherwise justified, or when the preparation is intended for administration by the intraocular, intracardiac or intracisternal routes (or other route giving access to the cerebrospinal fluid).

Where the active ingredient is susceptible to oxidative degradation a suitable antioxidant may be added and/or the air in the container may be evacuated or displaced by oxygen-free nitrogen or other suitable inert gas.

**Sterilisation.** Methods of sterilisation that may be used in the manufacture of Parenteral Preparations are described in Chapter 5.3.

**Containers.** Containers for Parenteral Preparations are made as far as possible from materials that (1) are sufficiently transparent to permit visual inspection of the contents, except for implants; (2) do not adversely affect the quality of the preparation under the ordinary conditions of handling, shipment, storage, sale and use; (3) do not permit diffusion into or across the walls of the container or yield foreign substances into the preparation. Parenteral Preparations may be supplied in glass ampoules, vials or bottles or in other containers such as plastic bottles or bags or in prefilled syringes the integrity of which is ensured by suitable means. Requirements concerning containers are given in Chapter 6.2.

Single dose containers are used for administration of the contents on one occasion only and are to be preferred for all parenteral preparations. They may be used for intrathecal, intracardiac, intracisternal or intravenous injectable preparations. They contain sufficient of the Parenteral Preparation to permit the withdrawal and administration of the nominal dose using normal technique. They must be used for all parenteral preparations administered at one time in volumes of 10 ml or more.

Multiple dose containers permit the withdrawal of successive portions of the contents without removal or destruction of the closure and without changing the strength, quality or purity of the remaining portion. They may be used for intramuscular, subcutaneous or intracutaneous administration, but no multiple dose container may contain a total volume of injection sufficient to permit the withdrawal of more than ten doses, unless otherwise stated in the individual monograph.

The period of time between the withdrawal of the first and final dose should not be unduly prolonged.

A multiple dose container for a sterile solid permits the addition of a suitable vehicle and withdrawal of portions of the resulting preparation in such a manner that the sterility of the product is maintained.

**Closures.** Vials or bottles are fitted with suitable closures that ensure a good seal, prevent the access of micro-organisms and other contaminants and usually permit the withdrawal of a part or the whole of the contents of the container without removal of the closure. The plastic or rubber materials of which the closure is composed must be compatible with the preparation and be sufficiently firm and elastic to allow the passage of a needle with minimal shedding of particles and to ensure that the puncture is resealed when the needle is withdrawn. Requirements concerning closures are given in Chapter 6.3.

Before use, closures should be washed with a suitable detergent and rinsed with and boiled in several changes of Purified Water. Closures made from rubber and synthetic materials are liable to absorb the ingredients of the parenteral preparation with which they are used, e.g., the preservative. When an antimicrobial preservative is used the closure, when necessary, should be placed in a solution of that preservative in Purified Water containing at least twice the concentration to be used in the preparation; the quantity of solution used should be sufficient to cover the closures and should be at least 2 ml for each g of the material. The vessel should then be closed and heated at an appropriate combination of time and temperature. After heating, the closures should be kept in the sealed container until required for use.

When the parenteral preparation with which the closures are to be used contains other added substances that are liable to be absorbed by the closure, these should be added to the solution in which the closures are to be heated in amounts equal to at least twice the concentration to be used in the parenteral preparation. Closures intended for containers of oily preparations should be made of oil-resistant materials.

**Inspection.** Good Manufacturing Practices require that each final container of a Parenteral Preparation be subjected
individually to a physical inspection whenever the nature of the container permits and that every container the contents of which show evidence of contamination with visible foreign material be rejected.

**Labelling.** Containers of Parenteral Preparations should be labelled in a manner that sufficient area of the container remains uncovered for its full length or circumference to permit inspection of the contents. The label of a Parenteral Preparation states (1) the name of the Parenteral Preparation; (2) the strength in terms of the amount of active ingredient in percentage or in a suitable dose-volume; (3) the name and proportion of or antimicrobial preservative added; (4) the conditions under which the preparation should be stored.

In the case of Parenteral Preparations like Powders for Injection and Concentrated Solutions for Injection wherein a diluent is intended to be added before use, the label also states (1) the composition of the recommended diluent; (2) the conditions under which the constituted preparation should be stored; (3) the period within which the constituted solution should be used if it has been stored under the recommended conditions of storage after constitution. In the case of Powders for Injection, the label also states the amount of diluent to be used to attain a specific concentration of the active ingredient in the solution or suspension so obtained whereas in the case of Concentrated Solutions for Injection, the amount of diluent to be used to attain a specific concentration and the final volume of the solution or suspension so obtained.

**Injections**

Injections are sterile solutions, emulsions or suspensions. They are prepared by dissolving, emulsifying or suspending the active ingredient(s) and any added substances in Water for Injection or in a suitable non-aqueous vehicle, or in a mixture of the two if they are miscible.

Injections that are emulsions should not show any evidence of separation and show a uniform appearance after shaking. The diameter of the globules of the dispersed phase of emulsions intended for intravenous injection must be decided with regard to the use of the preparation. Injections that are suspensions may show a sediment which is readily dispersible on shaking. The suspension remains sufficiently stable to enable a homogenous dose to be withdrawn from the container.

**Tests**

**Particulate matter.** Injections that are solutions, when examined under suitable conditions of visibility, are clear and practically free from particles that can be observed on visual inspection by the unaided eye. Injections that are supplied in containers with a nominal content of 100 ml or more comply with the test for particulate contamination (2.5.9).

**Uniformity of content.** Unless otherwise stated in the individual monograph, suspensions for injection that are presented in single dose containers and that contain less than 10 mg or less than 10 per cent of active ingredient comply with the following test. For suspensions for injection containing more than one active ingredient carry out the test for each active ingredient that corresponds to the above conditions.

The test for Uniformity of content should be carried out only after the content of active ingredient(s) in a pooled sample of the preparation has been shown to be within accepted limits of the stated content.

Determine the content of active ingredient(s) of each of 10 containers taken at random, using the method given in the monograph or by any other suitable analytical method of equivalent accuracy and precision. The preparation under examination complies with the test if the individual values thus obtained are all between 85 and 115 per cent of the average value. The preparation under examination fails to comply with the test if more than one individual value is outside the limits 85 to 115 per cent of the average value or if any one individual value is outside the limits 75 to 125 per cent of the average value. If one individual value is outside the limits 85 to 115 per cent but within the limits 75 to 125 per cent of the average value, repeat the determination using another 20 containers taken at random. The preparation under examination complies with the test if in the total sample of 30 containers not more than one individual value is outside the limits 85 to 115 per cent and none is outside the limits 75 to 125 per cent of the average value.

**NOTE —** The test for Uniformity of content is not applicable to suspensions for injection containing multivitamins and trace elements.

**Extractable volume.** Where the nominal volume does not exceed 5 ml, the containers comply with the requirements of Method 1 and where the nominal volume is greater than 5 ml, the containers comply with the requirements of Method 2. Suspensions should be shaken before the contents are withdrawn; oily injections may be warmed but should be cooled to 25° before carrying out the test.

**Method 1** — Use 6 containers, 5 for the test and 1 for rinsing the syringe used. Inspect the 5 containers to be used in the test visually and ensure that each contains approximately the same volume of the preparation.

Using a syringe with a capacity not exceeding twice the volume to be measured and fitted with a suitable needle, take up a small quantity of the liquid under examination from the container reserved for rinsing the syringe, and discharge it from the syringe whilst the needle is pointing upwards so as to expel any air. Withdraw as much as possible the contents of one of the containers reserved for the test and transfer, without emptying the needle, to a dry graduated cylinder of such
capacity that the total combined volume to be measured occupies not less than 40 per cent of the nominal volume of the cylinder. Repeat the procedure until the contents of the 5 containers have been transferred and measure the volume. The average content of the 5 containers is not less than the nominal volume and not more than 115 per cent of the nominal volume.

Method 2 — Transfer the contents of not less than 3 containers separately to dry graduated cylinders such that the volume to be measured occupies not less than 40 per cent of the nominal volume of the cylinder and measure the volume transferred. The contents of each container are not less than the nominal volume and not more than 110 per cent of the nominal volume.

Multiple dose containers labelled to yield a specific number of doses shall contain a sufficient excess to permit the withdrawal of the designated number of doses.

Sterility (2.2.11). Injections comply with the test for sterility.

Pyrogens. Unless otherwise stated in the individual monograph, when the volume to be injected in a single dose is 10 ml or more, Injections comply with the test for pyrogens (2.2.8), unless the test for bacterial endotoxins (2.2.3), is prescribed.

Infusions

Infusions are sterile aqueous solutions or emulsions with water as the continuous phase. They are free from pyrogens or bacterial endotoxins, are usually made isotonic with blood and do not contain any added antimicrobial preservatives. Intravenous Infusions that are emulsions do not show any evidence of phase separation. The diameter of the globules of the dispersed phase of emulsions must be decided with regard to the use of the preparation.

Tests

Intravenous Infusions comply with the requirements of tests stated under individual monographs and with the following requirements.

Particulate contamination. Intravenous Infusions that are solutions, when examined under suitable conditions of visibility, are clear and practically free from particles that can be observed on visual inspection by the unaided eye. Intravenous Infusions that are solutions and are supplied in containers with a nominal content of 100 ml or more comply with the test for particulate contamination (2.5.9).

Sterility (2.2.11). Intravenous Infusions comply with the test for sterility.

Pyrogens. Where no test for bacterial endotoxins (2.2.3) is prescribed, Intravenous Infusions comply with the test for pyrogens (2.2.8). Unless otherwise stated in the individual monograph inject 10 ml per kg of body weight into each animal.

Powders for injection

Powders for injection are sterile, solid substances (including freeze-dried materials) which are distributed in their final containers and which, when shaken with the prescribed volume of the appropriate sterile liquid, rapidly form clear and practically particle-free solutions or uniform suspensions.

Tests

Powders for injection comply with the requirements of tests stated under individual monographs and with the following requirements.

Uniformity of content. Unless otherwise stated in the individual monograph, Powders for injection that contain 10 mg or less than 10 mg or less than 10 per cent of active ingredient or that have a unit weight equal to or less than 50 mg comply with the test for Uniformity of content described under Injections. For Powders for injection containing more than one active ingredient carry out the test for each active ingredient that corresponds to the above conditions. The test is not applicable to Powders for injection containing multivitamins and trace elements.

The test for Uniformity of content should be carried out only after the content of active ingredient(s) in a pooled sample of the preparation has been shown to be within accepted limits of the stated content.

Uniformity of weight. For Powders for injection that are required to comply with the test for Uniformity of content of all active ingredients, the test for Uniformity of weight is not required.

Remove any adherent labels from a container and wash and dry the outside. Open the container and immediately weigh the container and its contents. Empty the container as completely as possible by gentle tapping, rinse if necessary with water and then with ethanol (95 per cent) and dry at 100° to 105° for 1 hour or, if the nature of the container precludes such treatment, dry at a lower temperature to constant weight. Allow to cool in a desiccator and weigh. The difference between the weights represents the weight of the contents. Repeat the procedure with a further 19 containers and determine the average weight. Not more than two of the individual weights deviate from the average weight by more than 10 per cent and none deviates by more than 20 per cent.

Clarity of solution. Constitute the injection as directed on the label.

a) The solid dissolves completely, leaving no visible residue as undissolved matter.

b) The constituted injection is not significantly less clear than an equal volume of the diluent or of water for injections contained in a similar container and examined in the same manner.
Particulate matter. Constitute the injection as directed on the label; the solution is essentially free from particles of foreign matter that can be seen on visual inspection.

Sterility (2.2.11). Powders for injection comply with the test for sterility.

Concentrated Solutions for injection

Concentrated Solutions for injection are sterile solutions that are intended to be administered by injection or by intravenous infusion only after dilution with a suitable liquid.

Tests

After dilution Concentrated Solutions for injection comply with the requirements of tests for Injections or Infusions as appropriate.

Implants

Implants are sterile solid preparations of size and shape suitable for implantation into body tissues so as to release the active ingredient over an extended period of time. They are normally presented individually in sterile containers.

Tests

Sterility (2.2.11). Implants comply with the test for sterility.

Pessaries

Pessaries are solid preparations containing one or more active ingredients and are suitable for vaginal insertion. They are normally intended for use as a single dose.

The active ingredients are dissolved or dispersed in a suitable basis containing one or more auxiliary substances that may be dispersible, soluble or insoluble in water. The auxiliary substances may be similar to the ones used for Suppositories or Tablets; such substances must be innocuous and therapeutically inert in the quantities present.

During manufacture, packaging, storage and distribution of pessaries, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 5.9.

Compressed Pessaries. Compressed Pessaries, also known as Vaginal Tablets, have the general characteristics of Uncoated Tablets but are usually large and of greater weight.

Storage. Store in well-closed containers, protected from moisture and from being crushed.

Moulded Pessaries. Moulded Pessaries are manufactured by pouring the liquefied mass containing the medicament(s) and auxiliary substances into moulds of suitable volume and cooling in order to solidify the mass. Auxiliary substances normally used are mixtures of mono-, di- and triglycerides of saturated fatty acids, macrogols, theobroma oil and gelatinous mixtures consisting of Gelatin, Glycerin and Water.

Moulded Pessaries are smooth and are usually ovoid in shape but may also be of various other shapes and of various volumes. When examined microscopically, their surfaces and longitudinal sections are normally of uniform texture except where the pessary consists of many layers.

Storage. Store in ventilated containers.

Shell Pessaries. Shell Pessaries, also known as Vaginal Capsules, are similar to Soft Capsules, differing only in their shape and size. They are commonly ovoid in shape, smooth and have a uniform appearance.

Storage. Store in well-closed containers.

Tests

Uniformity of container contents. Comply with the test for contents of packaged dosage forms (2.5.6).

Uniformity of content. The test is applicable to Pessaries that contain less than 10 mg or less than 10 per cent of active ingredient. For Pessaries containing more than one active ingredient carry out the test for each active ingredient that corresponds to the above conditions.

The test for Uniformity of content should be carried out only after the content of active ingredient(s) in a pooled sample of the pessaries has been shown to be within accepted limits of the stated content.

Carry out the test for Uniformity of content described under Capsules.

Uniformity of weight. This test is not applicable to Pessaries that are required to comply with the test for Uniformity of content for all active ingredients.

Weigh individually 20 pessaries, taken at random, and determine the average weight. Not more than two of the individual weights deviate from the average weight by more than 5 per cent and none deviates by more than 10 per cent.

Disintegration. This test is not necessarily applicable to Pessaries intended for modified release or for prolonged local action.

Carry out the disintegration test (2.5.1). Disintegration occurs in not more than 30 minutes for Compressed Pessaries and Shell Pessaries and in not more than 60 minutes for Moulded Pessaries.

Suppositories

Suppositories are solid preparations each containing one or more active ingredients and are suitable for rectal
administer. They are normally intended for use as a single
dose for local action or systemic absorption of the active
ingredients.

The active ingredients are ground and passed through a sieve,
if necessary, and dissolved or dispersed in a suitable basis
that may be soluble or dispersible in water or that may melt at
body temperature.

Suppositories may contain suitable auxiliary substances such
as adsorbents, diluents, lubricants, antimicrobial preservatives
and colouring agents permitted under the Drugs and Cosmetics
Rules, 1945.

Moulded Suppositories. Moulded Suppositories are
manufactured by liquefying by heating the mass containing
the medicament(s) and auxiliary substances and then pouring
the mass into moulds of suitable volume and cooling in order
to solidify the mass. In some cases, the solid medicated mass
may be cold-moulded by compression in a suitable matrix.

Moulded Suppositories have the characteristics of Moulded
Pessaries.

Shell Suppositories. Shell Suppositories, also known as Rectal
Capsules, are generally similar to Soft Capsules except that
they may have lubricating coatings.

Shell Suppositories have the characteristics of Shell Pessaries.

During manufacture, packaging, storage and distribution of
suppositories, suitable means shall be taken to ensure their
microbial quality; acceptance criteria for microbial quality are
given in Chapter 5.9.

Tests

Moulded Suppositories and Shell Suppositories comply with
the tests stated under Moulded Pessaries and Shell Pessaries
respectively.

Storage. Store in well-closed containers.

Tablets

NOTE — The provisions of this monograph do not necessarily
apply to tablets intended for use other than by oral
administration such as Vaginal preparations or Oromucosal
preparations, and to lozenges, oral pastes and oral gums.

Introduction

Tablets are solid dosage forms each containing a unit dose of
one or more medicaments. They are intended for oral
administration. Some tablets are swallowed whole or after being
crushed, some are dissolved or dispersed in water before
administration and some are retained in the mouth where the
active ingredient is liberated.

Because of their composition, method of manufacture or
intended use, tablets present a variety of characteristics and
consequently there are several categories of tablets.

Unless otherwise stated in the individual monograph, tablets
are uncoated. Where coating is permitted, the monograph
states “The tablets may be coated”. Where the monograph
directs coating the statement reads “The tablets are coated”.

Unless otherwise directed, tablets may be coated in one of
different ways.

Tablets are usually solid, right circular cylinders, the end
surfaces of which are flat or convex and the edges of which
may be bevelled. They may exist in other shapes like triangular,
rectangular, etc also. They may have lines or break-marks and
may bear a symbol or other markings. They are sufficiently
hard to withstand handling without crumbling or breaking.

Production

Tablets are obtained by compression of uniform volumes of
powders or granules by applying high pressures and using
punches and dies. The particles to be compressed consist of
one or more medicaments, with or without auxiliary substances
such as diluents, binders, disintegrating agents, lubricants,
glidants, permitted colours and substances capable of
modifying the behaviour of the medicaments in the digestive
tract. Such substances must be innocuous and therapeutically
inert in the quantities present.

In the production of tablets, measures are taken to ensure that
they have sufficient strength to avoid crumbling or breaking
on handling or subsequent handling. Chewing tablets are
manufactured to ensure that they are easily crushed by
chewing.

During manufacture, packaging, storage and distribution of
tablets, suitable means shall be taken to ensure their microbial
quality; acceptance criteria for microbial quality are given in
Chapter 5.9.

Tests

NOTE — Unless otherwise stated below or in the individual
monograph, the following tests apply to all categories of
tablets.

Uniformity of container contents. Tablets comply with the
test for contents of packaged dosage forms (2.5.6).

Content of active ingredients. Determine the amount of active
ingredient(s) by the method described in the Assay and
calculate the amount of active ingredient(s) per tablet. The
result lies within the range for the content of active
ingredient(s) stated in the monograph. This range is based on
the requirement that 20 tablets, or such other number as may
be indicated in the monograph, are used in the Assay. Where
20 tablets cannot be obtained, a smaller number, which must
Uniformity of weight (2.5.3). This test is not applicable to coated tablets other than film-coated tablets and to tablets that are required to comply with the test for uniformity of content for all active ingredients.

Dissolution (2.5.2). Where required, the requirements for this test are given in the individual monographs. Where a dissolution test is prescribed, the disintegration test may not be necessary.

Uncoated Tablets

Uncoated tablets may be single-layer tablets resulting from a single compression of particles or multi-layer tablets consisting of parallel layers obtained by successive compression of particles of different compositions. No treatment is applied to such tablets after compression. Any added substances are not specifically intended to modify the release of their active ingredient(s) in the digestive fluids.

The addition of flavouring agents to uncoated tablets other than multi-layer tablets is not official unless permitted in the individual monograph. Uncoated Tablets have the general characteristics of tablets. When a broken section of an uncoated tablet is examined under a lens, either a relatively uniform texture (single-layer tablets) or a stratified structure (multi-layer tablets) is seen; there are no signs of coating.

Tests

Disintegration (2.5.1). Use water as the liquid. Add a disc to each tube. Operate the apparatus for 15 minutes, unless otherwise stated in the individual monograph. Examine the state of the tablets. If the tablets fail to comply because of adherence to the discs, repeat the test on a further 6 tablets omitting the discs. The tablets comply with the test if all 6 tablets have disintegrated.

The test does not apply to chewable tablets.

Coated Tablets

Coated tablets are tablets covered with one or more layers of mixtures of various substances such as resins, gums, inactive and insoluble fillers, sugars, plasticisers, polyhydric alcohols, waxes, etc. The coating may also contain medicaments. In compression-coated tablets, the coating is applied by compressing around the tablets granules prepared from tablet excipients such as lactose, calcium phosphate, etc. Substances used as coatings are usually applied as a solution or suspension in conditions in which evaporation of the vehicle occurs. When the coating is thin, the tablets are described as film-coated.

Coated tablets may contain flavouring agents.

Coated tablets have a smooth, usually polished and often coloured, surface; a broken section examined under a lens shows a core surrounded by one or more continuous layers of a different texture.

Tests

Disintegration (2.5.1). For coated tablets other than film-coated tablets.

Use water as the liquid. Add a disc to each tube. Operate the apparatus for 60 minutes, unless otherwise stated in the individual monograph. Examine the state of the tablets. If any of the tablets has not disintegrated, repeat the test on a further 6 tablets, replacing water with 0.1 M hydrochloric acid. The tablets comply with the test if all 6 tablets have disintegrated in the acid medium.

For film-coated tablets.

Carry out the test described above but operate the apparatus for 30 minutes, unless otherwise stated in the individual monograph.
If coated tablets fail to comply because of adherence to the discs, repeat the test on a further 6 tablets omitting the discs. The tablets comply with the test if all 6 tablets have disintegrated.

*The test does not apply to chewable tablets.*

**Dispersible Tablets**

Dispersible tablets are uncoated or film-coated tablets that produce a uniform dispersion in water and may contain permitted flavouring and sweetening agents. However, if saccharin, including its sodium and potassium salts, is used as a sweetening agent, its concentration in dispersible tablets meant for paediatric use should be restricted so as to limit its intake to 5 mg/kg of body weight.

**Tests**

**Disintegration** (2.5.1). Determine at 24° to 26° and operate the apparatus for 3 minutes.

**Uniformity of dispersion.** Place 2 tablets in 100 ml of water and stir gently until completely dispersed. A smooth dispersion is obtained which passes through a sieve screen with a nominal mesh aperture of 710 mm (sieve number 22).

**Effervescent Tablets**

Effervescent tablets are uncoated tablets generally containing acidic substances and either carbonates or bicarbonates which react rapidly in the presence of water to release carbon dioxide. They are intended to be dissolved or dispersed in water before administration.

**Tests**

**Disintegration** (2.5.1). Place one tablet in a 250-ml beaker containing water at 20° to 30°; numerous gas bubbles are evolved. When the evolution of gas around the tablet or its fragments has ceased the tablet shall have disintegrated, being either dissolved or dispersed in the water so that no agglomerates of particles remain. Repeat the operation on a further 5 tablets. The tablets comply with the test if each of the 6 tablets disintegrates in the manner prescribed within 5 minutes, unless otherwise stated in the individual monograph.

**Modified-release Tablets**

Modified-release tablets (Sustained-release tablets) are coated or uncoated tablets containing auxiliary substances or prepared by procedures that, separately or together, are designed to modify the rate or the place at which the active ingredient is released.

Modified-release tablets include enteric-coated tablets, prolonged-release tablets and delayed-release tablets.

**Enteric-coated Tablets**

Enteric-coated tablets (Gastro-resistant tablets) are delayed-release tablets that are intended to resist the gastric fluid but to release their active ingredient(s) in the intestinal fluid. For this purpose substances such as cellulose acetate phthalate and anionic copolymers of methacrylic acid and its ethers are used for providing tablets with a gastric-resistant coating or for covering either granules or particles with gastric-resistant coating.

Enteric-coated tablets have the characteristics of Coated Tablets.

**Tests**

**Disintegration** (2.5.1). If the tablet has a soluble external coating, immerse the basket in water at room temperature for 5 minutes. Suspend the assembly in the beaker containing 0.1 M hydrochloric acid and operate without the discs for 120 minutes, unless otherwise stated in the individual monograph. Remove the assembly from the liquid. No tablet shows signs of cracks that would allow the escape of the contents of disintegration, apart from fragments of coating. Replace the liquid in the beaker with mixed phosphate buffer pH 6.8, add a disc to each tube and operate the apparatus for a further 60 minutes. Remove the assembly from the liquid. The tablets pass the test if all six have disintegrated.

**Dissolution** (2.5.2). For tablets prepared from granules or particles already covered with an enteric coating, the dissolution test is carried out to demonstrate the appropriate release of the active substance(s).

**Prolonged-release Tablets**

Prolonged-release tablets, also known as sustained-release tablets or extended-release tablets are tablets formulated in such a manner as to make the contained active ingredient available over an extended period of time after ingestion.

**Tests**

**Dissolution** (2.5.2). The test should be designed to demonstrate the appropriate release of the active substance(s). The manufacturer is expected to give specifications for drug release at 3 or more test-time points. The first point should be set after a testing period corresponding to a dissolved amount of typically 20 per cent to 30 per cent. The second point should define the dissolution pattern and should be set at around 50 per cent release. The final point should ensure almost complete release that is generally understood as more than 80 per cent release.

Carry out the test for the test-times indicated on the label of the product.
Soluble Tablets

Soluble tablets are uncoated tablets or film-coated tablets that are to be dissolved in water before use. The solution produced may be slightly opalescent due to added substances used in the manufacture of the tablets.

Tests

Disintegration (2.5.1). Soluble tablets disintegrate within 3 minutes. The test is carried out using water at 15° to 25°.

Tablets for Use in the Mouth

Tablets for use in the mouth are usually uncoated tablets formulated to be chewed or to effect a slow release and local action of the active ingredient (lozenges) or the release and absorption of the active ingredient under the tongue (sublingual tablets). Chewable tablets and lozenges may contain flavouring agents.
DRUG SUBSTANCES, DOSAGE FORMS
AND
PHARMACEUTICAL AIDS

A to ........................................................................................................................................
A

Abacavir Sulphate
Abacavir Oral Solution
Abacavir Tablets
Abacavir and Lamivudine Tablets
Abacavir, Lamivudin and Zidovudine Tablets
Acarbose
Acarbose Tablets
Acebutolol Hydrochloride
Acebutolol Tablets
Aceclofenac
Aceclofenac Tablets
Acetazolamide
Acetazolamide Tablets
Glacial Acetic Acid
Acetic Acid Ear Drops
Aciclovir
Aciclovir Intravenous Infusion
Aciclovir Tablets
Adenine
Adrenaline
Adrenaline Tartrate
Adrenaline Injection
Albendazole
Albendazole Tablets
Alginic Acid
Allopurinol
Allopurinol Tablets
Aloes
Alprazolam
Alprazolam Tablets
Aluminium Acetate Ear Drops
Aluminium Hydroxide Gel
Dried Aluminium Hydroxide Gel
Aluminium Sulphate
Amantadine Hydrochloride
Amantadine Capsules
Ambroxol Hydrochloride
Amikacin
Amikacin Sulphate
Amikacin Injection
Amiloride Hydrochloride
Amiloride Tablets
Aminocaproic Acid
Aminocaproic Acid Injection
Aminocaproic Acid Tablets
Aminophylline
Aminophylline Injection
Aminophylline Tablets
Amiodarone Hydrochloride
Amiodarone Tablets
Amitriptyline Hydrochloride
Amitriptyline Tablets
Amlodipine Besilate
Amlodipine Tablets
Ammonium Chloride
Amodiaquine Hydrochloride
Amodiaquine Tablets
Amoxycillin Sodium
Amoxycillin Capsules
Amoxycillin Injection
Amoxycillin Oral Suspension
Amoxycillin Trihydrate
Amoxycillin Dispersible Tablets  
Amoxycillin and Potassium Clavulanate Injection  
Amoxycillin and Potassium Clavulanate Oral Suspension  
Amoxycillin and Potassium Clavulanate Tablets  
Amphotericin B  
Amphotericin B Injection  
Ampicillin  
Ampicillin Capsules  
Ampicillin Sodium  
Ampicillin Injection  
Ampicillin Oral Suspension  
Ampicillin Dispersible Tablet  
Ampicillin Trihydrate  
Alpha Amylase  
Analgin  
Analgin Tablets  
Anticoagulant Citrate Dextrose Solution  
Anticoagulant Citrate Phosphate Dextrose Solution  
Anticoagulant Citrate Phosphate Dextrose Adenine Solution  
Arteether  
Artemether  
Artemisinin  
Ascorbic Acid  
Ascorbic Acid Injection  
Ascorbic Acid Tablets  
Aspartame  
Aspirin  
Aspirin Tablets  
Soluble Aspirin Tablets  
Aspirin And Caffeine Tablets  
Atenolol  
Atenolol Tablets  
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Abacavir Sulphate

\[
(C_{14}H_{18}N_{6}O)_2H_2SO_4 \quad \text{Mol. Wt. 670.8}
\]

Abacavir Sulphate is \([1S,4R]-4\{2\text{-amino-6-}(\text{cyclopropylamino})9\text{-H-purin-9-yl}\}\text{cyclopent-2-enyl}\} \text{methanol sulphate.}

Abacavir Sulphate contains not less than 98.0 per cent and not more than 102.0 per cent of \((C_{14}H_{18}N_{6}O)_2H_2SO_4\), calculated on the anhydrous basis.

**Description.** A white or almost white, crystalline powder.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with **abacavir sulphate RS** or with the reference spectrum of abacavir sulphate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. It gives reaction A of sulphates (2.3.1).

**Tests**

**Specific optical rotation** (2.4.22). –32.0° to –38.0°, determined in a 0.5 per cent w/v solution in methanol.

**Related substances.** Determine by liquid chromatography (2.4.14), as described in the Assay but using the following solutions.

**Test solution.** A 0.05 per cent w/v solution of the substance under examination in the mobile phase.

**Reference solution.** Dilute 1 ml of the test solution to 200 ml with the mobile phase.

Inject the test solution and the reference solution. Calculate the content of each impurity in the chromatogram obtained with the test solution by comparing the peak area of each peak with the area of the principal peak in the chromatogram obtained with the reference solution. The content of any individual impurity is not greater than 0.5 per cent and the sum of all the impurities is not greater than 1.5 per cent.

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.3 per cent.

**Water** (2.3.43). Not more than 1.5 per cent, determined on 0.2 g.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** A 0.01 per cent w/v solution of the substance under examination in the mobile phase.

**Reference solution.** A 0.01 per cent w/v solution of abacavir sulphate RS in the mobile phase.

**Chromatographic system**

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 10 volumes of methanol, 15 volumes of acetonitrile and 75 volumes of a buffer prepared by dissolving 1.15 g of ammonium dihydrogen phosphate and 2 g of tetrabutylammonium hydrogen sulphate in 1000 ml of water and adjusting the pH to 6.0 with triethylamine,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 214 nm,
- a 20 µl loop injector.

Inject alternately the test solution and the reference solution. Calculate the percentage content of \((C_{14}H_{18}N_{6}O)_2H_2SO_4\).

**Storage.** Store at a temperature not exceeding 30°.

Abacavir Oral Solution

Abacavir Sulphate Oral Solution

Abacavir Oral Solution contains a quantity of Abacavir Sulphate equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of abacavir \(C_{14}H_{18}N_{6}O\). It may contain one or more suitable buffers, colours, flavours, preservatives, stabilizers, sweeteners, and suspending agents.

**Identification**

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

**Tests**

**pH** (2.4.24). 4.6 to 5.0.
**Related substances.** Determine by liquid chromatography (2.4.14).

**NOTE — Prepare the solutions immediately before use.**

**Test solution.** Weigh a quantity of the oral solution containing 50 mg of abacavir, dissolve in 100 ml of the mobile phase and mix.

**Reference solution (a).** A solution of abacavir sulphate RS containing 0.05 per cent w/v of abacavir in the mobile phase.

**Reference solution (b).** Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 85 volumes of a buffer solution prepared by dissolving 1.15 g of ammonium dihydrogen phosphate and 2 g of tetrabutyl ammonium hydrogen sulphate in 1000 ml of water, adjusting the pH to 6.0 with triethylamine and filtering, and 15 volumes of acetonitrile,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 214 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

**Other tests.** Complies with the tests stated under Oral liquids.

**Assay.** Determine by liquid chromatography (2.4.14).

**NOTE — Prepare the solutions immediately before use.**

**Test solution.** Weigh accurately a quantity of the oral solution containing 60 mg of abacavir, dissolve in 100.0 ml of the mobile phase and mix. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

**Reference solution.** A 0.06 percent w/v solution of abacavir sulphate RS in the mobile phase and filter. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

**Chromatographic system**
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 85 volumes of a buffer solution prepared by dissolving 1.15 g of ammonium dihydrogen phosphate and 2 g of tetrabutyl ammonium hydrogen sulphate in 1000 ml of water and adjusting the pH to 6.0 with triethylamine and filtering, and 15 volumes of acetonitrile,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 214 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Determine the weight per ml of the oral solution (2.4.29) and calculate the content of $C_{14}H_{18}N_6O$ weight in volume.

**Storage.** Store at a temperature not exceeding 30°. Do not freeze.

**Labelling.** The label states the strength in terms of the equivalent amount of abacavir.

**Abacavir Tablets**

Abacavir Sulphate Tablets

Abacavir Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of abacavir, $C_{14}H_{18}N_6O$. The tablets may be coated.

**Identification**

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

**Tests**

**Dissolution** (2.5.2).

Apparatus. No 1

Medium. 900 ml of 0.1 M hydrochloric acid.

Speed and time. 75 rpm and 15 minutes.

Withdraw a suitable volume of the medium and filter, discarding the first few ml of the filtrate.

Determine by liquid chromatography (2.4.14).

**Test solution.** Dilute the filtrate, if necessary, with the dissolution medium.

**Reference solution.** A 0.075 per cent w/v solution of abacavir sulphate RS in the dissolution medium. Dilute 5 ml of the solution to 10 ml with the dissolution medium.
Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 40º,
- mobile phase: a mixture of 85 volumes of a buffer solution prepared by dissolving 1.15 g of ammonium dihydrogen phosphate and 2 g of tetrabutyl ammonium hydrogen sulphate in 1000 ml of distilled water and adjusting the pH to 6.0 with triethylamine, and 15 volumes of acetonitrile,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 214 nm,
- a 10 µl loop injector.

Inject alternatively the test solution and the reference solution.

D. Not less than 80 per cent of the stated amount of C₁₄H₁₈N₆O.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 50 mg of abacavir, disperse in 100 ml of the mobile phase and filter.

Reference solution (a). A solution of abacavir sulphate RS containing 0.05 per cent w/v of abacavir in the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 85 volumes of a buffer solution prepared by dissolving 1.15 g of ammonium dihydrogen phosphate and 2 g of tetrabutyl ammonium hydrogen sulphate in 1000 ml of water and adjusting the pH to 6.0 with triethylamine, and 15 volumes of acetonitrile,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 214 nm,
- a 20 µl loop injector.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and the reference solution.

Calculate the content of C₁₄H₁₈N₆O.

Storage. Store protected from moisture at a temperature not exceeding 30º.

Labelling. The label states the strength in terms of the equivalent amount of abacavir.

Abacavir and Lamivudine Tablets
Abacavir Sulphate and Lamivudine Tablets
Abacavir and Lamivudine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of abacavir, C₁₄H₁₈N₆O and lamivudine, C₈H₁₁N₃O₃S.

Identification
In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the principal peaks in the chromatogram obtained with the reference solution.

Tests
Dissolution (2.5.2).
Apparatus. No 1
Medium. 900 ml of 0.1 M hydrochloric acid.
Speed and time. 75 rpm and 30 minutes.
Withdraw a suitable volume of the medium and filter. Determine by liquid chromatography (2.4.14)

**Test solution.** The filtrate obtained as given above. Dilute the filtrate if necessary, with the dissolution medium.

**Reference solution.** Dissolve 75 mg of abacavir sulphate RS and 30 mg of lamivudine RS in 10 ml of methanol and dilute to 100 ml with the dissolution medium.

Use the chromatographic system described under Assay.

Inject the reference solution. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

D. Not less than 80 per cent of the stated amounts of C14H18N6O and C8H11N3O3S.

**Related substances**. Determine by liquid chromatography (2.4.14).

**Solvent mixture.** 95 volumes of mobile phase A and 5 volumes of mobile phase B.

**Test solution.** Weigh accurately a quantity of the powdered tablets containing 100 mg of abacavir and disperse in 100 ml of the solvent mixture and filter.

**Reference solution (a).** A solution containing 0.12 per cent w/v of abacavir sulphate RS and 0.05 per cent w/v of lamivudine RS in the solvent mixture.

**Reference solution (b).** Dilute 1 ml of reference solution (a) to 100 ml with the solvent mixture.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm, packed with octadecysilane bonded to porous silica (5 µm),
- column temperature 40º,
- mobile phase: A. a buffer solution prepared by dissolving 1.9 g of ammonium acetate in 900 ml of water, adjusting the pH to 3.8 with glacial acetic acid and diluting to 1000 ml with water,
  - B. methanol,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 277 nm,
- a 20 µl loop injector.

<table>
<thead>
<tr>
<th>Time (in min.)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>20</td>
<td>95</td>
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<td>5</td>
</tr>
<tr>
<td>50</td>
<td>95</td>
<td>5</td>
</tr>
</tbody>
</table>

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent) and the sum of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

**Other tests.** Comply with the tests stated under Tablets.

**Water** (2.3.43). Not more than 3.0 per cent, determined on 0.5 g.

**Assay.** Determine by liquid chromatography (2.4.14)

**Test solution.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 60 mg of abacavir, dissolve in 20 ml of 0.1 M hydrochloric acid and dilute to 100.0 ml with methanol. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

**Reference solution.** Dissolve 35 mg of abacavir RS and 15 mg of lamivudine RS in 15 ml of 0.1 M hydrochloric acid and dilute to 50.0 ml with methanol. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm, packed with octadecysilane bonded to porous silica (5 µm),
- column temperature 40º,
- mobile phase: a mixture of 50 volumes of a buffer solution prepared by dissolving 7.66 g of ammonium acetate in 1000 ml of a 0.5 per cent w/v solution of glacial acetic acid and 50 volumes of methanol,
- flow rate. 1 ml per minute,
- spectrophotometer set at 282 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the contents of C14H18N6O and C8H11N3O3S in the tablets.

**Storage.** Store protected from moisture, at a temperature not exceeding 30º.

**Abacavir, Lamivudine and Zidovudine Tablets**

Abacavir, Lamivudine and Zidovudine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the
stated amounts of abacavir, C14H18N6O, lamivudine, C8H11N3O3S and zidovudine, C10H13N5O4.

**Identification**

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

**Tests**

**Dissolution** (2.5.2).

Apparatus No. 1

Medium. 900 ml of 0.1 M hydrochloric acid.

Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

**Test solution.** Use the filtrate obtained as given above.

**Reference solution.** A solution containing 0.035 per cent w/v of abacavir sulphate RS, 0.015 per cent w/v lamivudine RS and 0.03 per cent w/v of zidovudine RS in the dissolution medium.

**Chromatographic system**

- a stainless steel column 5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm) (such as Restek’s Pinnacle II C-18),
- column temperature 50º,
- mobile phase: a mixture of 88 volumes of a buffer solution prepared by dissolving 1 g of octanesulphonic acid and 1 ml of triethylamine in 1000 ml of water and adjusting the pH to 2.5 with orthophosphoric acid, and 12 volumes of acetonitrile,
- flow rate. 2.5 ml per minute.
- spectrophotometer set at 272 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the resolution between lamivudine and zidovudine peaks is not less than 2.5, the column efficiency determined from lamivudine, zidovudine and abacavir peaks is not less than 700, 1200 and 2000 theoretical plates respectively, the tailing factor for lamivudine, zidovudine and abacavir peaks is not more than 2.0 and the relative standard deviation of replicate injections is not more than 2.0 per cent for each component.

Inject the test solution and the reference solution.

Calculate the contents of C14H18N6O, C8H11N3O3S and C10H13N5O4.

D. Not less than 70 per cent of the stated amounts of C14H18N6O, C8H11N3O3S and C10H13N5O4.

**Related substances.** Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

**Solvent mixture.** A 0.2 per cent v/v solution of orthophosphoric acid in a mixture of 70 volumes of water and 30 volumes of methanol.

**Test solution.** Weigh a quantity of the powdered tablets containing 75 mg of Lamivudine, disperse in 100 ml of the solvent mixture and filter.

**Reference solution (a).** A solution containing 0.18 per cent w/v of abacavir sulphate RS, 0.075 per cent w/v lamivudine RS and 0.15 per cent w/v of zidovudine RS in the solvent mixture.

**Reference solution (b).** Dilute 1 ml of the solution to 100 ml with the solvent mixture.

**Chromatographic system**

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm),
- mobile phase: A. a mixture of 70 volumes of methanol, 30 volumes of acetonitrile and 0.4 volume of tetrahydrofuran,
- B. a buffer solution pH 3.0 prepared by dissolving 6.8 g of potassium dihydrogen orthophosphate in 1000 ml of water, adjusting the pH to 3.0 with orthophosphoric acid and filtering,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 225 nm,
- a 10 µl loop injector.

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Mobile phase A</th>
<th>Mobile phase B</th>
<th>Flow rate ml per minute</th>
</tr>
</thead>
<tbody>
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<tr>
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</tr>
<tr>
<td>80</td>
<td>2</td>
<td>98</td>
<td>1</td>
</tr>
</tbody>
</table>

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 3000 and the tailing factor is not more than 1.5 for each component.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 3 times the area of any peak in the chromatogram obtained with the reference solution (3.0 per cent) and the sum of all the secondary peaks is not more than 5 times the area of any peak due to the reference solution (5.0 per cent).
Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of water and 50 volumes of methanol.

Test solution. Weigh accurately a quantity of the powdered tablets containing 150 mg of abacavir, dissolve in 100 ml of water, add 80 ml of methanol and dilute to 200.0 ml with methanol. Dilute 10.0 ml of the solution to 25.0 ml with the solvent mixture and filter.

Reference solution. A solution containing 0.35 per cent w/v of abacavir sulphate RS, 0.15 per cent w/v lamivudine RS and 0.30 per cent w/v of zidovudine RS in the solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm), (such as Kromasil C-18),
- column temperature 50º,
- mobile phase: a mixture of 65 volumes of a buffer solution prepared by dissolving 1 g of octane sulphonic acid and 1 ml of triethylamine in 1000 ml of water, adjusting the pH to 4.5 with orthophosphoric acid and filtering, and 35 volumes of methanol,
- flow rate. 1 ml per minute,
- spectrophotometer set at 272 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the column efficiency determined from the peak due to lamivudine is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 for each component and the relative standard deviation of replicate injections is not more than 2.0 per cent for each component.

Inject the test solution and the reference solution.

Calculate the contents of (C14H18N6O)2,H2SO4, C8H11N3O3S and C10H13N5O4 in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30º.

Acarbose

![Acarbose structure](image)

C25H43NO18    Mol. Wt. 646.0

Acarbose is O-4,6-dideoxy-4-[[1S,4R,5R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino-o-D-

Acarbose is O-4,6-dideoxy-4-[[1S,4R,5R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino-o-D-

glucopyranosyl-(1’4)-O-α-D-glucopyranosyl-(1→4)-D-glucopyranose, which is produced by certain strains of Actinoplanes utahensis.

Acarbose contains not less than 95.0 per cent and not more than 102.0 per cent of C25H43NO18, calculated on the anhydrous basis.

Description. A white or yellowish, amorphous powder, hygroscopic.

Identification
A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with acarbose RS or with the reference spectrum of acarbose.

B. In the Assay, the principal peak in the chromatogram obtained with test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 5.5 to 7.5, determined in 5.0 per cent w/v solution in carbon dioxide-free water (solution A).

Specific optical rotation (2.4.22). +168º to +183º, dilute 2 ml of solution A to 10 ml with water.

Light absorption (2.4.7). Absorbance of solution A at 425 nm, not more than 0.15.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.2 g of the substance under examination in 100 ml of water.

Reference solution (a). A 0.2 per cent w/v solution of acarbose RS in water.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with water.

Chromatographic system as described under Assay.

Inject reference solution (a). Test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

Heavy metals (2.3.13). 1 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.
**Water** (2.3.43). Not more than 4.0 per cent, determined on 0.3 g.

**Assay.** Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 10 mg of the substance under examination in 50.0 ml of water. Dilute 5.0 ml of the solution to 50.0 ml with water.

*Reference solution.* A 0.002 per cent w/v solution of acarbose RS in water.

Chromatographic system
- a stainless steel column 25 cm x 4 mm packed with aminopropylsilyl silica (5 µm),
- mobile phase: a mixture of 75 volumes of acetonitrile and 25 volumes of a solution containing 0.06 per cent w/v of potassium dihydrogen phosphate and 0.035 per cent w/v of disodium hydrogen phosphate dihydrate,
- flow rate: 2 ml per minute,
- spectrophotometer set at 210 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of C_{25}H_{43}NO_{18}.

**Storage.** Store protected from moisture.

---

**Acarbose Tablets**

Acarbose Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of acarbose, C_{25}H_{43}NO_{18}.

**Identification**

In the Assay, the chromatogram obtained with the test solution corresponds to the chromatogram obtained with the reference solution.

**Tests**

**Dissolution** (2.5.2).

Apparatus No. 1
Medium. 900 ml of water.
Speed and time. 100 rpm for 30 minutes.
Withdraw a suitable volume of the medium and filter.
Determine by liquid chromatography (2.4.14).

*Solvent mixture.* 15 volumes of phosphate buffer pH 3.0 and 85 volumes of acetonitrile.

Inject the reference solution. The test is not valid unless the relative standard deviation is not less than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of C_{25}H_{43}NO_{18}.

**Storage.** Store protected from light and moisture.

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**Acebutolol Hydrochloride**

![Acebutolol Hydrochloride](image)

C_{18}H_{28}N_{2}O_{4}, HCl  Mol. Wt. 372.9
Acebutolol Hydrochloride is (RS)-3’-acetyl-4’-(2-hydroxy-3-isopropylaminopropoxy)butyranilide hydrochloride.
Acebutolol Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of C$_{18}$H$_{28}$N$_2$O$_4$, HCl, calculated on the dried basis.

**Description.** A white or almost white, crystalline powder.

**Identification**

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with acebutolol hydrochloride RS or with the reference spectrum of acebutolol hydrochloride.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in 0.1 per cent v/v solution of hydrochloric acid shows absorption maxima at about 233 nm and 322 nm; absorbance at 233 nm, 0.55 to 0.61.

C. Determine by thin layer chromatography (2.4.17), coating the plate with silica gel GF 254.

**Mobile phase.** A mixture of 60 volumes of water, 40 volumes of methanol and 0.5 volumes of perchloric acid.

**Test solution.** Dissolve 0.1 g of the substance under examination in 100 ml of methanol.

Reference solution (a). A 0.1 per cent w/v solution of acebutolol hydrochloride RS in methanol.

Reference solution (b). A mixture of equal volumes of reference solution (a) and a 0.1 per cent w/v solution of pindolol RS in methanol.

Apply to the plate 10 µl of each solution. After development, dry the plates in a current of warm air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatograms obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b) and not more than two such spots are more intense than the spot in the chromatograms obtained with reference solution (d). Ignore any spot at the point of application.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Acebutolol Hydrochloride, shake with 40 ml of 0.1 M hydrochloric acid and add sufficient water to produce 100.0 ml, filter and dilute 10.0 ml of the filtrate to 100.0 ml with water. Dilute 10.0 ml of this solution to 100.0 ml with water and measure the absorbance of the resulting solution at the maximum at about 233 nm (2.4.7).

Calculate the content of C$_{18}$H$_{28}$N$_2$O$_4$, HCl taking 580 as the specific absorbance at 233 nm.

**Storage.** Store protected from light.

**Acebutolol Tablets**

Acebutolol Tablets contain Acebutolol Hydrochloride. The tablets may be coated.

Acebutolol Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of acebutolol hydrochloride, C$_{18}$H$_{28}$N$_2$O$_4$, HCl.

**Identification**

A. When examined in the range 220 nm to 360 nm (2.4.7), the solution obtained in the Assay, shows an absorption maximum at about 233 nm.
B. Determine by thin layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 60 volumes of water, 40 volumes of methanol and 0.5 volumes of perchloric acid.

**Test solution.** Shake a quantity of the powdered tablets containing 0.5 g of Acebutolol Hydrochloride with 30 ml of methanol in a 50-ml volumetric flask for 15 minutes, dilute to volume with methanol, centrifuge and use the clear supernatant liquid.

**Reference solution (a).** A 0.1 per cent w/v solution of acebutolol hydrochloride RS in methanol.

**Reference solution (b).** A mixture of equal volumes of reference solution (a) and a 0.1 per cent w/v solution of pindolol RS in methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

**Tests**

**Related substances.** Determine by thin layer chromatography (2.4.17), coating two plates with silica gel GF 254.

**Mobile phase (a).** The upper layer obtained by shaking together 50 volumes of water, 40 volumes of 1-butanol and 10 volumes of glacial acetic acid.

**Mobile phase (b).** A mixture of 90 volumes of 2-propanol and 10 volumes of glacial acetic acid.

**Test solution.** Shake a quantity of the powdered tablets containing 0.5 g of Acebutolol Hydrochloride with 30 ml of methanol in a 50-ml volumetric flask for 15 minutes, dilute to volume with methanol, centrifuge and use the clear supernatant liquid.

**Reference solution (a).** Dilute 1 ml of the test solution to 10 ml with methanol.

**Reference solution (b).** Dilute 3 ml of reference solution (a) to 100 ml with methanol.

**Reference solution (c).** A 0.1 per cent w/v solution of acebutolol hydrochloride RS in methanol.

**Reference solution (d).** Dilute 1 volume of reference solution (c) to 3 volumes with methanol.

Apply 20 µl of each solution on each plate. Develop two chromatograms using separately the two mobile phases. After development, dry the plates in a current of warm air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatograms obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b) and not more than two such spots are more intense than the spot in the chromatograms obtained with reference solution (d). Ignore any spot at the point of application.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Acebutolol Hydrochloride, shake with 40 ml of 0.1M hydrochloric acid and add sufficient water to produce 100.0 ml, filter and dilute 10.0 ml of the filtrate to 100.0 ml with water. Dilute 10.0 ml of this solution to 100.0 ml with water and measure the absorbance of the resulting solution at the maximum at about 233 nm (2.4.7). Calculate the content of C16H13Cl2NO4.HCl taking 580 as the specific absorbance at 233 nm.

**Storage.** Store protected from light.

**Aceclofenac**

\[
\text{C}_{16}\text{H}_{13}\text{Cl}_{2}\text{NO}_{4} \quad \text{Mol. Wt. 354.2}
\]

Aceclofenac is [(2,6-dichlorophenyl)amino] phenylacetoxycetic acid.

Aceclofenac contains not less than 99.0 per cent and not more than 101.0 per cent of C16H13Cl2NO4, calculated on the dried basis.

**Description.** A white or almost white, crystalline powder.

**Identification**

**Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with aceclofenac RS or with the reference spectrum of aceclofenac.

B. When examined in the range 220 nm to 370 nm (2.4.7), the 0.002 per cent w/v solution in methanol shows an absorption maximum at 275 nm.

C. Dissolve about 10 mg in 10 ml of ethanol. To 1 ml of the solution, add 0.2 ml of a mixture, prepared immediately before
use, of equal volumes of a 0.6 per cent solution of potassium ferricyanide and a 0.9 per cent solution of ferric chloride. Allow to stand protected from light for 5 minutes. Add 3 ml of a 1 per cent solution of hydrochloric acid. Allow to stand protected from light for 15 minutes. A blue colour develops and a precipitate is formed.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of 30 volumes of mobile phase A and 70 volumes of mobile phase B.

Test solution. Dissolve 50 mg of the substance under examination in 25 ml in solvent mixture.

Reference solution (a). A 0.2 per cent w/v solution of aceclofenac RS in solvent mixture.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with spherical end-capped octadecylsilane bonded to porous silica (5 µm), with a pore size of 10 µm and carbon loading of 19 per cent,
- mobile phase: A. a 0.112 per cent w/v solution of orthophosphoric acid adjusted to pH 7.0 using a 4.2 per cent solution of sodium hydroxide,
  B. 1 volume of water and 9 volumes of acetonitrile,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 275 nm,
- a 10 µl loop injector.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
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</table>

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1 g by drying in an oven at 105°C.

Assay. Weigh accurately about 0.3 g and dissolve in 40 ml of methanol. Titrate with 0.1 M sodium hydroxide. Determine the end point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.03542 g of C₁₆H₁₃Cl₂NO₄.

Storage. Store protected from light.

Aceclofenac Tablets

Aceclofenac Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of aceclofenac, C₁₆H₁₃Cl₂NO₄.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus. No. 1
Medium: 900 ml of phosphate buffer pH 7.5.
Speed and time. 50 rpm for 45 minutes.

Withdraw a suitable volume of the medium and filter. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with dissolution medium. Measure the absorbance of the resulting solution at the maximum at about 273 nm (2.4.7). Calculate the content of aceclofenac, C₁₆H₁₃Cl₂NO₄ in the medium from the absorbance obtained from a solution of known concentration of aceclofenac RS.

D. Not less than 70 per cent of the stated amount of C₁₆H₁₃Cl₂NO₄.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of powdered tablet containing 100 mg of Aceclofenac, disperse in 100 ml of mobile phase and filter.

Reference solution (a). A 0.1 per cent w/v solution of aceclofenac RS in the mobile phase.
Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with dimethylctysilislane (C8 alkyl chain) fully end capped stationary phase (5 µm) (such as Hypersil MOS),
- mobile phase: a mixture of 55 volumes of buffer pH 3.5 prepared by adding 1.2 ml of glacial acetic acid in 1000 ml of water; adjust the pH to 3.5 with dilute sodium hydroxide and filter, 22.5 volumes of acetonitrile and 22.5 volumes of tetrahydrofuran,
- flow rate. 1 ml per minute,
- spectrophotometer set at 275 nm,
- a 20 µl loop injector.

Inject reference solution (a). Test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of 55 volumes of acetonitrile and 45 volumes of water.

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of powdered tablet containing 100 mg of Aceclofenac, add about 60 ml of acetonitrile and sonicate for 10 minutes. Make up the volume to 100.0 ml with acetonitrile. Dilute 5.0 ml of the solution to 50.0 ml with solvent mixture.

Reference solution. Weigh about 25 mg of aceclofenac RS and add acetonitrile to dissolve and make up the volume to 25.0 ml with solvent mixture. Dilute 5.0 ml of the above solution to 50.0 ml with solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane stationary phase (5 µm) (such as Hypersil ODS),
- mobile phase: a mixture of 55 volumes of buffer solution prepared by adding 1.0 ml of glacial acetic acid in 1000 ml of water and 45 volumes of acetonitrile,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 275 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2500 theoretical plates. The tailing factor is not more than 2.0. The relative standard deviation for replicate injections is not more than 2.0 per cent. Inject the test solution and the reference solution.

Calculate the content of C16H13Cl2NO4.

Storage. Store protected from moisture, at a temperature not exceeding 25°.

Acetazolamide

\[
\text{C}_4\text{H}_6\text{N}_4\text{O}_3\text{S}_2 \quad \text{Mol. Wt. 222.2}
\]

Acetazolamide is N-(5-sulphamoyl-1,3,4-thiadiazol-2-yl) acetamide.

Acetazolamide contains not less than 98.5 per cent and not more than 101.0 per cent of \( \text{C}_4\text{H}_6\text{N}_4\text{O}_3\text{S}_2 \), calculated on the dried basis.

Description. A white to faintly yellowish-white, crystalline powder; odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with acetazolamide RS or with the reference spectrum of acetazolamide.

B. When examined in the range 230 nm to 260 nm (2.4.7), a 0.003 per cent w/v solution in 0.01 M sodium hydroxide shows an absorption maximum at about 240 nm; absorbance at about 240 nm, 0.49 to 0.53. When examined in the range 260 nm to 360 nm (2.4.7), a 0.00075 per cent w/v solution in 0.01 M sodium hydroxide shows an absorption maximum at about 292 nm; absorbance at about 292 nm, 0.43 to 0.47.

C. To about 20 mg in a test-tube add 4 ml of 2 M hydrochloric acid and 0.2 g of zinc powder and immediately place a piece of lead acetate paper over the mouth of the tube; the paper exhibits a brownish-black colour.

D. To about 25 mg add 5 ml of water, 4 drops of 1 M sodium hydroxide and 2 drops of cupric sulphate solution; a bluish-green colour or precipitate is produced.

Tests

Silver-reducing substances. Mix 5 g with 25 ml of ethanol (95 per cent), add 125 ml of water, 10 ml of nitric acid and 5 ml of 0.1 M silver nitrate, stir for 30 minutes and filter. Wash the
residue with water, mix the filtrate and washings and titrate the excess of silver nitrate in the mixture with 0.05 M ammonium thiocyanate using ferric ammonium sulphate solution as indicator; not less than 9.5 ml of 0.05 M ammonium thiocyanate is required.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A freshly prepared mixture of 50 volumes of 2-propanol, 30 volumes of ethyl acetate and 20 volumes of strong ammonia solution.

Test solution. Dissolve 0.5 g of the substance under examination in 100 ml of a mixture of equal volumes of ethanol (95 per cent) and ethyl acetate.

Reference solution. A 0.005 per cent w/v solution of the substance under examination in a mixture of equal volumes of ethanol (95 per cent) and ethyl acetate.

Apply to the plate 20 µl of each solution. Do not line the walls of the tank. Allow to saturate for 1 hour before development. After development, dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Heavy metals (2.3.13). 1.0 g dissolved in a mixture of 10 ml of 1 M sodium hydroxide and 15 ml of water complies with the limit test for heavy metals, Method C (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 2.5 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.4 g of the substance under examination and dissolve and 90 ml of dimethylformamide. Titrate with 0.1 M tetrabutylammonium hydroxide, determining the end-point potentiometrically (2.4.25). Take precautions to prevent absorption of atmospheric carbon dioxide. Carry out a blank titration.

1 ml of 0.1 M tetrabutylammonium hydroxide is equivalent to 0.02222 g of C₄H₆ N₄O₃S₂.

Storage. Store protected from light.

Acetazolamide Tablets

Acetazolamide Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of acetazolamide, C₄H₆ N₄O₃S₂.

Identification

A. To a quantity of the powdered tablets containing 0.5 g of Acetazolamide add 2 ml of 1 M sodium hydroxide, shake thoroughly and filter. Neutralise the filtrate with glacial acetic acid, filter and dry the resulting precipitate at 105°. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with acetazolamide RS or with the reference spectrum of acetazolamide.

B. Triturate a quantity of the powdered tablets containing 0.5 g of Acetazolamide with a mixture of 5 ml of water and 1 ml of 1 M sodium hydroxide, transfer to a test-tube, add 0.2 g of zinc powder and immediately place a piece of lead acetate paper over the mouth of the tube; the paper exhibits a brownish-black colour.

C. To a quantity of the powdered tablets containing 25 mg of Acetazolamide add 5 ml of water, 3 drops of 1 M sodium hydroxide and 2 drops of cupric sulphate solution; a bluish-green colour or precipitate is produced.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A freshly prepared mixture of 50 volumes of 2-propanol, 30 volumes of ethyl acetate and 20 volumes of strong ammonia solution.

Solvent mixture. A mixture of equal volumes of ethanol (95 per cent) and ethyl acetate.

Test solution. Shake a quantity of the powdered tablets containing 50 mg of Acetazolamide for 20 minutes with 10 ml of solvent mixture, filter and use the filtrate.

Reference solution. Dilute 1 volume of the test solution to 100 volumes with the same solvent mixture.

Apply to the plate 20 µl of each solution. Do not line the walls of the tank. Allow to saturate for 1 hour before development. After development, dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.4 g of Acetazolamide and add 90 ml of dimethylformamide. Titrate with 0.1 M tetrabutylammonium hydroxide, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M tetrabutylammonium hydroxide is equivalent to 0.02222 g of C₄H₆ N₄O₃S₂.

Storage. Store protected from light.
Glacial Acetic Acid

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{O} \\
\text{C}_2\text{H}_4\text{O}_2 \quad \text{Mol. Wt. 60.1}
\end{align*}
\]

Glacial Acetic acid contains not less than 99.0 per cent w/w and not more than 100.5 per cent w/w of \(\text{C}_2\text{H}_4\text{O}_2\).

**Description.** A crystalline mass or clear, colourless, volatile liquid.

**Identification**
A. A 10 per cent w/v solution is strongly acidic.
B. To 0.03 ml add 3 ml of water and neutralize with 2 M sodium hydroxide; the solution gives reaction C of acetates (2.3.1).

**Tests**

**Freezing point** (2.4.11). Not less than 14.8°.

**Residue on evaporation.** Not more than 0.01 per cent, determined on 20.0 g by evaporating to dryness on a water-bath and drying at 105°.

**Reducing substances.** To 5 ml add 10 ml of water and mix. To 5 ml of the resulting solution add 6 ml of sulphuric acid and cool. Add 2 ml of 0.0167 M potassium dichromate, allow to stand for 1 minute and add 25 ml of water and 1 ml of freshly prepared dilute potassium iodide solution. Titrate with 0.1 M sodium thiosulphate using 1 ml of starch solution as indicator. Not less than 1.0 ml of 0.1 M sodium thiosulphate is required.

**Heavy metals** (2.3.13). Dissolve the residue obtained in the test for Residue on evaporation by heating with two quantities, each of 15 ml, of water and add sufficient water to produce 50 ml (solution A). The solution complies with the limit test for heavy metals, Method D (5 ppm). Use 10 ml of lead standard solution (2 ppm Pb) to prepare the standard.

**Iron** (2.3.14). 5 ml of solution A diluted to 10 ml with water complies with the limit test for iron (5 ppm). Use 1.0 ml of iron standard solution (10 ppm Fe) to prepare the standard.

**Chlorides** (2.3.12). To 20 ml add sufficient water to produce 100 ml (solution B). 10 ml of solution B diluted to 15 ml with water complies with the limit test for chlorides (25 ppm). Use 10 ml of chloride standard solution (5 ppm Cl) to prepare the standard.

**Sulphates** (2.3.17). 15 ml of solution B complies with the limit test for sulphates (50 ppm).

**Assay.** Weigh accurately a conical flask with a ground-glass stopper containing 25 ml of water, add 1 ml of the substance under examination and reweigh. Titrate with 1 M sodium hydroxide using 0.5 ml of phenolphthalein solution of indicator.

1 ml of 1 M sodium hydroxide is equivalent to 0.06005 g of \(\text{C}_2\text{H}_4\text{O}_2\).

**Storage.** Store protected from light and moisture.

Acetic Acid Ear Drops

Acetic Acid Otic Solution

Acetic Acid Ear Drops is a solution of Glacial Acetic Acid in a suitable non-aqueous solvent.

Acetic Acid Ear Drops contain not less than 85.0 per cent and not more than 130.0 per cent of the stated amount of acetic acid, \(\text{C}_2\text{H}_4\text{O}_2\).

**Identification**
A. Dilute 5 ml with 10 ml of water and adjust to a pH of about 7 with 1 M sodium hydroxide. Add ferric chloride test solution, a deep red colour is produced, which is decolorized on the addition of hydrochloric acid.
B. Warm the solution with sulphuric acid and ethanol (95 per cent); a characteristic odour of ethyl acetate is evolved.

**Tests**

**pH** (2.4.24). 2.0 to 4.0, determined in a 50.0 per cent v/v solution.

**Other tests.** Comply with the tests stated under Ear Drops.

**Assay.** Transfer a volume containing about 0.1 g of Glacial Acetic Acid to a conical flask, add 5 ml of sodium chloride solution and about 40 ml of water. Titrate with 0.1 M sodium hydroxide, using 0.15 ml of phenolphthalein solution as indicator.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.006005 g of \(\text{C}_2\text{H}_4\text{O}_2\).

**Storage.** Store protected from light and moisture.

Aciclovir

Aciclovir

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{N} \\
\text{C}_6\text{H}_1\text{N}_5\text{O}_3 \quad \text{Mol. Wt. 225.2}
\end{align*}
\]

Aciclovir is 2-amino-9-[2-hydroxyethoxy)methyl]-1,9-dihydro-6H-purin-6-one.
Aciclovir contains not less than 98.5 per cent and not more than 101.0 per cent of C₈H₁₁N₅O₃, calculated on the anhydrous basis.

**Description.** A white or almost white, crystalline powder.

**Identification**

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with aciclovir RS.

**Tests**

**Appearance of solution.** A 1.0 per cent w/v solution in 0.1 M sodium hydroxide is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 80 volumes of dichloromethane, 20 volumes of methanol and 2 volumes of strong ammonia solution.

Prepare the following solutions immediately before use.

**Test solution.** Dissolve 1.0 g of the substance under examination in 100 ml of dimethyl sulphoxide.

**Reference solution.** A 0.005 per cent w/v solution of aciclovir impurity RS in dimethyl sulphoxide.

Apply to the plate 10 µl of each solution. Keep the spots compact by drying in a current of warm air and allow the plate to cool. Allow the mobile phase to rise 10 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot with Rf value greater than that of the principal spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 6.0 per cent, determined on 0.5 g.

**Assay.** Weigh accurately about 0.15 g and dissolve in 60 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02252 g of C₈H₁₁N₅O₃.

**Storage.** Store protected from light and moisture.

**Aciclovir Intravenous Infusion**

Acyclovir Intravenous Infusion; Acyclovir Sodium Intravenous Infusion

Aciclovir Intravenous Infusion is a sterile material consisting of aciclovir sodium, prepared from Aciclovir with the aid of a suitable alkali, with or without auxiliary substances. It is filled in a sealed container.

The infusion is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

**Storage.** The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Aciclovir Intravenous Infusion contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of aciclovir, C₈H₁₁N₅O₃.

**Description.** A white or almost white, crystalline powder.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injections) and with the following requirements.

**Identification**

A. When examined in the range 230 nm to 360 nm (2.4.7), the solution prepared in the Assay shows an absorption maximum at about 255 nm and a broad shoulder at about 274 nm.

B. In the test for Guanine, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. Gives reaction A of sodium salts (2.3.1).

**Tests**

**Appearance of solution.** Dissolve the contents of a sealed container in sufficient water for injection to produce a solution containing the equivalent of 2.5 per cent w/v solution of Aciclovir (solution A). The solution is not more opalescent than opalescence standard OS2 (2.4.1), and not more intensely coloured than reference solution BYS5 (2.4.1).

**pH** (2.4.24). 10.7 to 11.7, determined in solution A.

**Guanine.** Determine by thin-layer chromatography (2.4.17), coating the plate with cellulose F254. (Merck cellulose F plates are suitable).

**Mobile phase.** A mixture of 10 volumes of 1-propanol, 30 volumes of strong ammonia solution and 60 volumes of a 5 per cent w/v solution of ammonium sulphate.

**Test solution (a).** Dissolve a suitable quantity of the substance under examination in sufficient 0.1 M sodium hydroxide to produce a solution containing 0.5 per cent of Aciclovir.

**Test solution (b).** Dilute 1 volume of test solution (a) to 10 volumes with 0.1 M sodium hydroxide.
**Reference solution (a).** A 0.05 per cent w/v solution of aciclovir RS in 0.1 M sodium hydroxide.

**Reference solution (b).** A 0.005 per cent w/v solution of guanine in 0.1 M sodium hydroxide.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. Any secondary spot corresponding to guanine in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (1 per cent).

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 80 volumes of dichloromethane, 20 volumes of methanol and 2 volumes of strong ammonia solution.

Prepare the following solutions immediately before use.

**Test solution.** Dissolve a suitable quantity of the substance under examination in dimethyl sulfoxide to produce a solution containing 2.5 per cent of aciclovir.

**Reference solution.** Dilute 1 volume of the test solution to 200 volumes with dimethyl sulfoxide.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in a current of warm air and examine in ultraviolet light 254 nm. Any secondary spot with an Rf value greater than that of the principal spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (0.5 per cent).

**Bacterial endotoxins (2.2.3).** Not more than 0.174 Endotoxin Units per mg of aciclovir.

**Assay.** Dissolve a quantity of the mixed contents of 10 containers containing 0.10 g of Aciclovir in sufficient 0.1 M hydrochloric acid to produce 500.0 ml. Dilute 5.0 ml of the resulting solution to 100.0 ml with 0.1 M hydrochloric acid. Measure the absorbance of the resulting solution at the maximum at about 255 nm (2.4.7). Calculate the content of C8H11N5O3 taking 560 as the specific absorbance at 255 nm.

**Storage.** Store protected from moisture, in a sterile, tamper-evident container sealed so as to exclude micro-organisms, at a temperature not exceeding 30°.

**Labelling.** The label states (1) the quantity of aciclovir sodium in the sealed container in terms of the equivalent amount of Aciclovir; (2) the strength of the constituted solution in terms of the equivalent amount of Aciclovir in a suitable dose-volume.

---

**Aciclovir Tablets**

Aciclovir Tablets

Aciclovir Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of aciclovir, C8H11N5O3.

**Identification.**

A. When examined in the range 230 nm to 360 nm (2.4.7), the solution prepared in the Assay shows an absorption maximum at about 255 nm and a broad shoulder at about 274 nm.

B. In the test for Guanine, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

**Tests.**

**Guanine.** Determine by thin-layer chromatography (2.4.17), coating the plate with cellulose F254. (such as Merck cellulose F plates).

**Mobile phase.** A mixture of 10 volumes of 1-propanol, 30 volumes of strong ammonia solution and 60 volumes of a 5 per cent w/v solution of ammonium sulphate.

**Test solution (a).** Shake a quantity of the powdered tablets containing 0.25 g of Aciclovir with 25 ml of 0.1 M sodium hydroxide for 10 minutes. Add a sufficient quantity of 0.1 M sodium hydroxide to produce 50 ml. Allow to stand and allow any undissolved material to settle before application to the plate.

**Test solution (b).** Dilute 1 volume of test solution (a) to 10 volumes with 0.1 M sodium hydroxide.

**Reference solution (a).** A 0.05 per cent w/v solution of aciclovir RS in 0.1 M sodium hydroxide.

**Reference solution (b).** A 0.005 per cent w/v solution of guanine in 0.1 M sodium hydroxide.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. Any secondary spot corresponding to guanine in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (1 per cent).

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 80 volumes of dichloromethane, 20 volumes of methanol and 2 volumes of strong ammonia solution.

Prepare the following solutions immediately before use.
Adenine

C₅H₅N₅

Mol. Wt. 135.1

Adenine is 1H-purin-6-amine.

Adenine contains not less than 98.5 per cent and not more than 101.0 per cent of C₅H₅N₅, calculated on the dried basis.

Description. A white, crystalline powder; odourless.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with adenine RS or with the reference spectrum of adenine.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. To 1 g add 3.5 ml of propionic anhydride, boil for 15 minutes with stirring and cool. To the resulting crystalline mass add 15 ml of light petroleum (50° to 70°) and heat to boiling with vigorous stirring. Cool and filter. Wash the precipitate with two quantities, each of 5 ml, of light petroleum (50° to 70°). Dissolve the precipitate in 10 ml of water and boil for 1 minute. Allow the mixture to cool to 30° to 40° and filter. Dry the precipitate at 105° for 1 hour. The precipitate melts at 237° to 241° (2.4.21).

Tests

Appearance of solution. A 1.0 per cent w/v solution in dilute hydrochloric acid is clear (2.4.1) and colourless (2.4.1).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 40 volumes of 1-propanol, 40 volumes of ethyl acetate and 20 volumes of strong ammonia solution.

Test solution (a). Dissolve 0.1 g of the substance under examination in dilute acetic acid, with heating if necessary, and dilute to 10 ml with the same solvent.

Test solution (b). Dilute 1 ml of test solution (a) to 10.0 ml with dilute acetic acid.

Reference solution (a). Dissolve 10 mg of adenine RS in dilute acetic acid, with heating if necessary, and dilute to 10 ml with the same solvent.

Reference solution (b). Dilute 1 ml of test solution (b) to 20 ml with dilute acetic acid.

Reference solution (c). Dissolve 10 mg of adenine RS and 10 mg of adenosine RS in dilute acetic acid with heating if necessary and dilute to 10 ml with the same solvent.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry in a current of warm air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Chlorides (2.3.12). Suspend 2.5 g in 50 ml of water and boil for 3 minutes. Cool and dilute to 50 ml with water and filter. To 10 ml of the filtrate (solution A) add 1 ml of strong ammonia solution and 3 ml of dilute silver nitrate solution. Filter, wash
the precipitate with a little quantity of water and dilute the filtrate to 15 ml with water. The resulting solution complies with the limit test for chlorides (100 ppm).

Sulphates (2.3.17). Dilute 10 ml of solution A to 15 ml with water. The solution complies with the limit test for sulphates (300 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.1 g, dissolve in a mixture of 30 ml of anhydrous glacial acetic acid and 20 ml of acetic anhydride. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.01351 g of C₉H₁₃NO₃.

Storage. Store protected from light and moisture.

### Adrenaline

Epinephrine

![Image of Adrenaline structure]

C₉H₁₃NO₃  Mol. Wt. 183.3

Adrenaline is \((R)-1-(3,4\text{-dihydroxyphenyl})-2\text{-methylaminoethanol} \)

Adrenaline contains not less than 98.5 per cent and not more than 101.0 per cent of C₉H₁₃NO₃, calculated on the dried basis.

Description. A white or creamy-white, microcrystalline powder or granules. It gradually darkens on exposure to light and air, decomposition being faster in the presence of moisture and at higher temperatures.

Identification

Test A may be omitted if tests B, C and D are carried out. Test C may be omitted if tests A, B and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with adrenaline RS or with the reference spectrum of adrenaline.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.003 per cent w/v solution in 0.01 M hydrochloric acid shows an absorption maximum at about 280 nm; absorbance at about 280 nm, about 0.45.

C. To 1 ml of a neutral or faintly acid solution add dropwise a 0.25 per cent w/v solution of ferric chloride until an emerald-green colour is produced. Add sodium bicarbonate solution gradually; the solution changes first to blue and then to red.

D. To 1 ml of a 0.1 per cent w/v solution add 1 ml of a 1.0 per cent v/v solution of 2,5-diethoxytetrahydrofuran in glacial acetic acid. Heat at 80° for 2 minutes, cool in ice and add 3 ml of a 2.0 per cent w/v solution of 4-dimethylaminobenzaldehyde in a mixture of 19 volumes of glacial acetic acid and 1 volume of hydrochloric acid. Mix and allow to stand for 2 minutes. The solution becomes yellow and is similar to the one obtained by performing the test in the same manner but omitting the substance under examination (distinction from noradrenaline).

Tests

Specific optical rotation (2.4.22). –50.0° to –53.5°, determined in a freshly prepared 4.0 per cent w/v solution in 1 M hydrochloric acid.

Phenones. Absorbance of a 0.2 per cent w/v solution in 0.1 M hydrochloric acid at the maximum at about 310 nm, not greater than 0.20, calculated on the dried basis (2.4.7).

Noradrenaline. Dissolve 5 mg in 1 ml of a 0.5 per cent w/v solution of tartaric acid, add 4 ml of buffer pH 9.6, mix, add 1 ml of a freshly prepared 0.5 per cent w/v solution of sodium 1,2-naphthaquinone-4-sulphonate, mix and allow to stand for 30 minutes. Add 0.2 ml of a 1 per cent v/v solution of benzalkonium chloride solution, mix, add 15 ml of toluene previously washed with buffer pH 9.6 and filtered through a dry filter paper, shake for 30 minutes and allow to separate, centrifuging if necessary. Any red or purple colour in the toluene layer is not more intense than that produced by treating a solution of 0.40 mg of noradrenaline acid tartrate and 9 mg of noradrenaline-free adrenaline acid tartrate in 1 ml of water in a similar manner.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying over phosphorus pentoxide at a pressure not exceeding 0.7 kPa for 18 hours.

Assay. Weigh accurately about 0.3 g and dissolve in 50 ml of anhydrous glacial acetic acid, warming slightly, if necessary, to effect solution. Titrate with 0.1 M perchloric acid, using crystal violet solution as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.01832 g of C₉H₁₃NO₃.

Storage. Store protected from light in containers preferably filled with nitrogen.
Adrenaline Tartrate

Adrenaline Acid Tartrate; Adrenaline Bitartrate; Epinephrine Bitartrate.

\[
\text{C}_9\text{H}_{13}\text{NO}_3\cdot\text{C}_4\text{H}_6\text{O}_6 \quad \text{Mol. Wt. 333.3}
\]

Adrenaline tartrate is \((R)-1-(3,4\text{-dihydroxyphenyl})-2\text{-methylaminoethanol hydrogen tartrate.}

Adrenaline Tartrate contains not less than 98.0 per cent and not more than 101.0 per cent of \(\text{C}_9\text{H}_{13}\text{NO}_3\cdot\text{C}_4\text{H}_6\text{O}_6\), calculated on the dried basis.

Description. A white or greyish-white, crystalline powder; odourless. It darkens on exposure to air and light, decomposition being faster in the presence of moisture and at higher temperatures.

Identification

Dissolve about 1 g in 10 ml of \(\text{water}\) containing 0.1 g of \(\text{sodium metabisulphite}\), add a slight excess of \(\text{dilute ammonia solution}\) and allow to stand at about 4\(^\circ\) for 1 hour, filter and reserve the filtrate for test D. Wash the precipitate with three successive quantities, each of 2 ml, of \(\text{cold water}\), then with 5 ml of cold \(\text{ethanol (95 per cent)}\) and finally with 5 ml of cold \(\text{ethanol}\) and dry over \(\text{silica gel}\) at a pressure of 1.5 to 2.5 kPa for 3 hours. The residue comply with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with adrenaline \(\text{RS}\) or with the reference spectrum of adrenaline.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent \(\text{w/v} \) solution in \(0.01 \text{ M hydrochloric acid}\) shows an absorption maximum only at about 279 nm; absorbance at about 279 nm, about 0.4.

C. The filtrate reserved above gives the reactions of tartrates (2.3.1).

Tests

Appearance of solution. A 5.0 per cent \(\text{w/v} \) solution examined immediately after preparation is not more opalescent than opalescence standard OS2 (2.4.1) and not more intensely coloured than reference solution BYS4 (2.4.1).

Specific optical rotation (2.4.22). \(-50.0^\circ\) to \(-54.0^\circ\), determined in a freshly prepared 4.0 per cent \(\text{w/v} \) solution in \(1 \text{ M hydrochloric acid}\).

\(\text{pH}\) (2.24.4). 2.8 to 4.0, determined in a 1.0 per cent \(\text{w/v} \) solution.

Phenones. Absorbance of a 0.2 per cent \(\text{w/v} \) solution in 0.1 M \(\text{hydrochloric acid}\) at the maximum at about 310 nm, not more than 0.10, calculated on the dried basis (2.4.7).

Noradrenaline. Determine by thin-layer chromatography (2.4.17), coating the plate with \(\text{silica gel G}\).

Mobile phase. A mixture of 100 volumes of \(\text{acetone}\), 100 volumes of \(\text{dichloromethane}\) and 1 volume of \(\text{anhydrous formic acid}\).

Test solution. Dissolve 2.5 g of the substance under examination in 100 ml of \(\text{water}\).

Reference solution (a). A freshly prepared 0.125 per cent \(\text{w/v} \) solution of noradrenaline bitartrate \(\text{RS}\) in \(\text{water}\).

Reference solution (b). A freshly prepared 0.025 per cent \(\text{w/v} \) solution of noradrenaline bitartrate \(\text{RS}\) in \(\text{water}\).

Reference solution (c). A mixture of equal volumes of the test solution and reference solution (b).

Apply to the plate 6 µl of each of the test solution and reference solutions (a) and (b) and 12 µl of reference solution (c) as bands 20 mm by 2 mm.

Allow the applied bands to dry and spray them with a saturated solution of \(\text{sodium bicarbonate}\). Allow the plate to dry in air, spray the applied bands twice with \(\text{acetic anhydride}\), drying the plate between the two sprayings and heat the plate at 50\(^\circ\) for 90 minutes and develop the chromatograms. After removal of the plate, allow it to dry in air and spray with a freshly prepared mixture of 8 volumes of \(\text{methanol}\), 2 volumes of \(\text{ethylenediamine}\) and 2 volumes of a 0.5 per cent \(\text{w/v} \) solution of \(\text{potassium ferricyanide}\). Dry the plate at 60\(^\circ\) for 10 minutes and examine in ultraviolet light at 254 nm and 365 nm. Any band situated between the two most intense bands in the chromatogram obtained with the test solution is not more intense than the corresponding band in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (c) exhibits between the two most intense bands a clearly separated band corresponding to the most intense band in the chromatogram obtained with reference solution (a).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over \(\text{phosphorus pentoxide}\) at a pressure of 1.5 to 2.5 kPa for 18 hours.

Assay. Weigh accurately about 0.3 g and dissolve in 50 ml of \(\text{anhydrous glacial acetic acid}\), warming slightly, if necessary, to effect solution. Titrate with 0.1 M \(\text{perchloric acid}\), using \(\text{crystal violet solution}\) as indicator. Carry out a blank titration. 1 ml of 0.1 M \(\text{perchloric acid}\) is equivalent to 0.03333 g of \(\text{C}_9\text{H}_{13}\text{NO}_3\cdot\text{C}_4\text{H}_6\text{O}_6\).
Storage. Store protected from light in containers preferably filled with nitrogen.

Adrenaline Injection

Adrenaline Bitartrate Injection; Adrenaline Acid Tartrate Injection; Adrenaline Tartrate Injection; Epinephrine Tartrate Injection

Adrenaline Injection is a sterile, isotonic solution containing 0.18 per cent w/v of Adrenaline Tartrate in Water for Injection. Adrenaline Injection contains the equivalent of not less than 0.09 per cent and not more than 0.115 per cent w/v of adrenaline, \( \text{C}_9\text{H}_13\text{NO}_3 \).

Description. A clear, colourless or almost colourless solution.

Identification

A. To an appropriate quantity add sufficient 0.01M hydrochloric acid to produce a solution containing 0.005 per cent w/v of adrenaline. When examined in the range 230 nm to 360 nm (2.4.7), the solution shows an absorption maximum at about 279 nm; absorbance at about 279 nm, about 0.4.

B. To 1 ml add dropwise a 0.25 per cent w/v solution of ferric chloride until an emerald-green colour is produced. Add sodium bicarbonate solution gradually; the solution changes first to blue and then to red.

C. To 10 ml add 2 ml of disodium hydrogen phosphate solution and sufficient iodine solution to produce a brown colour. Add 0.1M sodium thiosulphate dropwise until excess iodine is removed; a red colour is produced.

Tests

Appearance of solution. Examine the injection in a clear glass test-tube against a white background; it is not pinkish and does not contain a precipitate. If any yellow colour is observed, it is not more intense than a reference solution prepared by diluting 0.4 ml of it with 100 ml of water, when viewed similarly.

\( \text{pH} \) (2.4.24). 2.8 to 3.6.

Noradrenaline. Determine by liquid chromatography (2.4.14). Test solution. Substance under examination.

Reference solution (a). A 0.0018 per cent w/v solution of noradrenaline acid tartrate in the mobile phase.

Reference solution (b). A solution containing 0.0018 per cent w/v of noradrenaline-free adrenaline acid tartrate and 0.0018 per cent w/v of noradrenaline acid tartrate in the mobile phase.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles 5 to 10 \( \mu \text{m} \) (such as Nucleosil ODS),
- mobile phase: Dissolve 4.0 g of tetramethylammonium hydrogen sulphate, 1.1 g of sodium heptanesulphonate and 2 ml of 0.1M disodium edetate in 1000 ml of 5 per cent v/v solution of methanol, with pH adjusted to 3.5 to 3.6 with 1M sodium hydroxide,
- flow rate. 2 ml per minute,
- spectrophotometer set at 205 nm,
- a 20 \( \mu \)l loop injector.

Inject reference solution (b). The test is not valid unless the resolution factor between the two principal peaks is not less than 2.0.

Inject the test solution and reference solution (a). The area of any peak corresponding to noradrenaline is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a).

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Extract 30.0 ml in a separator with three quantities, each of 25 ml, of carbon tetrachloride, shaking vigorously for 1 minute each time; reject the carbon tetrachloride extracts. Add 0.2 ml of starch solution and, dropwise with swirling, a solution prepared by dissolving 0.5 g of iodine and 1.5 g of potassium iodide in 25 ml of water, until the blue colour persists. Immediately add just sufficient 0.1M sodium thiosulphate to discharge the blue colour and proceed further without delay. Add 2.1 g of sodium bicarbonate and swirl until most of the sodium bicarbonate has dissolved. Using a syringe, rapidly inject 1.0 ml of acetic anhydride directly into the contents of the separator, insert the stopper, and shake vigorously until the evolution of carbon dioxide ceases (7 to 10 minutes), releasing the pressure when necessary through the stop-cock. Allow to stand for 5 minutes and extract with six successive quantities, each of 25 ml, of chloroform, filtering each extract into a beaker through a small plug of cotton wool moistened with chloroform. Remove the chloroform, heat the residue at 105° for 30 minutes, allow to cool and weigh. Dissolve the residue in 5.0 ml of chloroform, swirling to assist solution, and determine the specific optical rotation of the resulting solution (2.4.22), using a 2-dm tube. Calculate the percentage content of \( \text{C}_9\text{H}_13\text{NO}_3 \), in the injection from the expression 1.974 W (0.5 + 0.5 R/93), where \( W \) is the weight of the residue in g and \( R \) is its specific optical rotation (in degrees without regard to the sign).

Storage. Store protected from light, in a single dose or multiple dose container.
Labelling. The label states (1) the quantity of active ingredient in parts per 1000 or mg per ml in terms of equivalent amount of adrenaline; (2) that the injection should not be used if it is pinkish or darker than slightly yellow.

Albendazole

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{H}_3\text{C} & \quad \text{CH}_3
\end{align*}
\]

C\text{\textsubscript{12}}H\text{\textsubscript{15}}N\text{\textsubscript{3}}O\text{\textsubscript{2}}S \quad \text{Mol. Wt. 265.3}

Albendazole is methyl 5-propylthio-1\textsubscript{H}-benzimidazol-2-yl-carbamate.

Albendazole contains not less than 98.0 per cent and not more than 102.0 per cent of C\text{\textsubscript{12}}H\text{\textsubscript{15}}N\text{\textsubscript{3}}O\text{\textsubscript{2}}S, calculated on the dried basis.

Description. A white to pale buff-coloured powder.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with albendazole RS or with the reference spectrum of albendazole.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Melting point (2.4.21). 208° to 210°.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 60 volumes of chloroform, 10 volumes of ether and 10 volumes of glacial acetic acid.

Test solution (a). Dissolve 0.2 g of the substance under examination in sufficient glacial acetic acid to produce 10 ml.

Test solution (b). Dilute 1 ml of test solution (a) to 4 ml with glacial acetic acid.

Reference solution (a). Dilute 1 ml of test solution (a) to 200 ml with glacial acetic acid.

Reference solution (b). Dissolve 25 mg of albendazole RS in sufficient glacial acetic acid to produce 5 ml.

Apply to the plate 10 µl of each solution. After development, dry in a current of warm air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the principal spot in the chromatogram obtained with reference solution (a).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm)

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

Assay. Weigh accurately about 0.5 g and dissolve in 80 ml of anhydrous glacial acetic acid. Titrate with 0.1 \text{ M perchloric acid}, using crystal violet solution as indicator. Carry out a blank titration.

1 ml of 0.1 \text{ M perchloric acid} is equivalent to 0.02653 g of C\text{\textsubscript{12}}H\text{\textsubscript{15}}N\text{\textsubscript{3}}O\text{\textsubscript{2}}S

Storage. Store protected from light.

Albendazole Tablets

Albendazole Tablets contain Albendazole. The tablets may contain permitted flavouring agents.

Albendazole Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of albendazole, C\text{\textsubscript{12}}H\text{\textsubscript{15}}N\text{\textsubscript{3}}O\text{\textsubscript{2}}S.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 60 volumes of chloroform, 10 volumes of ether and 10 volumes of glacial acetic acid.

Test solution. Add a quantity of the powdered tablets containing 200 mg of Albendazole to 20 ml of a mixture of 18 volumes of chloroform and 1 volume of formic acid, warm the suspension on a water-bath for 15 minutes, cool and filter. Dilute 10 ml of the filtrate with an equal volume of glacial acetic acid.

Reference solution. Dissolve 25 mg of albendazole RS in sufficient glacial acetic acid to produce 5 ml.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.
Alginic Acid

Polymannuronic Acid

Alginic acid is a hydrophilic colloidal mixture of polyuronic acids, \([\left(\text{C}_6\text{H}_{10}\text{O}_5\right)_n]\), composed of residues of D-mannuronic acid and L-guluronic acid extracted with dilute alkali from various species of brown seaweeds (Fam. Phaeophyceae).

Alginic Acid contains not less than 19.0 per cent and not more than 25.0 per cent of carboxylic acid groups (COOH), calculated on the dried basis.

Description. A white to yellowish-white, fibrous powder; odourless.

Identification

A. To 5 ml of a 0.75 per cent w/v solution in 0.1 M sodium hydroxide add 1 ml of calcium chloride solution; a gelatinous precipitate is formed.

B. To 5 ml of the solution obtained in test A add 1 ml of 2 M sulphuric acid; a gelatinous precipitate is formed.

C. To about 5 mg in a test-tube add 5 ml of water, 1 ml of a freshly-prepared 1 per cent w/v solution of 1,3-naphthalenediol in ethanol (95 per cent) and 5 ml of hydrochloric acid. Heat the mixture to boiling, boil gently for 3 minutes and cool to about 15°. Transfer the contents of the test-tube to a small separator with the aid of 5 ml of water and extract with 15 ml of di-isopropyl ether; the di-isopropyl ether extract exhibits a deep purple colour which is more intense than that exhibited by a blank prepared in the same manner without the substance under examination.

Tests

pH (2.4.24). 1.5 to 3.5, determined in a 3.0 per cent w/v dispersion in water.

Arsenic (2.3.10). Mix 2.0 g with 5 ml of sulphuric acid, add a few glass beads and digest at a temperature not exceeding 120° until charring begins. Additional sulphuric acid may be added if necessary but the total volume of acid added should not exceed 10 ml. Add cautiously, dropwise, hydrogen peroxide solution (100 vol) allowing the reaction to subside and again heating between addition of drops. Discontinue heating if foaming becomes excessive. When the reaction has abated, heat cautiously rotating the flask occasionally. Maintain oxidising conditions at all times during the digestion by adding small quantities of the hydrogen peroxide solution whenever the mixture turns brown or darkens. Continue the digestion until the organic matter has been destroyed, gradually raising the temperature until fumes of sulphur trioxide are copiously evolved and the solution becomes colourless or has only a light straw colour. Cool, add cautiously 10 ml of water, mix, and again evaporate till there is strong fuming, repeating this procedure to remove any trace of hydrogen peroxide. Cool, add cautiously 10 ml of water, wash the sides of the flask with a few ml of water and dilute with water to 35 ml. The resulting solution complies with the limit test for arsenic (5 ppm).

Heavy metals (2.3.13). 0.5 g complies with the limit test for heavy metals, Method B (40 ppm). Use nitric acid \(Sp.\) in place of sulphuric acid \(Sp.\) to wet the sample.

Acid value. Not less than 230, calculated on the dried basis and determined in the following manner. Weigh accurately about 1.0 g and suspend in a mixture of 50 ml of water and 30 ml of a 4.4 per cent w/v solution of calcium acetate. Shake vigorously, allow the mixture to stand for 1 hour, add phenolphthalein solution and titrate with the liberated acetic acid with 0.1 M sodium hydroxide. Carry out a blank titration.

Calculate the acid value from the expression 5.611 \(A/W\), where \(A\) is the volume, in ml, of 0.1 M sodium hydroxide consumed and \(W\) is the weight, in g, of the sample.

Microbial contamination (2.2.9). 1 g is free from Escherichia coli and 10 g is free from salmonellae.

Total ash (2.3.19). Not more than 4.0 per cent, determined on 0.5 g by Method B.

Loss on drying (2.4.19). Not more than 15.0 per cent, determined on 0.1 g by drying in an oven at 105° for 4 hours.

Assay. Weigh accurately about 0.25 g, add 25 ml of water and 25.0 ml of 0.1 M sodium hydroxide and titrate with 0.1 M hydrochloric acid using 0.2 ml of dilute phenolphthalein solution as indicator.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.004502 g of carboxylic acid groups (COOH).
Allopurinol

\[
\text{C}_9\text{H}_7\text{N}_3\text{O} \quad \text{Mol. Wt. 136.1}
\]

Allopurinol is a tautomeric mixture of 1H-pyrazolo[3,4-d]pyrimidin-4-ol and 1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one.

Allopurinol contains not less than 98.0 per cent and not more than 101.0 per cent of \( \text{C}_9\text{H}_7\text{N}_3\text{O} \), calculated on the dried basis.

**Description.** A white or almost white, crystalline powder.

**Identification**

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with allopurinol RS.

B. Dissolve 0.1 g in 10 ml of 0.1 M sodium hydroxide and add sufficient 0.1 M hydrochloric acid to produce 100.0 ml; dilute 10.0 ml to 100.0 ml with 0.1 M hydrochloric acid and dilute 10.0 ml of this solution to 100.0 ml with 0.1 M hydrochloric acid. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 250 nm and a minimum at about 231 nm; ratio of the absorbance at the minimum at about 231 nm to that at the maximum at about 250 nm, 0.52 to 0.62.

C. Dissolve 50 mg in 5 ml of dilute sodium hydroxide solution, add 1 ml of alkaline potassium mercuri-iodide solution, heat to boiling and allow to stand; a flocculent yellow precipitate is produced.

D. Shake about 0.1 g with 5 ml of dilute sodium hydroxide solution, add 3 ml of lithium and sodium molybdophosphotungstate solution and 5 ml of a 20 per cent w/v solution of sodium carbonate; a grey-blue colour is produced.

**Tests**

**Appearance of solution.** A 5.0 per cent w/v solution in 2 M sodium hydroxide is clear, (2.4.1), and not more intensely coloured than reference solution YS6 or GYS4 (2.4.1).

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

*Mobile phase. A mixture of 60 volumes of 2-butanone, 20 volumes of 2-methoxyethanol and 20 volumes of strong ammonia solution.*

Test solution. A 2.5 per cent w/v solution of the substance under examination in strong ammonia solution.

Reference solution. A 0.005 per cent w/v solution of 5-aminopyrazole-4-carboxamide hemisulphate RS in strong ammonia solution.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Heavy metals** (2.3.13). Mix carefully 1.0 g in a silica crucible with 4 ml of a 25 per cent w/v solution of magnesium sulphate in 1 M sulphuric acid and heat cautiously to dryness. Ignite the residue at a temperature not exceeding 800° and continue heating until a white or greyish residue is obtained. Allow to cool, moisten with 0.2 ml of 1 M sulphuric acid, evaporate, ignite again and allow to cool. The total ignition period should be less than 2 hours. Dissolve the residue with two quantities, each of 5 ml, of 2 M hydrochloric acid. Add 2 drops of dilute phenolphthalein solution and strong ammonia solution dropwise until a pink colour is produced. Cool, add glacial acetic acid until the solution gets decolorised and add a further 0.5 ml. Filter, if necessary, and dilute the solution to 20 ml with water. The resulting solution complies with the limit test for heavy metals, Method D (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.2 g and dissolve with gentle heating, if necessary, in 50 ml of dimethylformamide. Titrate with 0.1 M tetrabutylammonium hydroxide, determining the end-point potentiometrically (2.4.25). Carry out a blank titration. 1 ml of 0.1 M tetrabutylammonium hydroxide is equivalent to 0.01361 g of \( \text{C}_9\text{H}_7\text{N}_3\text{O} \).

**Allopurinol Tablets**

Allopurinol Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of allopurinol, \( \text{C}_9\text{H}_7\text{N}_3\text{O} \).

**Identification**

A. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows an absorption maximum only at about 250 nm.

B. Shake a quantity of the powdered tablets containing about 0.1 g of Allopurinol with 5 ml of dilute sodium hydroxide solution, add 3 ml of lithium and sodium molybd-
phosphotungstate solution and 5 ml of a 20 per cent w/v solution of sodium carbonate; a grey-blue colour is produced.

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

*Mobile phase.* A mixture of 60 volumes of 2-butanone, 20 volumes of 2-methoxyethanol and 20 volumes of strong ammonia solution.

*Test solution.* Shake a quantity of the powdered tablets containing about 0.25 g of Allopurinol with 10 ml of strong ammonia solution and filter.

*Reference solution.* A 0.005 per cent w/v solution of 5-aminopyrazole-4-carboxamide hemisulphate RS in strong ammonia solution

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Disintegration** (2.5.1). 30 minutes.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Allopurinol and shake with 20 ml of 0.05 M sodium hydroxide for 15 to 20 minutes, add 75 ml of 0.1 M hydrochloric acid shake for 10 minutes, add sufficient 0.1 M hydrochloric acid to produce 250.0 ml, filter and dilute 5.0 ml of the filtrate to 250.0 ml with 0.1 M hydrochloric acid. Measure the absorbance of the resulting solution at the maximum at about 250 nm (2.4.7) using 0.1 M hydrochloric acid as the blank.

Calculate the content of C9H7N3O, taking 563 as the specific absorbance at 250 nm.

**Aloes**

Aloes is the dried juice of the leaves of *Aloe barbadensis Miller* (*A. vera* Linn), known in commerce as Curacao Aloes or Barbados Aloes, or of *A. ferox* Miller and hybrids of this species with *A. africana* Miller and *A. spicata* Baker, known in commerce as Cape Aloes (Fam. Liliaceae). Indian Aloes of commerce is obtained from *A. barbadensis*.

Aloes contains not less than 50.0 per cent of water-soluble extractive. Curacao Aloes contains not less than 18.0 per cent and Cape Aloes not less than 28.0 per cent of hydroxyanthracene derivatives, calculated as anhydrous barbaloin.

**Description.** Unground Curacao Aloes — Brownish-black, opaque masses; fractured surface uneven, waxy and somewhat resinous; odour, strong and characteristic.

Unground Cape Aloes — Dark-brown or greenish-brown to olive-brown masses; fractured surface shiny and conchoidal; odour, strong and characteristic.

**Identification**

Mix 0.5 g with 50 ml of water; boil until nearly dissolved, cool, add 0.5 g of silica gel and filter. On the filtrate carry out the following tests.

A. Heat 5 ml with 0.2 g of borax until dissolved, add a few drops of this solution to a test-tube nearly filled with water; a green fluorescence is produced.

B. Mix 2 ml with 2 ml of bromine water; a pale yellow precipitate is produced. The supernatant liquid is violet with Curacao Aloes; no such violet colour appears with Cape Aloes.

C. Mix 5 ml with 2 ml of nitric acid; with Cape Aloes a reddish-yellow colour is produced; with Socotrine Aloes a pale brownish-yellow colour is produced; with Cape Aloes a yellowish-brown colour passing rapidly to green is produced.

D. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

*Mobile phase.* A mixture of 100 volumes of ethyl acetate, 17 volumes of methanol and 13 volumes of water.

*Test solution.* Heat 0.5 g, in powder, with 20 ml methanol to boiling on a water-bath, shake well, decant the supernatant liquid, keep at 4° and use within 24 hours.

*Reference solution.* Dissolve 50 mg of barbaloin in 10 ml methanol.

Apply to the plate 5 µl of each solution as bands 20 mm x 3 mm. Allow the mobile phase to rise 15 cm. Dry the plate in a current of air, spray with a 10 per cent w/v solution of potassium hydroxide in methanol and examine in ultraviolet light at 365 nm. The chromatogram obtained with the reference solution shows a yellow band with an Rf value of 0.4 to 0.5. In the case of Curacao Aloes, the chromatogram obtained with the test solution shows a yellow fluorescent band corresponding to that due to barbaloin in the chromatogram obtained with the reference solution and in the lower part a light blue fluorescent band (corresponding to aloesine). In the case of Cape Aloes, the test solution shows a yellow fluorescent band corresponding to that due to barbaloin in the chromatogram obtained with the reference solution and in the lower part two yellow fluorescent bands (due to aloinosides A and B) as well as a blue fluorescent band (due to aloesine). Heat the plate at 110° for 5 minutes. In the case of Curacao Aloes, with the test solution a violet fluorescent band appears just below the yellow band corresponding to barbaloin while in the case of Cape Aloes no such violet band appears.
Tests

Ethanol-insoluble substances. Weigh accurately about 1.0 g, in fine powder, and add to 50 ml of ethanol (95 per cent) in a flask. Reflux the mixture for 15 minutes. Remove the source of heat and set aside for 1 hour, shaking frequently, filter through a small dried and tared filter paper or suitable filtering crucible and wash the residue on the filter with ethanol (95 per cent) till the washings are colourless. The residue after drying to constant weight at 105°C weighs not more than 0.1 g.

Water-soluble extractive. Weigh accurately about 2.0 g, in fine powder, and macerate with about 60 to 70 ml of water in a flask. Shake the mixture at 30-minute intervals for 8 hours and allow to stand for a further 16 hours without shaking. Filter, wash the flask and the residue with small portions of water, passing the washings through the filter until the filtrate measures 100 ml. Evaporate 50 ml of this filtrate to dryness in a tared dish on a water-bath and dry at 105°C for 3 hours; the residue weighs not less than 0.5 g.

Total ash (2.3.19). Not more than 0.5 per cent, determined on 1.0 g by Method A.

Loss on drying (2.4.19). Not more than 12 per cent, determined on 1.0 g by drying in an oven at 105°C.

Assay. Moisten 0.2 g, in fine powder, with 2 ml of methanol, add 5 ml of water at about 60°C, mix, add a further 75 ml of water at about 60°C, shake for 30 minutes, cool, filter through a filter paper, washing the flask with 20 ml of water and add sufficient water to the combined filtrate and washings to produce 1000.0 ml. Transfer 10.0 ml of the solution to a flask containing 1 ml of a 60 per cent w/v solution of ferric chloride hexahydrate and 6 ml of hydrochloric acid, heat in a water-bath under a reflux condenser for 4 hours so that the water level is always above that of the liquid in the flask, cool, transfer the solution to a separating funnel, rinsing the flask successively with 4 ml of 1 M sodium hydroxide and 4 ml of water and adding the rinsings to the contents of the separating funnel. Extract with three quantities, each of 20 ml, of carbon tetrachloride and wash the combined carbon tetrachloride layers with two quantities, each of 100 ml, of water, discarding the washings. Dilute the organic phase to 100.0 ml with carbon tetrachloride, evaporate 20.0 ml carefully to dryness on a water-bath and dissolve the residue in 10.0 ml of 1 M sodium hydroxide. Immediately measure the absorbance of the resulting solution at the maximum at about 440 nm and at about 500 nm (2.4.7). Calculate the content of anhydrous barbaloin, taking 200 as at the maximum at about 440 nm and at about 500 nm (2.4.7). Immediately measure the absorbance

Alprazolam

C_{17}H_{13}ClN_{4}  Mol. Wt. 308.8
Alprazolam is 8-chloro-1-methyl-6-phenyl-4H-1,2,4-triazolo[4,3-a][1,4]benzodiazepine.
Alprazolam contains not less than 98.0 per cent and not more than 102.0 per cent of C_{17}H_{13}ClN_{4}, calculated on the dried basis.
Description. A white to off-white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with alprazolam RS or with the reference spectrum of alprazolam.
B. Dissolve 10.0 mg in water and dilute to 500.0 ml with the same solvent. Dilute 20.0 ml of this solution to 100.0 ml with water. When examined in the range 210 nm to 360 nm (2.4.7), the solution shows an absorption maximum at about 220 nm.
C. Melts at about 225°C (2.4.21).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.
Mobile phase. A mixture of 50 volumes of chloroform, 50 volumes of acetone, 50 volumes of ethyl acetate and 5 volumes of methanol.
Test solution. Dissolve 2 g in sufficient chloroform to produce 50 ml.
Reference solution. A 0.012 per cent w/v solution of alprazolam RS in chloroform.
Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in a current of air. Repeat the development process a second time and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).
Sulphated ash (2.3.18). Not more than 0.5 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure of 0.7 kPa for 16 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately about 25 mg and dissolve in sufficient acetonitrile to produce 100.0 ml. Dilute 10.0 ml of this solution to 100.0 ml with acetonitrile.

Reference solution. A solution containing 0.0025 per cent w/v of alprazolam RS in acetonitrile.

Chromatographic system  
- a stainless steel column 25 cm x 4.6 mm, packed with porous silica particles 3 to 10 µm,  
- mobile phase: a mixture of 850 volumes of acetonitrile, 80 volumes of chloroform, 50 volumes of l-butanol, 20 volumes of water and 0.5 volume of glacial acetic acid,  
- flow rate. 2 ml per minute,  
- spectrophotometer set at 254 nm,  
- a 10 µl or 20 µl loop injector.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the percentage content of C17H13ClN4.

Alprazolam Tablets

Alprazolam Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of alprazolam, C17H13ClN4.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Uniformity of content. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

Test solution. Transfer one tablet to a container, add 0.4 ml of water on to the tablet, allow the tablet to stand for 2 minutes and swirl the container to disperse the tablet. Add sufficient acetonitrile to produce a solution containing 0.0025 per cent w/v of alprazolam. Shake to mix and centrifuge, if necessary.

Calculate the percentage content of C17H13ClN4 in the tablets.

Storage. Store protected from light.

Aluminium Acetate Ear Drops

Aluminium Acetate Otic Drops; Aluminium Acetate Solution; Burow’s Solution.

Aluminium Sulphate 255 g  
Calcium Carbonate 100 g  
Tartaric Acid 45 g  
Glacial Acetic Acid 82.5 ml  
Purified Water sufficient to produce 1000 ml

Dissolve the Aluminium Sulphate in 600 ml of Purified Water, add Glacial Acetic Acid followed by Calcium Carbonate mixed with the remainder of the Purified Water and allow to stand for not less than 24 hours in a cool place, stirring occasionally. Filter, add the Tartaric Acid to the filtrate and mix.
Aluminium Gel Ear Drops contain not less than 1.7 per cent w/v and not more than 1.9 per cent w/v of aluminium, Al.

**Description.** A clear solution.

**Tests**

**Weight per ml** (2.4.29). 1.06 g to 1.08 g.

**Other tests.** Comply with the tests stated under Ear Drops.

**Assay.** Dilute 10.0 ml to 100.0 ml with water. To 10.0 ml of the resulting solution add 40.0 ml of 0.5 M disodium edetate, 90 ml of water and 0.15 ml of methyl red solution. Neutralise by the addition of 1 M sodium hydroxide dropwise and warm on a water-bath for 30 minutes. Cool, add 1 ml of 2 M nitric acid and 5 g of hexamine and titrate with 0.05 M lead nitrate using 0.5 ml of xylol orange solution as indicator.

1 ml of 0.05 M disodium edetate is equivalent to 0.001349 g of Al.

**Storage.** Store protected from light, in well-filled containers.

### Aluminium Hydroxide Gel

**Aluminium Hydroxide Suspension; Aluminium Hydroxide Mixture**

Aluminium Hydroxide Gel is an aqueous suspension of hydrated aluminium oxide together with varying quantities of basic aluminium carbonate and bicarbonate. It may contain Glycerin, Sorbitol, Sucrose or Saccharin as sweetening agents and Peppermint Oil or other suitable flavours. It may also contain suitable antimicrobial agents.

**Description.** A white, viscous suspension, translucent in thin layers; small amounts of clear liquid may separate on standing.

**Identification**

A solution in dilute hydrochloric acid gives the reactions of aluminium salts (2.3.1).

**Tests**

**pH** (2.4.24). 5.5 to 8.0.

**Arsenic** (2.3.10). Dilute 10.0 g in 18 ml of brominated hydrochloric acid, add 42 ml of water and remove the excess bromine with a few drops of stannous chloride solution AsT. The resulting solution complies with the limit test for arsenic (1 ppm).

**Heavy metals** (2.3.13). Dissolve 5.0 g in 10 ml of dilute hydrochloric acid, filter if necessary, and dilute to 25 ml with water. The resulting solution complies with the limit test for heavy metals, Method A (10 ppm).

**Chlorides** (2.3.12). Dissolve 0.5 g in 5 ml of dilute nitric acid, boil, cool, dilute to 100 ml with water and filter. 20 ml of the filtrate complies with the limit test for chlorides (0.25 per cent).

**Sulphates** (2.3.17). Dissolve 1.0 g in 5 ml of dilute hydrochloric acid with the aid of heat. Cool and dilute to 100 ml with water. Mix well and filter, if necessary. To 5 ml of the filtrate add 2 ml of dilute hydrochloric acid; the solution complies with the limit test for sulphates (0.3 per cent).

**Neutralising capacity.** Disperse 5.0 g in 100 ml of water, heat to 37°, add 100.0 ml of 0.1 M hydrochloric acid previously heated to 37° and stir continuously, maintaining the temperature at 37°; the pH of the solution, at 37°, after 10, 15 and 20 minutes, is not less than 1.8, 2.3 and 3.0 respectively and at no time is more than 4.5. Add 10.0 ml of 0.5 M hydrochloric acid previously heated to 37°, stir continuously for 1 hour maintaining the temperature at 37° and titrate with 0.1 M sodium hydroxide to pH 3.5.

**Microbial contamination** (2.2.9). Total viable aerobic count, not more than 100 micro-organisms per ml, determined by plate count. 1 ml is free from Escherichia coli.

**Assay.** Weigh accurately about 5.0 g and dissolve in 3 ml of hydrochloric acid by warming on a water-bath; cool to below 20° and dilute to 100.0 ml with water. To 20.0 ml of this solution, add 40.0 ml of 0.05 M disodium edetate, 80 ml of water, and 0.15 ml of methyl red solution and neutralise by the dropwise addition of 1 M sodium hydroxide. Warm on a water-bath for 30 minutes, add 3 g of hexamine and titrate with 0.05 M lead nitrate using 0.5 ml of xylol orange solution as indicator.

1 ml of 0.05 M disodium edetate is equivalent to 0.002549 g of Al₂O₃.

**Storage.** Store at a temperature not exceeding 30°. Do not freeze.

### Dried Aluminium Hydroxide Gel

**Dried Aluminium Hydroxide; Hydrated Aluminium Oxide**

Dried Aluminium Hydroxide Gel consists largely of hydrated aluminium oxide together with varying quantities of basic aluminium carbonate and bicarbonate.

Dried Aluminium Hydroxide Gel contains not less than 47.0 per cent and not more than 60.0 per cent of Al₂O₃.

**Description.** A white, light, amorphous powder containing some aggregates; odourless; tasteless.
Identification

A solution in *dilute hydrochloric acid* gives the reactions of aluminium salts (2.3.1).

Tests

**pH** (2.4.24). Not more than 10.0, determined in a 4.0 per cent w/v suspension in carbon dioxide-free water.

**Arsenic** (2.3.10). Dissolve 2 g in 18 ml of *brominated hydrochloric acid*, add 42 ml of water and remove the excess of bromine with a few drops of stannous chloride solution AsT. The resulting solution complies with the limit test for arsenic (5 ppm).

**Heavy metals** (2.3.13). Dissolve 0.33 g in 10 ml of *dilute hydrochloric acid* with the aid of heat, filter if necessary, and dilute to 25 ml with water. The resulting solution complies with the limit test for heavy metals Method A (60 ppm).

**Chlorides** (2.3.12). Dissolve 0.1 g in 10 ml of *dilute nitric acid*, boil, cool, dilute to 100 ml with water and filter. 20 ml of the filtrate complies with the limit test for chlorides (1.25 per cent).

**Sulphates** (2.3.17). Dissolve 0.5 g in 5 ml of *dilute hydrochloric acid*, boil, cool, dilute to 200 ml with water and filter. 5 ml of the filtrate complies with the limit test for sulphates (1.2 per cent).

**Neutralising capacity**. Pass a sufficient quantity, triturated if necessary, through a sieve of nominal mesh aperture of 150 µm. Weigh accurately 0.5 g of the sifted material and add to 200.0 ml of 0.05 M *hydrochloric acid* previously heated to 37° and stir continuously, maintaining the temperature at 37°; the pH of the solution, at 37°, after 10, 15 and 20 minutes, is not less than 1.8, 2.3 and 3.0 respectively and at no time is more than 4.5. Add 10.0 ml of 0.3 M *hydrochloric acid* previously heated to 37°, stir continuously for 1 hour maintaining the temperature at 37° and titrate with 0.1 M *sodium hydroxide* to pH 3.5.

Not more than 35.0 ml of 0.1 M *sodium hydroxide* is required and the pH of the solution at 37° at no time is more than 4.5.

**Microbial contamination** (2.2.9). 1 g is free from *Escherichia coli*.

**Assay**. Weigh accurately about 0.4 g and dissolve in a mixture of 3 ml of *hydrochloric acid* and 3 ml of water by warming on a water-bath, cool to below 20° and dilute to 100.0 ml with water. To 20.0 ml of this solution, add 40.0 ml of 0.05 M *disodium edetate*, 80 ml of water, and 0.15 ml of *methyl red solution* and neutralise by the dropwise addition of 1 M *sodium hydroxide*. Warm on a water-bath for 30 minutes, add 3 g of *hexamine* and titrate with 0.05 M *lead nitrate* using 0.5 ml of *xylenol orange solution* as indicator.

1 ml of 0.05 M *disodium edetate* is equivalent to 0.002549 g of Al₂O₃.

Storage. Store protected from moisture.

Aluminium Sulphate

Al₂(SO₄)₃.xH₂O   Mol. Wt. 342.1 (anhydrous)

Aluminium Sulphate contains not less than 51.0 per cent and not more than 59.0 per cent of Al₂(SO₄)₃. It contains a variable quantity of water of crystallisation.

**Description**. Colourless, lustrous crystals or a white, crystalline powder or masses; odourless.

**Identification**

A. Dissolve 2.5 g in sufficient water to produce 50 ml (solution A). The solution gives reaction A of aluminium salts (2.3.1). B. Solution A gives reaction A of sulphates (2.3.1).

**Tests**

**Appearance of solution**. Solution A is not more opaque than opalescence standard OS3 (2.4.1), and is colourless (2.4.1).

**pH** (2.4.24). 2.5 to 4.0, determined in a 2.0 per cent w/v solution.

**Alkalis and alkaline-earth metals**. To 20 ml of solution A add 100 ml of water, heat and add 0.1 ml of *methyl red solution*. Add 6 M ammonia until the colour changes to yellow, dilute to 150 ml with water, heat to boiling and filter. Evaporate 75 ml of the filtrate to dryness on a water-bath and ignite. The weight of the residue does not exceed 2 mg (0.4 per cent).

**Ammonium salts**. Heat 1 g with 10 ml of *sodium hydroxide solution* on a water-bath for 1 minute; the odour of ammonia is not perceptible.

**Arsenic** (2.3.10). Dissolve 3.3 g in 50 ml of water and add 10 ml of stannated hydrochloric acid AsT. The resulting solution complies with the limit test for arsenic (3 ppm).

**Heavy metals** (2.3.13). 0.5 g dissolved in 1 ml of *dilute acetic acid* and sufficient water to produce 25 ml complies with the limit test for heavy metals, Method A (40 ppm).

**Iron** (2.3.14). 8 ml of solution A diluted to 10 ml complies with the limit test for iron, but using 0.3 ml in place of 0.1 ml of thioglycollic acid (100 ppm).

**Assay**. Weigh accurately about 0.6 g and dissolve in 2 ml of 1 M *hydrochloric acid* and 50 ml of water. Add 50.0 ml of 0.05 M *disodium edetate* and neutralise to *methyl red solution* with 1 M *sodium hydroxide*. Heat the solution to boiling, leave on a water-bath for 10 minutes, cool rapidly and add about 50 mg of *xylenol orange mixture* and 5 g of *hexamine*. Titrate with 0.05 M *lead nitrate*. Carry out a blank titration.
1 ml of 0.05 M disodium edetate is equivalent to 0.008554 g of \( \text{Al}_2(\text{SO}_4)_3 \).

**Amantadine Hydrochloride**

C_{10}H_{17}N.HCl  
Mol. Wt. 187.7

Amantadine Hydrochloride is tricyclo[3.3.1.1^{3,7}]dec-1-ylamine hydrochloride.

Amantadine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of C_{10}H_{17}N.HCl, calculated on the anhydrous basis.

**Description.** A white or almost white, crystalline powder; sublimes when heated.

**Identification**

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Dissolve 0.1 g in 5 ml of water, add 0.5 ml of 5 M sodium hydroxide, extract with 5 ml of dichloromethane, filter the dichloromethane layer through anhydrous sodium sulphate with 2 ml of dichloromethane and evaporate the solution to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with amantadine hydrochloride RS treated in the same manner or with the reference spectrum of amantadine.

B. Dissolve 0.2 g in 1 ml of 0.1 M hydrochloric acid and add 1 ml of a 50 per cent w/v solution of sodium nitrite; a white precipitate is produced.

C. 1 ml of a 10 per cent w/v solution in carbon dioxide-free water gives reaction A of chlorides (2.3.1).

**Tests**

**Appearance of solution.** A 10.0 per cent w/v solution in carbon dioxide-free water is clear (2.4.1) and not more intensely coloured than reference solution YS7 (2.4.1).

**pH** (2.4.24). 3.0 to 5.5, determined in a 20.0 per cent w/v solution.

**Heavy metals** (2.3.13). A solution prepared by dissolving 1.0 g in 1 ml of dilute acetic acid and sufficient water to produce 25 ml complies with the limit test for heavy metals, Method A (20 ppm).

**Related substances.** Determine by gas chromatography (2.4.13).

**Test solution.** Dissolve 0.1 g of the substance under examination in 2 ml of water, add 2 ml of a 20 per cent w/v solution of sodium hydroxide and 2 ml of chloroform and shake for 10 minutes. Separate the chloroform layer, dry over anhydrous sodium sulphate and filter.

Chromatographic system

- a glass column 1.8 m x 2 mm, packed with material prepared in the following manner. Mix 19.5 g of silanised diatomaceous support (such as Chromosorb G/AW/DMCS) with 60 ml of a 0.33 per cent w/v solution of potassium hydroxide in methanol and evaporate the solvent under reduced pressure while slowly rotating the mixture. Dissolve over a 5-hour period 0.4 g of low-vapour pressure hydrocarbons (type L) (such as Apiezon L) in 60 ml of toluene, add this solution to the prepared silanised diatomaceous support and evaporate the solvent under reduced pressure while slowly rotating the mixture,

- temperature: column. Allow the temperature to increase from 100° to 200° at a constant rate of 6° per minute, inlet port. 220°, detector. 300°,

- flow rate. 30 ml per minute of the carrier gas.

Inject 1 µl or other suitable volume of the test solution. Record the chromatogram for at least 2.5 times the retention time of the principal peak.

The area of any secondary peak is not greater than 0.3 per cent and the sum of the areas of any secondary peaks is not greater than 1 per cent by normalisation.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 0.5 per cent, determined on 2.0 g.

**Assay.** Weigh accurately about 0.15 g, dissolve in a mixture of 5.0 ml of 0.01 M hydrochloric acid and 50 ml of ethanol (95 per cent) and titrate with 0.1 M sodium hydroxide determining the end-point potentiometrically (2.4.25). Record the volume used between the two inflections.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.01877 g of C_{10}H_{17}N.HCl.

**Amantadine Capsules**

Amantadine Hydrochloride Capsules

Amantadine Capsules contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of amantadine hydrochloride, C_{10}H_{17}N.HCl.
Identification

To the contents of three capsules add 6 ml of pentane and shake well. Collect the undissolved solids on a sintered-glass filter, wash with two portions, each of 3 ml, of pentane and dry in air. The residue comply with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with amantadine hydrochloride RS treated in the same manner or with the reference spectrum of amantadine.

B. Dissolve 0.2 g in 1 ml of 0.1 M hydrochloric acid and add 1 ml of a 50 per cent w/v solution of sodium nitrite; a white precipitate is produced.

Tests

Other tests. Comply with the tests stated under Capsules.

Assay. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 0.12 g of Amantadine Hydrochloride and warm in a mixture of 30 ml of anhydrous glacial acetic acid and 10 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid, using crystal violet solution as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.01877 g of C_{10}H_{17}N,HCl.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Ambroxol Hydrochloride

C_{13}H_{18}Br_{2}N_{2}O,HCl

Ambroxol hydrochloride is trans-4-[(2-amino-3,5-dibromobenzyl)amino]cyclohexanol hydrochloride.

Ambroxol Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of C_{13}H_{18}Br_{2}N_{2}O,HCl, calculated on the dried basis.

Description. A white or yellowish crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ambroxol hydrochloride RS or with the reference spectrum of ambroxol hydrochloride.

B. Dissolve 25 mg in 2.5 ml of water, add 1.0 ml of dilute ammonia and allow to stand for 5 minutes. Acidify the aqueous layer with dilute nitric acid and filter. The filtrate gives reaction A of chlorides (2.3.1).

Tests

pH (2.4.24). 4.5 to 6.0, determined in a 1.0 per cent w/v solution in carbon dioxide-free water.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 250 ml of water and dilute to 50 ml with the same solvent.

Reference solution (a). Dissolve 5 mg of ambroxol hydrochloride RS in 250 ml of water. Dilute 5 ml of the solution to 100 ml with the mobile phase.

Reference solution (b). Dissolve 5 mg of the substance under examination in 0.2 ml of methanol and add 0.04 ml of a mixture of 1 volume of formaldehyde solution and 99 volumes of water. Heat at 60º for 5 minutes. Evaporate to dryness under a current of nitrogen. Dissolve the residue in 5 ml of water and dilute to 20 ml with the mobile phase.

Chromatographic system – a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),

– mobile phase: a mixture of equal volumes of acetonitrile and a buffer solution prepared by dissolving 1.32 g of ammonium phosphate in 900 ml of water, adjusting the pH to 7.0 with phosphoric acid and diluting to 1000 ml with water,

– flow rate. 1 ml per minute,

– spectrophotometer set at 248 nm,

– a 20 µl loop injector.

Inject reference solution (b). The test is not valid unless the resolution between the secondary peak (trans-4-(6,8-dibromo-1,4-dihydroquinazolin-3(2H)-yl)cyclohexanol) and the ambroxol peak is at least 4.0.

Inject the test solution and reference solution (a). Continue the chromatography for 3 times the retention time of the principal peak in the chromatogram obtained with the test solution. The area of any secondary peak in the chromatogram obtained with the test solution is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). The sum of the areas of all the secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.01 per cent).
Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.3 g in 70 ml of ethanol. Titrate with 0.1 M sodium hydroxide, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.04146 g of C_{13}H_{18}Br_{2}N_{2}O, HCl.

Storage. Store protected from light.

Amikacin

\[
\text{C}_{22}\text{H}_{43}\text{N}_{5}\text{O}_{13} \quad \text{Mol. Wt. 585.6}
\]

Amikacin is \((S)-O-3\text{-amino}-3\text{-deoxy-}\alpha\text{-D-glucopyranosyl-}(1\rightarrow 6)-O-[6\text{-amino}-6\text{-deoxy-}\alpha\text{-D-glucopyranosyl}(1\rightarrow 4)]-N^\prime\text{-}(4\text{-amino-2-hydroxy-1-oxobutyl})-2\text{-deoxy-}\alpha\text{-D-streptamine.}

Amikacin contains not less than 900 µg of C_{22}H_{43}N_{5}O_{13} per mg, calculated on the anhydrous basis.

Description. A white crystalline powder; almost odourless.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 60 volumes of methanol, 30 volumes of strong ammonia solution and 25 volumes of chloroform.

Test solution. A 0.6 per cent w/v solution of the substance under examination.

Reference solution (a). A 0.6 per cent w/v solution of amikacin RS.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 3 µl of each solution. After development, allow the plate to dry in air, heat it at 110° for 15 minutes and immediately spray it with a 1 per cent w/v solution of ninhydrin in a mixture of 100 volumes of 1-butanol and 1 volume of pyridine. The principal pink-coloured spot in the chromatogram obtained with the test solution corresponds to those in the chromatograms obtained with reference solutions (a) and (b).

B. To 1 ml of a 1 per cent w/v solution add 1 ml of 2 M sodium hydroxide, mix and add 2 ml of a 1 per cent w/v solution of cobalt nitrate; a violet colour is produced.

C. To a solution of 50 mg in 5 ml of water add 4 ml of a 0.035 per cent w/v solution of anthrone in sulphuric acid; a bluish-violet colour is produced.

Tests

pH (2.4.24). 9.5 to 11.5, determined in a 1.0 per cent w/v solution in carbon dioxide-free water.

Specific optical rotation (2.4.22). +97° to +105°, determined in a 2.0 per cent w/v solution.

Sulphated ash (2.3.18). Not more than 1.0 per cent, the charred residue being moistened with 2 ml of nitric acid and 5 drops of sulphuric acid.

Water (2.3.43). Not more than 8.5 per cent, determined on 0.2 g.

Assay. Determine by the microbiological assay of antibiotics, Method B (2.2.10), and express the result in µg of Amikacin, C_{22}H_{43}N_{5}O_{13}, per mg.

Amikacin Sulphate

\[
\text{C}_{22}\text{H}_{43}\text{N}_{5}\text{O}_{13}\cdot 1.8\text{H}_{2}\text{SO}_{4} \quad \text{Mol. Wt. 762.1}
\]

\[
\text{C}_{22}\text{H}_{43}\text{N}_{5}\text{O}_{13}\cdot 2\text{H}_{2}\text{SO}_{4} \quad \text{Mol. Wt. 781.8}
\]

Amikacin Sulphate is \((S)-O-3\text{-amino}-3\text{-deoxy-}\alpha\text{-D-glucopyranosyl-}(1\rightarrow 6)-O-[6\text{-amino}-6\text{-deoxy-}\alpha\text{-D-glucopyranosyl}(1\rightarrow 4)]-N^\prime\text{-}(4\text{-amino-2-hydroxy-1-oxobutyl})-2\text{-deoxy-}\alpha\text{-D-streptamine sulphate (1:2 or 1:1.8)(salt).}
Amikacin Sulphate having a molar ratio of Amikacin to H$_2$SO$_4$ of 1:2 contains the equivalent of not less than 674 µg and not more than 786 µg of C$_{22}$H$_{43}$N$_5$O$_{13}$ per mg, calculated on the dried basis. Amikacin Sulphate having a molar ratio of Amikacin to H$_2$SO$_4$ of 1:1.8 contains the equivalent of not less than 691 µg and not more than 806 µg of C$_{22}$H$_{43}$N$_5$O$_{13}$ per mg, calculated on the dried basis.

**Description.** A white to yellowish-white crystalline powder; almost odourless.

**Identification**

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 60 volumes of methanol, 30 volumes of strong ammonia solution and 25 volumes of chloroform.

**Test solution.** A 0.6 per cent w/v solution of the substance under examination.

**Reference solution (a).** A 0.6 per cent w/v solution of amikacin RS.

**Reference solution (b).** A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 3 µl of each solution. After development, allow the plate to dry in air, heat it at 110° for 15 minutes and immediately spray it with a 1 per cent w/v solution of ninhydrin in a mixture of 100 volumes of 1-butanol and 1 volume of pyridine. The principal pink-coloured spot in the chromatogram obtained with the test solution corresponds to those in the chromatograms obtained with reference solutions (a) and (b).

B. To 1 ml of a 1 per cent w/v solution add 1 ml of 2 M sodium hydroxide, mix and add 2 ml of a 1 per cent w/v solution of cobalt nitrate; a violet colour is produced.

C. To a solution of 50 mg in 5 ml of water add 4 ml of a 0.035 per cent w/v solution of anthrone in sulphuric acid; a bluish-violet colour is produced.

**Tests**

**pH** (2.4.24). 2.0 to 4.0 (1:2 salt), or 6.0 to 7.3 (1:1.8 salt), determined in a 1.0 per cent w/v solution in carbon dioxide-free water.

**Specific optical rotation** (2.4.22). +76.0° to +84.0°, determined in a 2.0 per cent w/v solution.

**Sulphated ash** (2.3.18). Not more than 1.0 per cent, the charred residue being moistened with 2 ml of nitric acid and 5 drops of sulphuric acid.

**Loss on drying** (2.4.19). Not more than 13.0 per cent, determined on 0.1 g by drying in an oven over phosphorus pentoxide at 110° at a pressure not exceeding 0.7 kPa for 3 hours.

**Assay.** Determine by the microbiological assay of antibiotics, Method B (2.2.10), and express the result in µg of amikacin, C$_{22}$H$_{43}$N$_5$O$_{13}$, per mg.

**Labelling.** The label states (1) whether the molar ratio of amikacin to H$_2$SO$_4$ of the contents is 1:2 or 1:1.8; (2) whether the material is intended for use in the manufacture of parenteral preparations.

**Amikacin Injection**

Amikacin Sulphate Injection

Amikacin Injection is a sterile solution of Amikacin Sulphate in Water for Injections or of Amikacin in Water for Injections prepared with the aid of Sulphuric Acid.

Amikacin Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of amikacin, C$_{22}$H$_{43}$N$_5$O$_{13}$.

**Identification**

Dilute the injection to obtain a solution containing 6 mg of amikacin per ml (test solution). The test solution complies with the following tests.

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 60 volumes of methanol, 30 volumes of strong ammonia solution and 25 volumes of chloroform.

**Reference solution (a).** A 0.6 per cent w/v solution of amikacin RS.

**Reference solution (b).** A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 3 µl of each solution. After development, allow the plate to dry in air, heat it at 110° for 15 minutes and immediately spray it with a 1 per cent w/v solution of ninhydrin in a mixture of 100 volumes of 1-butanol and 1 volume of pyridine. The principal pink-coloured spot in the chromatogram obtained with the test solution corresponds to those in the chromatograms obtained with reference solutions (a) and (b).

B. To 1.5 ml of the test solution add 1 ml of 2 M sodium hydroxide, mix and add 2 ml of a 1 per cent w/v solution of cobalt nitrate; a violet colour is produced.

C. To 1.5 ml of the test solution add 3.5 ml of water mix and add 4 ml of a 0.035 per cent w/v solution of anthrone in sulphuric acid; a bluish-violet colour is produced.

**Tests**

**pH** (2.4.24). 3.5 to 5.5.
**Bacterial Endotoxins** (2.2.3). Not more than 0.33 Endotoxin unit per mg of amikacin.

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** Dilute the injection to obtain a solution containing 1 mg of amikacin per ml. Determine by the microbiological assay of antibiotics, Method B, (2.2.10) and express the result in mg of amikacin, C$_{18}$H$_{43}$N$_{5}$O$_{13}$ per ml.

**Labelling.** The label states the quantity of Amikacin Sulphate contained in the sealed container in terms of the equivalent amount of amikacin.

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**Amiloride Hydrochloride**

![Chemical Structure of Amiloride](https://example.com/structure.png)

C$_{6}$H$_{8}$ClN$_{7}$O$_{2}$HCl, 2H$_{2}$O  
Mol. Wt. 302.1

Amiloride Hydrochloride is N-amidino-3,5-diamino-6-chloropyrazine-2-carboxamide hydrochloride dihydrate.

Amiloride Hydrochloride contains not less than 98.0 per cent and not more than 101.0 per cent of C$_{6}$H$_{8}$ClN$_{7}$O$_{2}$HCl, calculated on the anhydrous basis.

**Description.** A pale yellow to greenish-yellow powder.

**Identification**

_Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out._

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with amiloride hydrochloride RS or with the reference spectrum of amiloride hydrochloride.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with a suitable silica gel.

Mobile phase. A freshly prepared mixture of 88 volumes of dioxan, 6 volumes of dilute ammonia solution and 6 volumes of water.

Test solution. Dissolve 0.2 g of the substance under examination in sufficient methanol to produce 50 ml.

Reference solution. A 0.4 per cent w/v solution of amiloride hydrochloride RS in methanol.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Dissolve 10 mg in 10 ml of water and add 10 ml of a 20 per cent w/v solution of cetrimide, 0.25 ml of 2 M sodium hydroxide and 1 ml of bromine water; a greenish-yellow colour is produced. Add 2 ml of 2 M hydrochloric acid; the solution becomes deep yellow and exhibits a blue fluorescence in ultraviolet light at 365 nm.

D. A 5 per cent w/v solution gives the reactions of chlorides (2.3.1).

**Tests**

**Free acid.** Dissolve 1.0 g in 100 ml of a mixture of equal volumes of methanol and water and titrate with 0.1 M sodium hydroxide determining the end-point potentiometrically (2.4.25); not more than 0.3 ml is required.

**Related substances.** Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of 1 volume of acetonitrile and 3 volumes of water.

Test solution (a) Dissolve 0.2 g of the substance under examination in 100 ml of solvent mixture.

Test solution (b). Dilute 1 ml of test solution (a) to 100 ml with the same solvent mixture.

Test solution (c). Dilute 10 ml of test solution (b) to 100 ml with the same solvent mixture.

Reference solution. A 0.001 per cent w/v solution of methyl 3,5-diamino-6-chloropyrazine-2-carboxylate RS in the same solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm) (such as Nucleosil C18),
- mobile phase: a mixture of 745 volumes of water, 250 volumes of acetonitrile and 5 volumes of tetramethylammonium hydroxide solution (10 per cent), the pH of the mixture being adjusted to 7.0 with a mixture of 1 volume of phosphoric acid and 9 volumes of water,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject the reference solution and adjust the concentration of acetonitrile so that the retention time of methyl 3,5-diamino-6-chloropyrazine-2-carboxylate is 5 to 6 minutes (an increase in the concentration of acetonitrile reduces the retention time). Inject 20 µl of test solution (b) and adjust the concentrations of tetramethylammonium hydroxide and orthophosphoric acid so that the retention time of amiloride is 9 to 12 minutes.
keeping the pH at 7.0 (an increase in the concentrations reduces the retention time).

Inject each of test solution (a) and the reference solution and allow the chromatography to proceed for 5 times the retention time of amiloride. In the chromatogram obtained with test solution (a) the sum of the areas of any secondary peaks is not greater than the area of the peak due to methyl 3,5-diamino-6-chloro-pyrazine-2-carboxylate in the chromatogram obtained with the reference solution. Ignore any peak with an area less than 10 per cent of the area of the peak due to methyl 3,5-diamino-6-chloropyrazine-2-carboxylate in the chromatogram obtained with the reference solution.

Inject test solution (c). The test is not valid if the signal-to-noise ratio of the peak due to amiloride in the chromatogram obtained with this solution is less than 5.0.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 11.0 to 13.0 per cent, determined on 0.2 g.

Assay. Weigh accurately about 0.25 g and dissolve in a mixture of 100 ml of anhydrous glacial acetic acid and 15 ml of dioxan and add 10 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02661 g of C6H8ClN7O,HCl.

Storage. Store protected from light.

Amiloride Tablets

Amiloride Hydrochloride Tablets

Amiloride Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous amiloride hydrochloride, C6H8ClN7O,HCl.

Identification

A. Extract a quantity of the powdered tablets containing 0.5 g of anhydrous amiloride hydrochloride with 100 ml of 0.1 M hydrochloric acid and filter. When examined in the range 230 nm to 380 nm (2.4.7), the solution shows absorption maxima at about 285 nm and at about 363 nm.

B. Carry out the method described under Related substances using the following solutions.

Test solution. Shake a quantity of the powdered tablets containing 10 mg of anhydrous amiloride hydrochloride with 10 ml of methanol and centrifuge.

Reference solution. A 0.1 per cent w/v solution of amiloride hydrochloride RS in methanol.

The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), using a precoated silica gel plate (such as Merck silica gel 60 plates).

Mobile phase. A freshly prepared mixture of 90 volumes of dioxan and 12 volumes of 3 M ammonia.

Test solution. Shake a quantity of the powdered tablets containing 17.5 mg of anhydrous amiloride hydrochloride with 5 ml of methanol and centrifuge.

Reference solution (a). A 0.002 per cent w/v solution of methyl 3,5-diamino-6-chloropyrazine-2-carboxylate RS in methanol.

Reference solution (b). A 0.0008 per cent w/v solution of methyl 3,5-diamino-6-chloropyrazine-2-carboxylate RS in methanol.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. Any spot corresponding to methyl 3,5-diamino-6-chloropyrazine-2-carboxylate in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a). Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Uniformity of content. Comply with the test stated under Tablets.

Powder one tablet and transfer to a 100-ml volumetric flask, add 60 ml of 0.1 M hydrochloric acid, and shake by mechanical means for 30 minutes. Dilute with 0.1 M hydrochloric acid to volume, mix, and centrifuge a portion of the mixture. Dilute an accurately measured portion of the clear supernatant liquid quantitatively to obtain a solution containing about 10 µg of amiloride hydrochloride per ml. Measure the absorbance of the resulting solution at the maximum at about 363 nm (2.4.7).

Calculate the content of C6H8ClN7O, HCl taking 692 as the specific absorbance at 363 nm.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and finely powder 20 tablets. Weigh accurately a quantity of the powder containing about 10 mg of anhydrous amiloride hydrochloride, transfer to a 100-ml volumetric flask, add 60 ml of 0.1 M hydrochloric acid, and shake by
mechanical means for 30 minutes. Dilute with 0.1 M hydrochloric acid to volume, mix, and centrifuge a portion of the mixture. Dilute an accurately measured portion of the clear supernatant liquid quantitatively to obtain a solution containing about 10 µg of amiloride hydrochloride per ml. Measure the absorbance of the resulting solution at the maximum at about 363 nm (2.4.7).

Calculate the content of C$_6$H$_8$ClN$_7$O$_2$HCl taking 692 as the specific absorbance at 363 nm.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of anhydrous amiloride hydrochloride.

### Aminocaproic Acid

![Aminocaproic Acid](image)

C$_6$H$_{13}$NO$_2$  
Mol. Wt. 131.2

Aminocaproic Acid is 6-aminohexanoic acid.

Aminocaproic Acid contains not less than 98.5 per cent and not more than 101.0 per cent of C$_6$H$_{13}$NO$_2$, calculated on the dried basis.

**Description.** Colourless crystals or a white, crystalline powder.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with aminocaproic acid RS.

B. Determine by thin-layer chromatography (2.4.7), coating the plate with silica gel G.

**Mobile phase.** A mixture of 25 volumes of ethanol (95 per cent), 3 volumes of water and 4 volumes of strong ammonia solution.

**Test solution.** Dissolve 0.25 g of the substance under examination in 100 ml of water.

**Reference solution.** A 0.25 per cent w/v solution of aminocaproic acid RS.

Apply to the plate 2 µl of each solution. After development, remove the plate, spray it with a 0.25 per cent w/v solution of ninhydrin in a mixture of equal volumes of methanol and pyridine and heat at 105° for 2 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

### Tests

**Appearance of solution.** A 20.0 per cent w/v solution remains clear for 24 hours (2.4.1), and is colourless (2.4.1).

**pH** (2.4.24). 7.5 to 8.0, determined in a 20.0 per cent w/v solution.

**Stability.** Place 20.0 g evenly spread in a shallow dish about 9 cm in diameter, cover and allow to stand at 100° ± 2° for 72 hours. Dissolve in sufficient water to produce 100.0 ml. Prepare a 20.0 per cent w/v solution of the substance under examination but without the above treatment. Measure the absorbances (2.4.7) of the two solutions at the maximum at about 287 nm and at about 450 nm. Absorbance of the solution prepared from the exposed substance being examined at the maximum at about 287 nm is not more than 0.15 and of the solution of the substance under examination without the above treatment, at the maximum at about 287 nm is not more than 0.10. Absorbance of both solutions at the maximum at about 450 nm is not more than 0.03.

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.2 g, dissolve in about 100 ml of anhydrous glacial acetic acid with gentle heat to effect solution, cool and add 15 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid, using crystal violet solution as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.01312 g of C$_6$H$_{13}$NO$_2$.

### Aminocaproic Acid Injection

Aminocaproic Acid Injection is a sterile solution of Aminocaproic Acid in Water for Injections.

Aminocaproic Acid Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of aminocaproic acid, C$_6$H$_{13}$NO$_2$.

**Identification**

To a volume containing 0.4 g of Aminocaproic Acid add 2 ml of ether, stir, add 2 ml of methanol, stir again and allow to stand; the crystals after drying on a water-bath comply with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with aminocaproic acid RS.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.
Mobile phase. A mixture of 25 volumes of ethanol (95 per cent), 3 volumes of water and 4 volumes of strong ammonia solution.

Test solution. Dissolve 0.25 g of the substance under examination in 100 ml of water.

Reference solution. A 0.25 per cent w/v solution of aminocaproic acid RS.

Apply to the plate 2 µl of each solution. After development, remove the plate, spray it with a 0.25 per cent w/v solution of ninhydrin in a mixture of equal volumes of methanol and pyridine and heat at 105° for 2 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 6.0 to 7.6.

Bacterial endotoxins (2.2.3). Not more than 0.05 Endotoxin Unit per mg of aminocaproic acid.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. To a volume containing 0.2 g of Aminocaproic Acid add 10 ml of ethanol and evaporate to dryness on a water-bath. Dissolve the residue in 100 ml of anhydrous glacial acetic acid by gentle heating, if necessary, cool and add 15 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid, using crystal violet solution as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.01312 g of C₆H₁₃NO₂.

Aminocaproic Acid Tablets

Aminocaproic Acid Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of aminocaproic acid, C₆H₁₃NO₂.

Identification

Triturate 2 tablets with 10 ml of water and filter into 100 ml of acetone. Swirl the mixture and allow to stand for 15 minutes to complete crystallisation. Filter through a medium porosity, sintered-glass filter and wash the crystals with 25 ml of acetone. Apply vacuum to remove the solvent, dry at 105° for 30 minutes and cool. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with aminocaproic acid RS.

B. Determine by thin-layer chromatography (2.4.7), coating the plate with silica gel G.

Aminophylline

Theophylline and Ethylenediamine

Aminophylline is a stable mixture or combination of theophylline and ethylenediamine. It may be anhydrous or may contain not more than two molecules of water of hydration.

Aminophylline contains the equivalent of not less than 84.0 per cent and not more than 87.4 per cent of theophylline, C₇H₈N₄O₂, and the equivalent of not less than 13.5 per cent of ethylenediamine, C₂H₄N₂.
and not more than 15.0 per cent of ethylenediamine, C\textsubscript{2}H\textsubscript{8}N\textsubscript{2}, both calculated on the anhydrous basis.

**Description.** A white or slightly yellowish granules or powder; odour, slightly ammoniacal. On exposure to air it gradually loses ethylenediamine and absorbs carbon dioxide with liberation of free theophylline. Even in the absence of light, it is gradually decomposed on exposure to a humid environment, the degradation being faster at higher temperatures.

**Identification**

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

Dissolve 1 g in 10 ml of water and add 2 ml of dilute hydrochloric acid dropwise, with shaking. Separate the precipitate by filtration and reserve the filtrate for test D. Wash the precipitate with successive small quantities of cold water, recrystallise from hot water and dry at 100° to 105°. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with theophylline RS.

B. To 10 mg of the residue obtained in test A add 1 ml of hydrochloric acid in a porcelain dish and 0.1 g of potassium chlorate and evaporate to dryness on a water-bath; invert the dish over a vessel containing a few drops of dilute ammonia solution; the residue acquires a purple colour. Add a few drops of dilute sodium hydroxide solution; the colour is discharged.

C. Saturate in water a portion of the residue obtained in test A and add tannic acid solution; a precipitate soluble in excess of the reagent is produced.

D. The filtrate complies with the following test.

To the filtrate reserved above add 0.2 ml of benzoyl chloride, make alkaline with 2 M sodium hydroxide and shake vigorously. Filter, wash the precipitate with 10 ml of water, dissolve in 5 ml of hot ethanol (95 per cent) and add 5 ml of water. The precipitate, after washing with water and drying at 100° to 105° melts at 248° to 252° (2.4.21).

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17) coating the plate with silica gel GF254.

*Mobile phase.* A mixture of 40 volumes of 1-butanol, 30 volumes of acetone, 30 volumes of chloroform and 10 volumes of strong ammonia solution.

*Test solution.* Dissolve 0.2 g of the substance under examination in 2 ml of water with the aid of heat and dilute to 10 ml with methanol.

*Reference solution.* Dilute 1 volume of the test solution to 200 volumes with methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Heavy metals** (2.3.13). A 8 per cent w/v solution complies with the limit test for heavy metals, Method A (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 1.5 per cent (for anhydrous), determined on 2.0 g dissolved in 20 ml of pyridine. 3.0 to 8.0 per cent (for hydrate), determined on 0.5 g.

**Assay.** *For theophylline* — Weigh accurately about 0.25 g, add 50 ml of water and 8 ml of dilute ammonia solution and warm gently on a water-bath until complete solution is effected. Add 20.0 ml of 0.1 M silver nitrate, mix and boil for 15 minutes. Cool to between 5° and 10° for 20 minutes, filter at a pressure not exceeding 2.75 kPa and wash the precipitate with three quantities, each of 10 ml, of water. Acidify the combined filtrate and washings with nitric acid and add an excess of 3 ml of the acid. Cool, add 2 ml of ferric ammonium sulphate solution, and titrate with 0.1 M ammonium thiocyanate.

1 ml of 0.1 M silver nitrate is equivalent to 0.01802 g of C\textsubscript{7}H\textsubscript{8}N\textsubscript{4}O\textsubscript{2}.

*For ethylenediamine* — Weigh accurately about 0.25 g and dissolve in 30 ml of water. Titrate with 0.1 M hydrochloric acid using methyl orange solution as indicator.

1 ml of 0.1 M hydrochloric acid is equivalent to 0.003005 g of C\textsubscript{2}H\textsubscript{8}N\textsubscript{2}.

**Storage.** Store protected from light and from atmospheric carbon dioxide.

## Aminophylline Injection

**Theophylline and Ethylenediamine Injection**

Aminophylline Injection is a sterile solution of Aminophylline in Water for Injections or is a sterile solution of Theophylline in a solution of Ethylenediamine Hydrate in Water for Injections free from carbon dioxide. Aminophylline Injection may contain an excess of ethylenediamine but no other substance may be added.

Aminophylline Injection contains theophylline, C\textsubscript{7}H\textsubscript{8}N\textsubscript{4}O\textsubscript{2}, equivalent to not less than 73.25 per cent and not more than 88.25 per cent of the stated amount of aminophylline, and not more than 0.295 g of ethylenediamine, C\textsubscript{2}H\textsubscript{8}N\textsubscript{2}, for each g of anhydrous theophylline, C\textsubscript{7}H\textsubscript{8}N\textsubscript{4}O\textsubscript{2}, determined in the Assay for theophylline.
**Aminophylline Tablets**

Theophylline and Ethylenediamine Tablets

Aminophylline Tablets contain theophylline, C$_7$H$_8$N$_4$O$_2$, equivalent to not less than 80.6 per cent and not more than 90.8 per cent of the stated amount of aminophylline, and ethylenediamine, C$_2$H$_8$N$_2$, equivalent to not less than 10.9 per cent of the stated amount of aminophylline.

**Identification**

Shake a quantity of the powdered tablets containing 0.5 g of aminophylline with 25 ml of water and filter. To the filtrate add 1 ml of dilute hydrochloric acid with constant stirring. Separate the precipitate by filtration and reserve the filtrate. Wash the precipitate with 25 ml of water, and add an excess of 3 ml of the reagent. The filtrate complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with theophylline RS.

B. To 10 mg add 1 ml of hydrochloric acid in a porcelain dish and 0.1 g of potassium chlorate and evaporate to dryness on a water-bath; invert the dish over a vessel containing a few drops of dilute ammonia solution; the residue acquires a purple colour. Add a few drops of dilute sodium hydroxide solution; the colour is discharged.

C. Saturate a portion in water and add tannic acid solution; a precipitate soluble in excess of the reagent is produced.

The filtrate complies with the following test.

Add 0.2 ml of benzoyl chloride, make alkaline with 2 M sodium hydroxide and shake vigorously. Filter, wash the precipitate with 10 ml of water, dissolve in 5 ml of hot ethanol (95 per cent) and add 5 ml of water. The precipitate, after washing with water and drying at 100° to 105° melts at 248° to 252°.

**Tests**

**pH** (2.4.24). 8.8 to 10.0.

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** For theophylline — Measure accurately a volume containing about 0.25 g of aminophylline and dilute with water to 40 ml. Add 8 ml of dilute ammonia solution and 20.0 ml of 0.1 M silver nitrate, mix and boil for 15 minutes. Cool to between 5° and 10° for 20 minutes, filter at a pressure not exceeding 2.75 kPa and wash the precipitate with three quantities, each of 10 ml, of water. Acidify the combined filtrate and washings with nitric acid and add an excess of 3 ml of the acid. Cool, add 2 ml of ferric ammonium sulphate solution, and titrate with 0.1 M ammonium thiocyanate.

1 ml of 0.1 M silver nitrate is equivalent to 0.01802 g of C$_7$H$_8$N$_4$O$_2$.

For ethylenediamine — To a volume containing about 0.25 g of aminophylline, add sufficient water to produce 30 ml. Titrate with 0.1 M hydrochloric acid using methyl orange solution as indicator.

1 ml of 0.1 M hydrochloric acid is equivalent to 0.003005 g of C$_2$H$_8$N$_2$.

Calculate the amount of C$_2$H$_8$N$_2$ present for each g of C$_7$H$_8$N$_4$O$_2$ found.

**Storage.** Store in single dose containers, from which carbon dioxide has been excluded. Do not allow contact with metals.

**Labelling.** The label states (1) the strength in terms of the equivalent amount of anhydrous aminophylline in a suitable dose-volume; (2) the route of injection; (3) that the injection is not to be used if crystals have separated.

**Aminophylline Tablets**

Theophylline and Ethylenediamine Tablets

Aminophylline Tablets contain theophylline, C$_7$H$_8$N$_4$O$_2$, equivalent to not less than 80.6 per cent and not more than 90.8 per cent of the stated amount of aminophylline, and ethylenediamine, C$_2$H$_8$N$_2$, equivalent to not less than 10.9 per cent of the stated amount of aminophylline.

**Identification**

Shake a quantity of the powdered tablets containing 0.5 g of aminophylline with 25 ml of water and filter. To the filtrate add 1 ml of dilute hydrochloric acid with constant stirring. Separate the precipitate by filtration and reserve the filtrate. Wash the precipitate with a small portion of cold water, recrystallise from hot water and dry at 100° to 105°. The crystalline powder complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with theophylline RS.

B. To 10 mg add 1 ml of hydrochloric acid in a porcelain dish and 0.1 g of potassium chlorate and evaporate to dryness on a water-bath; invert the dish over a vessel containing a few drops of dilute ammonia solution; the residue acquires a purple colour. Add a few drops of dilute sodium hydroxide solution; the colour is discharged.

C. Saturate a portion in water and add tannic acid solution; a precipitate soluble in excess of the reagent is produced.

The filtrate complies with the following test.

Add 0.2 ml of benzoyl chloride, make alkaline with 2 M sodium hydroxide and shake vigorously. Filter, wash the precipitate with 10 ml of water, dissolve in 5 ml of hot ethanol (95 per cent) and add 5 ml of water. The precipitate, after washing with water and drying at 100° to 105° melts at 248° to 252°.

**Tests**

**pH** (2.4.24). 8.8 to 10.0.

**Other tests.** Complies with the tests stated under Tablets.

**Assay.** For theophylline — Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablets
containing about 0.5 g of aminophylline, transfer to a 200-ml volumetric flask with the aid of a mixture of 50 ml of water and 15 ml of dilute ammonia solution and allow to stand for 30 minutes with frequent shaking, warming to about 50°, if necessary. Cool, add water to volume and mix. Centrifuge the mixture, and pipette a volume of the clear supernatant liquid equivalent to about 0.25 g of aminophylline into a flask, dilute with sufficient water to produce 40 ml and add 8 ml of dilute ammonia solution. Add 20.0 ml of 0.1 M silver nitrate, mix and boil for 15 minutes. Cool to between 5° and 10° for 20 minutes, filter at a pressure not exceeding 2.75 kPa and wash the precipitate with three quantities, each of 10 ml, of water. Acidify the combined filtrate and washings with nitric acid and add an excess of 3 ml of the acid. Cool, add 2 ml of ferric ammonium sulphate solution, and titrate with 0.1 M ammonium thiocyanate.

1 ml of 0.1 M silver nitrate is equivalent to 0.01802 g of C$_7$H$_8$N$_4$O$_2$.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount anhydrous aminophylline.

**Amiodarone Hydrochloride**

![Chemical Structure](image)

C$_{25}$H$_{29}$I$_2$NO$_3$·HCl  Mol. Wt. 681.8

Amiodarone Hydrochloride is 2-butylbenzofuran-3yl-4-(2-diethylaminoethoxy)-3,5-diiodophenyl ketone hydrochloride.

Amiodarone Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of C$_{25}$H$_{29}$I$_2$NO$_3$·HCl, calculated on the dried basis.

**Description.** A white or almost white, fine crystalline powder.

**Identification**

- **Text B may be omitted if tests A and C are carried out. Test A may be omitted if tests B and C are carried out.**
- **A.** Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with amiodarone hydrochloride RS.
- **B.** In the test for Related substances the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).
- **C.** Gives reaction A of chlorides (2.3.1).

**Tests**

**Appearance of solution.** A 5.0 per cent w/v solution is clear (2.4.1), and not more intensely coloured than reference solution GY5 (2.4.1).

**pH** (2.4.24). 3.2 to 3.8, determined in 5.0 per cent w/v solution, prepared by dissolving in carbon dioxide-free water at 80° and cooling.

**Related substances.** Determine by thin layer chromatography (2.4.17), coating the plate with silica gel GF254.

All the solutions should be protected from light and should be used immediately after preparation.

**Mobile phase.** A mixture of 5 volumes of anhydrous formic acid, 10 volumes of methanol and 85 volumes of dichloromethane.

**Test solution (a).** Dissolve 10 g of the substance under examination in 100 ml of dichloromethane.

**Test solution (b).** Dissolve 0.5 g of the substance under examination in 100 ml of dichloromethane.

**Reference solution (a).** A 0.5 per cent w/v solution of amiodarone hydrochloride RS in dichloromethane.

**Reference solution (b).** A 0.05 per cent w/v solution of the substance under examination in dichloromethane.

**Reference solution (c).** A 0.025 per cent w/v solution of the substance under examination in dichloromethane.

**Reference solution (d).** A 0.02 per cent w/v of (2-chloroethyl) diethylamine hydrochloride RS in dichloromethane.

Apply to the plate 5 µl of each solution. After development, dry in a current of cold air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.05 per cent) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (d) (0.25 per cent). Spray with potassium iodo bismuthate solution. Examine immediately in daylight. Any spot corresponding to (2-chloroethyl) diethylamine hydrochloride in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (d) (0.2 per cent).

**Iodides.** Dissolve 1.5 g in 40 ml of water at 80° by shaking until completely dissolved. Cool and dilute to 50 ml with water (Solution A).

To 15 ml of solution A add 1 ml of 0.1 M hydrochloric acid and 1 ml of 0.05 M potassium iodate and dilute to 20 ml with water. Allow to stand protected from light for 4 hours (Solution B).
1. To 15 ml of solution A add 1 ml of 0.1 M hydrochloric acid, 1 ml of an 88.2 ppm solution of potassium iodide and 1 ml of 0.05 M potassium iodate and dilute to 20 ml with water. Allow to stand protected from light for 4 hours (Solution 2). Measure the absorbances of solutions (1) and (2) at the maximum at about 420 nm, using as the blank a mixture of 15 ml of solution A and 1 ml of 0.1 M hydrochloric acid diluted to 20 ml with water (2.4.7). The absorbance of solution (1) is not greater than half the absorbance of solution (2) (150 ppm).

**Heavy metals** (2.3.13). 10 g complies with the limit test for heavy metals, Method C (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying at 100° at a pressure not exceeding 0.3 kPa for 4 hours.

**Assay.** Weigh accurately about 0.6 g and dissolve in a mixture of 5.0 ml of 0.01 M hydrochloric acid and 75 ml of ethanol (95 per cent). Titrate with 0.1 M sodium hydroxide, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.06818 g of C₂₅H₂₉I₂NO₃, HCl.

**Storage.** Store protected from light, at a temperature not exceeding 30°.

### Amiodarone Tablets

Amiodarone Hydrochloride Tablets

Amiodarone Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of amiodarone hydrochloride, C₂₅H₂₉I₂NO₃, HCl.

**Identification**

A. Shake a quantity of the powdered tablets containing about 0.3 g of Amiodarone Hydrochloride with 25 ml of dichloromethane, filter and evaporate the filtrate to dryness. To the residue, add 2 ml of 1 M sodium hydroxide and extract with 25 ml of ether. Dry the extract over anhydrous sodium sulphate, filter and evaporate to dryness. Dry the residue obtained under reduced pressure over phosphorus pentoxide and dissolve in 2.5 ml of dichloromethane. The solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with amiodarone hydrochloride RS.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

### Tests

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 85 volumes of dichloromethane, 10 volumes of methanol and 5 volumes of anhydrous formic acid.

**Test solution.** Shake a quantity of the powdered tablets containing 50 mg of Amiodarone Hydrochloride with 20 ml of methanol and filter.

**Reference solution (a).** Dilute 1 volume of the test solution to 200 volumes with methanol.

**Reference solution (b).** A 0.00125 per cent w/v solution of 2-butyl-3-(4-hydroxy-3, 5-diiodobenzoyl) benzofuran RS in methanol.

Apply separately to the plate (pre-washed with the mobile phase and dried in air before use) 10 µl of each solution. After development, dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the test solution any spot corresponding to 2-butyl-3 (4-hydroxy-3, 5-diiodobenzoyl)benzofuran is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets.

Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh accurately a quantity of the powdered tablets containing about 0.1g of Amiodarone Hydrochloride, add 70 ml of methanol, mix with the aid of ultrasound for 15 minutes, cool and dilute to 100.0 ml with the same solvent and filter. Dilute 10.0 ml of the filtrate to 100.0 ml with the mobile phase.

**Reference solution.** Dissolve 0.1g of amiodarone hydrochloride RS in 70 ml of methanol, cool and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of the resulting solution to 100.0 ml with the mobile phase.

**Chromatographic system**

- a stainless steel column 7.5 cm x 3.9 mm, packed with very finely divided silica gel consisting of porous spherical particles with chemically bonded nitrile group (4 µm), (such as Nova-Pack CNHP),
- mobile phase: a mixture of 45 volumes of 0.01 M sodium perchlorate and 55 volumes of acetonitrile, the pH of the mixture being adjusted to 3.0 with 2 M phosphoric acid,
- flow rate. 1 ml per minute,
- spectrophotometer set at 244 nm, 20 µl loop injector.
Inject the reference solution. The relative standard deviation for replicate injections is not more than 2.0 per cent.
Inject the test solution and the reference solution. Calculate the content of C₂₅H₂₉I₂NO₃, HCl in the tablets.

**Amitriptyline Hydrochloride**

C₂₀H₂₃N.HCl, HCl
Mol. Wt. 313.9

Amitriptyline Hydrochloride is 3-(10,11-dihydro-5H-dibenzo[a,d]cyclohept-5-ylidene)propyldimethylamine hydrochloride.

Amitriptyline Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of C₂₀H₂₃N.HCl, calculated on the dried basis.

**Description.** Colourless crystals or a white or almost white powder; almost odourless.

**Identification**

*Test A may be omitted if tests B, C, and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with amitriptyline hydrochloride RS or with the reference spectrum of amitriptyline hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0012 per cent w/v solution in methanol shows an absorption maximum only at about 239 nm; absorbance at about 239 nm, about 0.55.

C. To about 50 mg dissolved in 3 ml of water add 1 drop of a 2.5 per cent w/v solution of quinhydrone in methanol; no red colour is produced within 15 minutes (distinction from nortriptyline).

D. Gives the reactions of chlorides (2.3.1).

**Tests**

**Appearance of solution.** Dissolve 1.25 g in sufficient water to produce 25 ml. The solution is clear (2.4.1) and not more intensely coloured than reference solution BS8 (2.4.1).

**pH** (2.4.24). 4.5 to 6.0, determined in a 1.0 per cent w/v solution.

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Related substances.** Determine by thin-layer chromatography (2.4.17), protected from light, coating the plate with silica gel G.

*Mobile phase.* A mixture of 85 volumes of cyclohexane, 15 volumes of ethyl acetate and 3 volumes of diethylamine.

*Test solution.* Weigh accurately about 0.2 g of the substance under examination and dissolve in sufficient chloroform to produce 10 ml.

*Reference solution (a).* A 0.001 per cent w/v solution of dibenzosuberone RS in chloroform.

*Reference solution (b).* A 0.004 per cent w/v solution of cyclobenzaprine hydrochloride RS in chloroform.

Apply to the plate 10 μl of each solution. Allow the mobile phase to rise 14 cm in an unlined tank. Dry the plate in air until the odour of the solvent is no longer detectable, spray with a freshly prepared mixture of 4 volumes of formaldehyde solution and 96 volumes of sulphuric acid, heat at 105° for 10 minutes and examine in ultraviolet light at 365 nm. Any spots in the chromatogram obtained with the test solution corresponding to dibenzosuberone and cyclobenzaprine hydrochloride are not more intense than the spots in the chromatograms obtained with reference solutions (a) and (b) respectively and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (b).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 1.0 g and dissolve in 50 ml of anhydrous glacial acetic acid, warm slightly, if necessary, to effect solution. Cool, add 10 ml of mercuric acetate solution and titrate with 0.1 M perchloric acid, using crystal violet solution as indicator to a green end-point. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.03139 g of C₂₀H₂₃N.HCl.

**Amitriptyline Tablets**

Amitriptyline Hydrochloride Tablets

Amitriptyline Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of amitriptyline hydrochloride, C₂₀H₂₃N.HCl. The tablets are coated.
Identification

A. Shake a quantity of the powdered tablets containing about 5 mg of Amitriptyline Hydrochloride with 20 ml of methanol and filter. To 1 ml of the filtrate add 1 ml of a 2.5 per cent w/v solution of sodium bicarbonate, 1 ml of a 2 per cent w/v solution of sodium periodate and 1 ml of a 0.3 per cent w/v solution of potassium permanganate, allow to stand for 15 minutes, acidify with dilute sulphuric acid and extract with 10.0 ml of 2,2,4-trimethylpentane. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum only at about 265 nm.

B. Triturate a quantity of the powdered tablets containing 0.1 g of Amitriptyline Hydrochloride with 10 ml of chloroform, filter and evaporate the filtrate to a low volume. Add 0.1 g of Amitriptyline Hydrochloride with 10 ml of 15 minutes, acidify with dilute sulphuric acid and extract with 10.0 ml of 2,2,4-trimethylpentane. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum only at about 265 nm.

C. The precipitate obtained in test B gives reaction A of chlorides (2.3.1).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), protected from light, coating the plate with silica gel G

Mobile phase. A mixture of 85 volumes of cyclohexane, 15 volumes of ethyl acetate and 3 volumes of diethylamine.

Test solution. Extract a quantity of the powdered tablets containing 20 mg of Amitriptyline Hydrochloride with 5 ml of a mixture of 9 volumes of ethanol (95 per cent) and 1 volume of 2 M hydrochloric acid centrifuge and use the supernatant liquid, evaporated to dryness and dissolve in 10 ml of chloroform.

Reference solution (a). A 0.001 per cent w/v solution of dibenzosuberone RS in chloroform.

Reference solution (b). A 0.004 per cent w/v solution of cyclobenzaprine hydrochloride RS in chloroform.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 14 cm in an unlined tank. Dry the plate in air until the odour of the solvent is no longer detectable, spray with a freshly prepared mixture of 4 volumes of formaldehyde solution and 96 volumes of sulphuric acid, heat at 105° for 10 minutes and examine in ultraviolet light at 365 nm. Any spots in the chromatogram obtained with the test solution corresponding to dibenzosuberone and cyclobenzaprine hydrochloride are not more intense than the spots in the chromatograms obtained with reference solutions (a) and (b) respectively and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (b).

Uniformity of content (For tablets containing 10 mg or less). Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14)

Test solution. Powder one tablet, shake with 2.5 ml of 0.1 M hydrochloric acid until completely disintegrated, add 5 ml of methanol, shake for 30 minutes, dilute the suspension to 10 ml with methanol, centrifuge and use the clear supernatant liquid.

Reference solution. Weigh accurately 25.0 mg of amitriptyline hydrochloride RS dissolve in 10 ml of methanol and dilute to 25.0 ml with methanol (50 per cent).

Chromatographic system

– a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (10 µm),
– mobile phase: 0.03 M sodium hexanesulphonate in a mixture of equal volumes of acetonitrile and water, adjusted to pH 4.5 by the addition of glacial acetic acid,
– flow rate. 2 ml per minute,
– spectrophotometer set at 239 nm,
– a 20 µl loop injector.

Calculate the content of C20H23N,HCl in the tablet.

Dissolution (2.5.2).

Apparatus. No 2

Medium. 900 ml of 0.1 M hydrochloric acid.

Speed and time. 100 rpm and 45 minutes.

Use one tablet in the vessel for each test.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 239 nm (2.4.7). Calculate the content of C20H23N,HCl in the medium from the absorbance obtained from a solution of known concentration of amitriptyline hydrochloride RS in the same medium.

D. Not less than 75 per cent of the stated amount of C20H23N,HCl.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. When tablets are film-coated, shake 20 tablets with 50 ml of 0.1 M hydrochloric acid until completely disintegrated, add 100 ml of methanol, shake for 30 minutes, dilute the suspension to 200.0 ml with methanol, centrifuge and dilute a volume of the supernatant liquid equivalent to 25 mg of Amitriptyline Hydrochloride to 100.0 ml with methanol (50 per cent).
When tablets are sugar-coated, weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Amitriptyline Hydrochloride, shake with 50 ml of 0.1 M hydrochloric acid for 30 minutes, add 100 ml of methanol, shake for 30 minutes, dilute the mixture to 200.0 ml with water, centrifuge and use the supernatant liquid.

Reference solution. Dissolve 50 mg of amitriptyline hydrochloride RS in 10 ml of methanol and dilute to 200.0 ml with methanol (50 per cent).

Follow the procedure described under Uniformity of content. Calculate the content of C₂₀H₂₃N,HCl in the tablets.

Amlodipine Besilate

C₂₆H₃₁ClN₂O₈S  Mol. Wt. 567.1

Amlodipine Besilate is 3-ethyl 5-methyl (4RS)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzene sulphonate.

Amlodipine Besilate contains not less than 97.0 per cent and not more than 102.0 per cent of C₂₆H₃₁ClN₂O₈S, calculated on the anhydrous basis.

Description. A white or almost white powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with amlodipine besilate RS or with the reference spectrum of amlodipine besilate.

B. In test A for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. When examined in the range 300 nm to 400 nm (2.4.7), a 0.005 per cent w/v solution in a 1 per cent v/v solution of 0.1 M hydrochloric acid in methanol shows an absorption maximum at about 360 nm. The specific absorbance at the maximum is 113 to 121.

Tests

Optical rotation (2.4.22). ±0.10° to +0.10°, determined in a 1.0 per cent w/v solution in methanol.

Related substances. A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. The upper layer of a mixture of 25 volumes of glacial acetic acid, 25 volumes of water and 50 volumes of methyl isobutyl ketone.

Test solution (a). Dissolve 0.14 g of the substance under examination in 2 ml of methanol.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with methanol.

Reference solution (a). Dissolve 70 mg of amlodipine besilate RS in 1 ml of methanol.

Reference solution (b). Dilute 1 ml of reference solution (a) to 10 ml with methanol.

Reference solution (c). Dilute 3 ml of test solution (b) to 100 ml with methanol.

Reference solution (d). Dilute 1 ml of test solution (b) to 100 ml with methanol.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate at 80°C for 15 minutes and examine in ultraviolet light at 254 nm and 365 nm. The chromatogram obtained with reference solution (a) shows two clearly separated minor spots with Rf values of about 0.18 and 0.22. In the chromatogram obtained with reference solution (a) any spot, other than the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.3 per cent) and at most 2 spots are more intense than the spot in the chromatogram obtained with reference solution (d) (0.1 per cent).

B. Determine by liquid chromatography (2.4.14).

Test solution (a). Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Test solution (b). Dilute 5.0 ml of test solution (a) to 100.0 ml with the mobile phase.

Reference solution (a). A solution containing 0.005 per cent w/v of amlodipine besilate RS in the mobile phase.

Reference solution (b). Dilute 3 ml of test solution (a) to 100 ml with the mobile phase and dilute 5 ml of the solution to 50 ml with the mobile phase.
Reference solution (c). Dissolve 5 mg of the substance under examination in 5 ml of strong hydrogen peroxide solution. Heat at 70° for 45 minutes.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 15 volumes of acetonitrile, 35 volumes of methanol and 50 volumes of a solution prepared by dissolving 7.0 ml of triethylamine in 1000 ml of water and adjusting the pH to 3.0 with phosphoric acid,
- flow rate. 1 ml per minute,
- spectrophotometer set at 237 nm,
- a 10 µl loop injector.

The relative retention time between amlodipine and 3-ethyl 5-methyl(4RS)-4-(2-chlorophenyl)-6-methyl-2-[2-[2-(methylcarbamoyl)benzoyl]amino]ethoxy]methyl]-1,4-dihydropyridine-3,5-dicarboxylate (impurity A) is about 0.5.

Inject reference solution (c). The test is not valid unless the resolution between the peaks corresponding to amlodipine and impurity A is at least 4.5.

Inject test solution (a) and reference solutions (b) and (c). Continue the chromatography for 3 times the retention time of amlodipine. The area of any peak corresponding to impurity A multiplied by 2 is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). The sum of the areas of all the other secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) Ignore any peak due to benzene sulphonate (relative retention about 0.2) and any peak with an area 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.03 per cent).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 3.0 g.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject alternately test solution (b) and reference solution (a).

Calculate the content of C_{26}H_{31}ClN_{2}O_{8}S.

Storage. Store protected from moisture.

Amlodipine Tablets

Amlodipine Besilate Tablets

Amlodipine Tablets contain Amlodipine Besilate.

Amlodipine Tablets contain less than 90.0 per cent and not more than 110.0 per cent of the stated amount of amlodipine, C_{20}H_{25}ClN_{2}O_{5}.
retention about 0.2) and any peak with an area 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Dissolution (2.5.2).**

Apparatus No. 1

Medium. 900 ml of 0.01 M hydrochloric acid.

Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the dissolution medium if necessary, at the maximum at about 239 nm (2.4.7). Calculate the content of C$_{20}$H$_{25}$ClN$_2$O$_5$ in the medium from the absorbance obtained from a solution of known concentration of amlodipine besilate RS in the same medium.

D. Not less than 70 per cent of the stated amount of C$_{20}$H$_{25}$ClN$_2$O$_5$.

**Uniformity of content.** Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14) as described under Related substances using the following solutions.

Test solution. Powder one tablet and dissolve in 50 ml of methanol, dilute with sufficient methanol to get a solution containing 0.002 per cent w/v of amlodipine, shake for 10 minutes and filter through a glass-fibre filter paper.

Reference solution. A solution of amlodipine besilate RS in methanol equivalent to 0.002 per cent w/v of amlodipine.

Calculate the content of C$_{20}$H$_{25}$ClN$_2$O$_5$ in the tablet.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject alternately test solution (b) and reference solution (a). Calculate the content of C$_{20}$H$_{25}$ClN$_2$O$_5$ in the tablets.

**Storage.** Store protected from moisture.

**Labelling.** The label states the strength in terms of the equivalent amount of amlodipine.

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**Ammonium Chloride**

NH$_4$Cl  
Mol. Wt. 53.5

Ammonium Chloride contains not less than 99.0 per cent and not more than 100.5 per cent of NH$_4$Cl, calculated on the dried basis.

**Description.** Colourless crystals or a white, crystalline powder.

**Identification**

Gives the reactions of ammonium salts and of chlorides (2.3.1).

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**Tests**

**Appearance of solution.** A 10.0 per cent solution is clear (2.4.1) and colourless (2.4.1).

**pH (2.4.24).** 4.5 to 6.0, determined in a 5.0 per cent solution.

**Arsenic (2.3.10).** Dissolve 2.5 g in 50 ml of water and add 10 ml of stannated hydrochloric acid. The resulting solution complies with the limit test for arsenic (4 ppm).

**Heavy metals (2.3.13).** 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

**Iron (2.3.14).** 2.0 g complies with the limit test for iron (20 ppm).

**Calcium.** To 0.2 ml of ethanolic calcium standard solution (100 ppm Ca) add 1 ml of a 4 per cent w/v solution of ammonium oxalate. After 1 minute add 1 ml of 2 M acetic acid and 15 ml of a solution made by diluting 5 ml of a 10 per cent solution of the substance under examination with 10 ml of water and shake. Compare any opalescence produced with that of a standard prepared in a similar manner but using a mixture of 10 ml of calcium standard solution (10 ppm Ca) and 5 ml of water instead of the solution of the substance under examination (200 ppm).

**Sulphates (2.3.17).** 1.0 g complies with the limit test for sulphates (150 ppm).

**Thiocyanate.** Acidify 10 ml of a 10 per cent w/v solution with hydrochloric acid and add a few drops of ferric chloride solution; no red colour is produced.

**Sulphated ash (2.3.18).** Not more than 0.1 per cent.

**Loss on drying (2.4.19).** Not more than 1.0 per cent determined on 1.0 g by drying in an oven at 105°C.

**Assay.** Weigh accurately about 0.1 g, dissolve in 20 ml of water and add a mixture of 5 ml of formaldehyde solution, previously neutralised to dilute phenolphthalein solution, and 20 ml of water. After 2 minutes, titrate slowly with 0.1 M sodium hydroxide using a further 0.2 ml of dilute phenolphthalein solution as indicator.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.005349 g of NH$_4$Cl.

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**Amodiaquine Hydrochloride**

Amodiaquine Hydrochloride

N \[\text{N} \overset{\text{O}}{\text{H}} \overset{\text{CH}_3}{\text{C}}_{20} \text{H}_25 \text{Cl}_n O_5 , 2\text{HCl}, 2\text{H}_2O \]  
Mol. Wt. 464.9
Amodiaquine Hydrochloride is 4-(7-chloro-4-quinolylamino)-2-(diethylaminomethyl)phenol dihydrochloride dihydrate.

Amodiaquine Hydrochloride contains not less than 98.0 per cent and not more than 101.5 per cent of C₂₀H₂₂ClN₃O₂HCl, calculated on the anhydrous basis.

Description. A yellow, crystalline powder; odourless or almost odourless.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests C and D may be omitted if tests A, B and E are carried out.

A. Dissolve 20 mg in 10 ml of water and add 1 ml of strong ammonia solution. Extract with two quantities, each of 25 ml, of chloroform, wash the combined chloroform extracts with water, dry with anhydrous sodium sulphate, evaporate the chloroform and dry the residue at 105° for 2 hours. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with amodiaquine RS.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0015 per cent w/v solution in 0.1 M hydrochloric acid shows an absorption maximum at about 343 nm; absorbance at 343 nm, about 0.55.

C. To 1 ml of a 2 per cent w/v solution add 0.5 ml of cobalt thiocyanate solution; a green precipitate is produced.

D. To 20 ml of a 2 per cent w/v solution, add 1 ml of dilute ammonia solution. Shake and filter; the filtrate gives the reactions of chlorides (2.3.1).

E. The undried material melts at about 158° (2.4.21).

Tests

pH (2.4.24). 3.6 to 4.6, determined in a 2.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 90 volumes of chloroform saturated with strong ammonia solution and 10 volumes of ethanol.

Test solution. Add to 200 mg of the substance under examination in a glass-stoppered test-tube 10 ml of chloroform saturated with strong ammonia solution, shake vigorously for 2 minutes, allow the solids to settle and decant the supernatant liquid.

Reference solution (a). Prepare in the same manner as the test solution but using 200 mg of amodiaquine hydrochloride RS and 10 ml of chloroform saturated with strong ammonia solution.

Reference solution (b). Dilute 1 volume of reference solution (a) with sufficient chloroform saturated with strong ammonia solution to obtain 200 volumes.

Apply to the plate 10 μl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a) and no secondary spot in the chromatogram obtained with the test solution is more intense than the principal spot in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 6.0 to 10.0 per cent, determined on 0.5 g.

Assay. Weigh accurately about 0.3 g and dissolve in sufficient 0.1 M hydrochloric acid to produce 200.0 ml. Dilute 10.0 ml to 1000.0 ml with 0.1 M hydrochloric acid. Measure the absorbance of the resulting solution at the maximum at about 343 nm (2.4.7), using 0.1 M hydrochloric acid as the blank.

Calculate the content of C₂₀H₂₂ClN₃O₂HCl from the absorbance obtained by carrying out the Assay simultaneously on amodiaquine hydrochloride RS.

Amodiaquine Tablets

Amodiaquine Hydrochloride Tablets

Amodiaquine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of amodiaquine, C₂₀H₂₂ClN₃O₂.

Identification

A. Extract the powdered tablets with water and filter. To 1 ml of the filtrate add 0.5 ml of cobalt thiocyanate solution; a green precipitate is produced.

B. The powdered tablets give the reactions of chlorides (2.3.1).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 90 volumes of chloroform saturated with strong ammonia solution and 10 volumes of ethanol.

Test solution. Shake a quantity of the powdered tablets containing 40 mg of Amodiaquine Hydrochloride with 20 ml of water for 1 minute, add 25 ml of chloroform and 1 ml of strong ammonia solution and shake vigorously for 2 minutes. Filter the chloroform extract through a cotton plug previously soaked in chloroform, evaporate the filtrate to dryness and dissolve the residue in 2 ml of chloroform saturated with strong ammonia solution.
Reference solution (a). Prepare in the same manner as the test solution but using 200 mg of *amodiaquine hydrochloride RS* and 10 ml of chloroform saturated with strong ammonia solution.

Reference solution (b). Dilute 1 volume of reference solution (a) with sufficient chloroform saturated with strong ammonia solution to obtain 200 volumes.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a) and no secondary spot in the chromatogram obtained with the test solution is more intense than the principal spot in the chromatogram obtained with reference solution (b).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.3 g of amodiaquine, add 100 ml of 0.1 M hydrochloric acid and heat on a water-bath for about 15 minutes with occasional stirring. Cool, transfer to a 200-ml graduated flask and dilute to volume with 1 M hydrochloric acid. To 10.0 ml of the clear supernatant liquid in a separator, add 10 ml of 0.1 M hydrochloric acid and extract with 20 ml of chloroform. Discard the chloroform extract. Add 4.5 ml of 1 M sodium hydroxide and extract with four quantities, each of 25 ml, of chloroform. Extract the combined chloroform solutions with three quantities, each of 50 ml, of 0.1 M hydrochloric acid and dilute with sufficient 0.1 M hydrochloric acid to produce 200.0 ml. Dilute 10.0 ml with sufficient 0.1 M hydrochloric acid to produce 100.0 ml. Measure the absorbance of the resulting solution at the maximum at about 343 nm (2.4.7), using 0.1 M hydrochloric acid as the blank.

Calculate the content of $C_{16}H_{18}N_3NaO_5S$, 2HCl from the absorbance obtained by carrying out the Assay simultaneously on *amodiaquine hydrochloride RS*. Multiply the result by 0.830 to get the equivalent quantity of $C_{20}H_{22}ClN_3O$.

Labelling. The label states the strength in terms of the equivalent amount of amodiaquine.

Amoxycillin Sodium

Amoxycillin Sodium is sodium (6R)-6-(α-D-4-hydroxyphenylglycylamino)penicillanate.

Amoxycillin Sodium contains not less than 85.0 per cent and not more than 100.5 per cent of $C_{16}H_{18}N_3NaO_5S$, calculated on the anhydrous basis.

Description. A white or almost white powder; very hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amoxycillin sodium RS* or with the reference spectrum of amoxycillin sodium.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. A 5 per cent w/v solution gives the reactions of sodium salts (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution is not more opalescent than opalescence standard OS2 (2.4.1) when examined immediately after preparation. The solution may initially show a pink colour and its absorbance after 5 minutes at about 430 nm is not more than 0.20 (2.4.7).

pH (2.4.24). 8.0 to 10.0, determined in a 10.0 per cent w/v solution.

Specific optical rotation (2.4.22). +240° to +290°, determined in a 0.25 per cent w/v solution in a 0.4 per cent w/v solution of *potassium hydrogen phthalate*.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

N,N-Dimethylaniline (2.3.21). Not more than 20 ppm, determined by Method A.

Sodium chloride. Not more than 2.0 per cent, calculated on the anhydrous basis, determined by the following method. Weigh accurately about 1.0 g, dissolve in 50 ml of *distilled water*, add 10 ml of 2 M *nitric acid* and titrate with 0.1 M *silver nitrate*, determining the end-point potentiometrically (2.4.25) using a silver indicator electrode and a mercury-mercurous sulphate reference electrode or any other suitable electrode.

1 ml of 0.1 M *silver nitrate* is equivalent to 0.005845 g of NaCl.

2-Ethylhexanoic acid. Not more than 2.0 per cent, determined by the following method.

Determine by gas chromatography (2.4.13).

Test solution. Prepare a 1.0 per cent w/v solution of *valeric acid* (internal standard) in *hexane* (solution A). Dissolve 1.0 g
of the substance under examination in 5 ml of water in a glass-
stoppered flask, add 3 ml of 2 M hydrochloric acid, 1 ml of
solution A and 5 ml of hexane, shake vigorously for 1 minute,
centrifuge if necessary and use the clear supernatant layer.

Reference solution (a). Prepare in the same manner as the test
solution but using an extra 1 ml of hexane in place of solution A.

Reference solution (b). Prepare in the same manner as the test
solution but using 20 mg of 2-ethylhexanoic acid suspended
in 5 ml of water in place of the substance under examination.

Chromatographic system
- a glass column 1.8 m x 4 mm, packed with a support
impregnated with a stationary phase suitable for the
separation of free fatty acids (such as a column
containing 10 per cent of SP 1200 and 1 per cent of
phosphoric acid on Chromosorb W AW, 80-100 mesh),
- temperature: column, 145°,
- inlet port and detector. 150°,
- flow rate. 45 ml per minute of the carrier gas.

Water (2.3.43). Not more than 4.0 per cent, determined on
0.4 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 6.8 g of monobasic potassium
phosphate in 100 ml of water and adjust the pH to about 5.0
with a 4.5 per cent w/v solution of potassium hydroxide.

Test solution. Transfer an accurately weighed quantity
containing about 120 mg of Amoxycillin to a 100-ml volumetric
flask, dissolve in the solvent mixture and dilute to 100.0 ml
with the solvent mixture. Use this solution within 6 hours.

Reference solution. Weigh accurately a suitable quantity
of amoxycillin trihydrate RS, dissolve in the solvent mixture by
shaking and mixing if necessary, with the aid of ultrasound
and dilute to obtain a solution having a known concentration
of about 1.2 mg per ml. Use this solution within 6 hours.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with
octadecylsilane chemically bonded to porous silica or
ceramic microparticles (5 µm),
- mobile phase: a mixture of 96 volumes of acetonitrile
and 4 volumes of the solvent mixture,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the
capacity factor is between 1.1 and 2.8, the column efficiency
is not less than 1700 theoretical plates, the tailing factor is not
more than 2.5 and the relative standard deviation for replicate
injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.
Calculate the percentage content of C_{16}H_{18}N_{3}NaO_{5}S by
multiplying the percentage content of C_{16}H_{19}N_{3}O_{5}S by 1.060.

Amoxycillin Sodium intended for use in the manufacture of
parenteral preparations without a further appropriate
procedure for the removal of bacterial endotoxins complies
with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.25 Endotoxin
Unit per mg of amoxycillin.

Amoxycillin Sodium intended for use in the manufacture of
parenteral preparations without a further appropriate
sterilisation procedure complies with the following
additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture, at a temperature not
exceeding 30°. If it is intended for use in the manufacture of
parenteral preparations, the container should be sterile, tamper-
evident and sealed so as to exclude micro-organisms.

Labelling. The label states whether or not the material is
intended for use in the manufacture of parenteral preparations.

Amoxycillin Capsules

Amoxycillin Trihydrate Capsules; Amoxicillin Trihydrate
Capsules; Amoxicillin Capsules

Amoxycillin Capsules contain not less than 90.0 per cent and
not more than 110.0 per cent of the stated amount of
amoxycillin, C_{16}H_{19}N_{3}O_{5}S.

Identification

Shake a quantity of the contents of the capsules containing
0.5 g of amoxycillin with 5 ml of water for 5 minutes, filter,
wash the residue first with ethanol and then with ether and
dry at a pressure not exceeding 0.7 kPa for 1 hour. The residue
complies with the following tests.

Test A may be omitted if test B is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6).
Compare the spectrum with that obtained with amoxycillin
trihydrate RS or with the reference spectrum of amoxycillin
trihydrate.

B. In the Assay, the principal peak in the chromatogram
obtained with the test solution corresponds to the peak in the
chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus. No 1

Medium. 900 ml of water.
Speed and time. 100 rpm and 60 minutes.

Use one capsule in the vessel for each test.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Dilute the filtrate, if necessary, with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 272 nm (2.4.7). Similarly measure the absorbance of a standard solution of known concentration of amoxicillin trihydrate RS at about 272 nm and calculate the content of $C_{16}H_{19}N_{3}O_{5}S$.

D. Not less than 80 per cent of the stated amount of $C_{16}H_{19}N_{3}O_{5}S$.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 6.8 g of monobasic potassium phosphate in 100 ml of water and adjust the pH to about 5.0 with a 4.5 per cent w/v solution of potassium hydroxide.

Test solution. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 100 mg of amoxicillin, add about 80 ml of the solvent mixture and dissolve by shaking for 15 minutes and mixing if necessary, with the aid of ultrasound. Dilute to 100.0 ml with the solvent mixture and filter. Use this solution within 6 hours.

Reference solution. Weigh accurately a suitable quantity of amoxicillin trihydrate RS, dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound and dilute to obtain a solution having a known concentration of about 1.2 mg per ml. Use this solution within 6 hours.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica particles or ceramic microparticles (5 µm),
- mobile phase: a mixture of 96 volumes of acetonitrile and 4 volumes of the solvent mixture,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the capacity factor is between 1.1 and 2.8, the column efficiency is not less than 1700 theoretical plates, the tailing factor is not more than 2.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the content of $C_{16}H_{19}N_{3}O_{5}S$ in the capsules.

Storage. Store protected from moisture.

Labelling. The label states the quantity of the active ingredient in terms of the equivalent amount of amoxycillin.

Amoxycillin Injection

Amoxicillin Sodium Injection; Amoxycillin Sodium Injection

Amoxycillin Injection is a sterile material consisting of Amoxicillin Sodium with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Amoxycillin Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of amoxicillin, $C_{16}H_{19}N_{3}O_{5}S$.

Description. A white or almost white powder; very hygroscopic.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with amoxicillin sodium RS or with the reference spectrum of amoxicillin sodium.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. A 5 per cent w/v solution gives the reactions of sodium salts (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution is not more opalescent than opalescence standard OS2 (2.4.1) when examined immediately after preparation. The solution may initially show a pink colour and its absorbance after 5 minutes at about 430 nm is not more than 0.20 (2.4.7).

pH (2.4.24). 8.0 to 10.0, determined in a 10.0 per cent w/v solution.

Specific optical rotation (2.4.22). $+240^\circ$ to $+290^\circ$, determined in a 0.25 per cent w/v solution in a 0.4 per cent w/v solution of potassium hydrogen phthalate.
Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

N,N-Dimethylaniline (2.3.21). Not more than 20 ppm, determined by Method A.

Sodium chloride. Not more than 2.0 per cent, calculated on the anhydrous basis, determined by the following method. Weigh accurately about 1.0 g, dissolve in 50 ml of distilled water; add 10 ml of 2 M nitric acid and titrate with 0.1 M silver nitrate, determining the end-point potentiometrically (2.4.25) using a silver indicator electrode and a mercury-mercurous sulphate reference electrode or any other suitable electrode.

1 ml of 0.1 M silver nitrate is equivalent to 0.005845 g of NaCl.

Bacterial endotoxins (2.2.3). Not more than 0.25 Endotoxin Unit per mg of amoxicillin.

Water (2.3.43). Not more than 4.0 per cent, determined on 0.4 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 6.8 g of monobasic potassium phosphate in 100 ml of water and adjust the pH to about 5.0 with a 4.5 per cent w/v solution of potassium hydroxide.

Test solution. Determine the weight of the contents of 10 containers. Transfer an accurately weighed quantity of the mixed contents of the 10 containers containing about 100 mg of amoxicillin to a 100-ml volumetric flask, add 80 ml of the solvent mixture and dissolve by shaking and mixing if necessary, with the aid of ultrasound. Dilute to 100.0 ml with the solvent mixture and filter. Use this solution within 6 hours.

Reference solution. Dissolve an accurately weighed quantity of amoxicillin trihydrate RS in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound and dilute to obtain a solution having a known concentration of about 1.2 mg per ml. Use this solution within 6 hours.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 96 volumes of acetonitrile and 4 volumes of the solvent mixture,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the capacity factor is between 1.1 and 2.8, the column efficiency is not less than 1700 theoretical plates, the tailing factor is not more than 2.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the percentage content of $C_{16}H_{19}N_3O_5S$ in the injection.

Storage. Store protected from moisture, in a sterile, tamper-evident container sealed so as to exclude micro-organisms, at a temperature not exceeding 30°.

Labelling. The label states the quantity of Amoxicillin Sodium contained in the sealed container in terms of the equivalent amount of amoxicillin.

Amoxicillin Oral Suspension

Amoxicillin Oral Suspension is a mixture consisting of Amoxicillin Trihydrate with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of Water just before issue.

Amoxicillin Oral Suspension contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of amoxicillin $C_{16}H_{19}N_3O_5S$.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of amoxicillin $C_{16}H_{19}N_3O_5S$.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

The constituted suspension complies with the tests stated under Oral liquids and with the following tests.

Tests

pH (2.4.24). 4.0 to 7.0.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 6.8 g of monobasic potassium phosphate in 100 ml of water and adjust the pH to about 4.5 with a 4.5 per cent w/v solution of potassium hydroxide.

Test solution. Transfer an accurately weighed quantity containing 120 mg of amoxicillin to a 100-ml volumetric flask, dissolve in the solvent mixture and dilute to 100.0 ml with the solvent mixture and filter.
Reference solution. Dissolve an accurately weighed quantity of amoxycillin trihydrate RS in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound and dilute to obtain a solution having a known concentration of about 1.2 mg per ml. Use this solution within 6 hours.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 96 volumes of acetonitrile and 4 volumes of the solvent mixture,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the capacity factor is between 1.1 and 2.8, the column efficiency is not less than 1700 theoretical plates, the tailing factor is not more than 2.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Determine the weight per ml of the oral suspension (2.4.29) and calculate the content of C₁₆H₁₉N₃O₅S weight in volume.

Repeat the procedure using a portion of the constituted suspension that has been stored at the temperature and for the period stated on the label.

Labelling. The label states (1) the quantity of active ingredient in terms of the equivalent amount of amoxicillin; (2) the temperature of storage and the period during which the constituted suspension may be expected to be satisfactory for use.

Amoxycillin Trihydrate

C₁₆H₁₉N₃O₅S·3H₂O  Mol. Wt 419.5

Amoxycillin Trihydrate is (6R)-6-(α-4-hydroxyphenyl-0-glycylamino)penicillanic acid trihydrate.

Amoxycillin Trihydrate contains not less than 95.0 per cent and not more than 100.5 per cent of C₁₆H₁₉N₃O₅S, calculated on the anhydrous basis.

Description. A white or almost white, crystalline powder.
Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 96 volumes of acetonitrile and 4 volumes of the solvent mixture,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the capacity factor is between 1.1 and 2.8, the column efficiency is not less than 1700 theoretical plates, the tailing factor is not more than 2.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the percentage content of C₁₆H₁₉N₃O₅S.

Storage. Store at a temperature not exceeding 30°.

Labelling. The label states (1) the strength in terms of the equivalent amount of amoxycillin; (2) that the tablets should be dispersed in water immediately before use.

Amoxycillin Dispersible Tablets

Amoxycillin Trihydrate Dispersible Tablets; Dispersible Amoxycillin Tablets

Amoxycillin Dispersible Tablets contain Amoxycillin Trihydrate in a suitable dispersible base.

Amoxycillin Dispersible Tablets contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of amoxycillin, C₁₆H₁₉N₃O₅S.

Identification

Shake a quantity of the powdered tablets containing 0.5 g of amoxycillin with 5 ml of water for 5 minutes, filter, wash the residue first with ethanol and then with ether and dry for 1 hour at a pressure not exceeding 0.7 kPa. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with amoxycillin trihydrate RS or with the reference spectrum of amoxycillin trihydrate.

Tests

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets.

Determine by liquid chromatography (2.4.14).

Amoxycillin and Potassium Clavulanate Injection

Amoxicillin and Potassium Clavulanate injection

Amoxicillin and Potassium Clavulanate injection is a sterile material consisting of Amoxycillin Sodium and Potassium Clavulanate with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).
**Storage.** The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Amoxycillin and Potassium Clavulanate Injection contains not less than 90.0 per cent and not more than 107.5 per cent of the stated amounts of amoxycillin, C₁₆H₁₉N₃O₅S and of clavulanic acid, C₇H₈NO₅.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

**Identification**

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel F254 (such as Merck silica gel 60 F254 plates).

**Mobile phase.** A mixture of 1 volume of butan-1-ol, 2 volumes of a 0.1 per cent w/v solution of disodium edetate in mixed phosphate buffer pH 4.0, 6 volumes of glacial acetic acid and 10 volumes of butyl acetate.

**Test solution.** Shake a quantity of the contents of the sealed container containing 0.4 g of clavulanic acid in 100 ml of a mixture of 4 volumes of methanol and 6 volumes of 0.1 M mixed phosphate buffer pH 7.0 and filter.

**Reference solution.** A solution containing 0.4 per cent w/v of lithium clavulanate RS and 0.8 per cent w/v of amoxycillin trihydrate RS in a mixture of 4 volumes of methanol and 6 volumes of 0.1 M mixed phosphate buffer pH 7.0.

Apply to the plate 1 µl of each of the solutions after impregnating the plate by spraying it with a 0.1 per cent w/v solution of disodium edetate in mixed phosphate buffer pH 4.0 and allowing to dry overnight and activating the plate by heating at 105º for 1 hour just before use. After development, allow it to dry in air and examine in ultraviolet light at 254 nm. The principal spots in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

B. In the Assay, the retention time of the two principal peaks in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

**Tests**

**pH** (2.4.24). 8.0 to 10.0, determined in a solution containing 10 per cent w/v of amoxycillin.

**Bacterial endotoxins** (2.2.3). Not more than 0.25 EU per mg of amoxycillin.

**Water** (2.3.43). Not more than 3.5 per cent, determined on 0.5 g.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Determine the weight of the contents of 10 containers. Dissolve, with shaking, a quantity of the mixed contents of the 10 containers containing about 60 mg of amoxycillin in water and dilute to 100.0 ml with the same solvent, mix and filter.

**Reference solution.** A solution containing 0.06 per cent w/v of amoxycillin trihydrate RS and 0.012 per cent w/v of clavulanic acid RS in water.

**Chromatographic system**

- a stainless steel column 30 cm × 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 95 volumes of buffer solution prepared by dissolving 7.8 g of monobasic sodium phosphate in 900 ml of water, adjust the pH to 4.4 with 10 M sodium hydroxide or orthophosphoric acid and 5 volumes of methanol,
- flow rate. 2 ml per minute,
- spectrophotometer set at 220 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to amoxycillin and clavulanic acid is not less than 3.5, the tailing factor is not more than 1.5, the column efficiency is not less than 550 theoretical plates for both component and the relative standard deviation is not more than 2.0. Inject alternately the test solution and the reference solution.

Calculate the content of C₁₆H₁₉N₃O₅S and C₇H₈NO₅.

1 mg of C₇H₈LiNO₅ is equivalent to 0.9711 mg of C₇H₈NO₅.

**Labelling.** The label states the quantity of Amoxycillin Sodium contained in it, in terms of the equivalent amount of amoxycillin, and the quantity of Potassium Clavulanate, in terms of the equivalent amount of clavulanic acid.

**Amoxicillin and Potassium Clavulanate Oral Suspension**

Amoxicillin and Potassium Clavulanate oral suspension is a mixture of Amoxicillin Trihydrate and Potassium Clavulanate with buffering agents and other excipients. It contains a suitable flavouring agent.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of Water just before use.

Amoxicillin and Potassium Clavulanate Oral Suspension contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of amoxycillin, C₁₆H₁₉N₃O₅S and C₇H₈NO₅.
not less than 90.0 per cent and not more than 125.0 per cent of the stated amount of clavulanic acid, C₈H₉NO₅.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amounts of amoxycillin, C₁₆H₁₉N₃O₅S and clavulanic acid, C₈H₉NO₅.

**Identification**

In the Assay, the retention time of the two principal peaks in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

**Tests**

**Water** (2.3.43). Not more than 7.5 per cent where the label indicates that after reconstitution as directed, the suspension contains an amount of amoxycillin that is less than 40 mg per ml; not more than 8.5 per cent where the label indicates that after reconstitution as directed, the suspension contains an amount of amoxycillin that is equal to or more than 40 mg per ml and is less than or equal to 50 mg per ml; not more than 11.0 per cent where the label indicates that after reconstitution as directed, the suspension contains an amount of amoxycillin that is more than 50 mg per ml and is less than or equal to 80 mg per ml; not more than 12.0 per cent where the label indicates that after reconstitution as directed, the suspension contains an amount of amoxycillin that is more than 80 mg per ml.

The constituted suspension complies with the tests stated under Oral liquids and with the following tests.

**pH** (2.4.24). 3.8 to 6.6.

**Assay**. Determine by liquid chromatography (2.4.14).

**Test solution**. Transfer an accurately weighed quantity containing about 50 mg of amoxycillin to a 100-ml volumetric flask, dissolve in water, dilute to 100.0 ml with the same solvent and filter. Use the filtrate as the test solution within 1 hour.

**Reference solution**. A solution containing 0.05 per cent w/v of amoxycillin trihydrate RS and 0.02 per cent w/v of lithium clavulanate RS in water.

Chromatographic system
- a stainless steel column 30 cm x 4 mm, packed with octadecylsilsane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 95 volumes of pH 4.4 sodium phosphate buffer and 5 volumes of methanol,
- flow rate. 2 ml per minute,
- spectrophotometer set at 220 nm,
- a 20 µl loop injector.

Inject the reference solution. The relative retention times are about 0.5 for clavulanic acid and 1.0 for amoxycillin. The resolution between the amoxycillin and clavulanic acid peaks is not less than 3.5. The test is not valid unless the column efficiency determined from each analyte peak is not less than 550 theoretical plates, the tailing factor for each analyte peak is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Determine the weight per ml of the oral suspension (2.4.29) and calculate the content of C₁₆H₁₉N₃O₅S and C₈H₉NO₅ weight in volume.

1 mg of C₈H₈LiNO₅ is equivalent to 0.9711 mg of C₈H₉NO₅.

Repeat the procedure using a portion of the constituted suspension that has been stored at the temperature and for the period stated on the label.

**Storage**. Store protected from moisture.

**Labelling**. The label states the quantity of Amoxycillin Trihydrate contained in it, in terms of the equivalent amount of amoxycillin, and the quantity of Potassium Clavulanate, in terms of the equivalent amount of clavulanic acid.

**Amoxycillin and Potassium Clavulanate Tablets**

Amoxicillin and Potassium Clavulanate Tablets contain Amoxycillin Trihydrate and Potassium Clavulanate.

Amoxicillin and Potassium Clavulanate Tablets contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amounts of amoxycillin, C₁₆H₁₉N₃O₅S and clavulanic acid, C₈H₉NO₅.

**Identification**

In the Assay, the retention time of the two principal peaks in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

**Tests**

**Disintegration** (2.5.1). 30 minutes, for tablets labelled for veterinary use only, simulated gastric fluid being substituted for water in the test.

**Dissolution** (2.5.2). (Tablets labelled for veterinary use only are exempt from this requirement).

Apparatus. No 1

Medium. 900 ml of water.

Speed and time. 75 rpm and 30 minutes or 45 minutes where the Tablets are labelled as chewable.
Withdraw a suitable volume of the medium and filter. Carry out the method described under Assay.

D. Not less than 85 per cent of the stated amount of \( \text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_5\text{S} \) and not less than 80 per cent of the stated amount of \( \text{C}_8\text{H}_9\text{N}_3\text{O}_5 \).

For tablets labelled as chewable. Not less than 80 per cent of the stated amount of \( \text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_5\text{S} \) and \( \text{C}_8\text{H}_9\text{N}_3\text{O}_5 \) is dissolved in 45 minutes.

**Uniformity of content.** Comply with the test stated under Tablets, determining the content of clavulanic acid in the tablets.

Follow the chromatographic procedure described under Assay using the following test solution.

Powder one tablet and transfer to a 100 ml flask. Dissolve in water and dilute to 100.0 ml with the same solvent and filter. Further dilute to obtain a solution containing 0.05 per cent w/v of amoxycillin. Use the solution within 1 hour.

Calculate the content of \( \text{C}_8\text{H}_9\text{N}_3\text{O}_5 \) in the tablet.

**Water (2.3.43).** Not more than 7.5 per cent, where the labelled amount of amoxycillin in each tablet is 250 mg or less; not more than 10.0 per cent where the labelled amount of amoxycillin in each tablet is more than 250 mg but less than or equal to 500 mg; not more than 11.0 per cent where the labelled amount of amoxycillin in each tablet is more than 500 mg. Where the tablets are labelled as chewable, not more than 6.0 per cent where the labelled amount of amoxycillin in each tablet is 125 mg or less; not more than 8.0 per cent where the labelled amount of amoxycillin in each tablet is more than 125 mg. Where the tablets are labelled for veterinary use only, not more than 10.0 per cent.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablet containing about 50 mg of amoxycillin, dissolve in water, dilute to 100.0 ml with water and filter. Use the filtrate as the test solution within 1 hour.

**Reference solution.** A solution containing 0.05 per cent w/v of amoxycillin trihydrate RS and 0.02 per cent w/v of lithium clavulanate RS in water.

Chromatographic system
- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 95 volumes of 0.78 per cent w/v solution of sodium phosphate, adjusted to pH 4.4 with orthophosphoric acid and 5 volumes of methanol,
- flow rate. 2 ml per minute,
- spectrophotometer set at 220 nm,
- a 20 µl loop injector.

Inject the reference solution. The relative retention times are about 0.5 for clavulanic acid and 1.0 for amoxycillin. The resolution between the amoxycillin and clavulanic acid peaks is not less than 3.5. The test is not valid unless the column efficiency determined from each analyte peak is not less than 550 theoretical plates, the tailing factor for each analyte peak is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the content of \( \text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_5\text{S} \) and \( \text{C}_8\text{H}_9\text{N}_3\text{O}_5 \) in the tablets.

1 mg of \( \text{C}_7\text{H}_5\text{Li}_3\text{N}_3\text{O}_5 \) is equivalent to 0.9711 mg of \( \text{C}_8\text{H}_9\text{N}_3\text{O}_5 \).

**Storage.** Store protected from moisture.

**Labelling.** The label includes the word “chewable” in juxtaposition to the official name in the case of Chewable Tablets. The label also indicates that Chewable Tablets may be chewed before being swallowed or may be swallowed whole. Tablets intended for veterinary use only are so labelled.

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**Amphotericin B**

Amphotericin B is a mixture consisting mainly of amphotericin B which is


and other antifungal polyenes produced by the growth of certain strains of *Streptomyces nodosus* or by any other means.

Amphotericin B has a potency of not less than 750 Units per mg, calculated on the dried basis.

**Description.** A yellow to orange powder; practically odourless. Even in the absence of light, it is gradually decomposed in a humid environment, degradation being faster at higher temperatures. In solutions, it is inactivated in the presence of light and at low pH values.
Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with amphotericin B RS or with the reference spectrum of amphotericin B.

B. Dissolve 25 mg in 5 ml of dimethyl sulphoxide, add sufficient methanol to produce 50 ml, and dilute 2 ml to 200 ml with methanol. When examined in the range 300 nm to 450 nm (2.4.7), the resulting solution shows absorption maxima at about 362 nm, 381 nm, and 405 nm. The ratio of the absorbance at the maximum at about 362 nm to the absorbance at the maximum at about 381 nm, 0.5 to 0.6; the ratio of the absorbance at the maximum at about 381 nm to the absorbance at the maximum at about 405 nm, about 0.9.

C. To 1 ml of a 0.05 per cent w/v solution in dimethyl sulfoxide add 5 ml of phosphoric acid to form a lower layer; a blue ring is immediately formed at the junction of the liquids. Mix; the mixture becomes intensely blue. Add 15 ml of water and mix; the solution becomes pale straw-coloured.

Tests

pH (2.4.24). 6.0 to 8.0, determined in a 3.0 per cent w/v suspension in water; for parenteral use, 3.5 to 6.0.

Tetraenes. Not more than 15.0 per cent (for parenteral use, not more than 10.0 per cent), determined by the following method. Weigh accurately about 50 mg, dissolve in 5 ml of dimethyl sulfoxide, dilute to 50.0 ml with methanol and dilute 4.0 ml of the resulting solution to 50.0 ml with methanol (solution 1). Prepare solution (2) in a similar manner using 50 mg of amphotericin B RS, accurately weighed, instead of the substance under examination. For solution (3) dissolve 25 mg of nystatin RS, accurately weighed, in 25 ml of dimethyl sulfoxide, dilute to 250.0 ml with methanol and dilute 4.0 ml to 50.0 ml with methanol. Using as the blank a 0.8 per cent v/v solution of dimethyl sulfoxide in methanol, measure the absorbances of solutions (1), (2) and (3) at the maxima at about 282 nm and about 304 nm (2.4.7).

Calculate the specific absorbances for the substance under examination, amphotericin B RS and nystatin RS at both wavelengths and calculate the content of tetraenes from the expression

$$\frac{25 W_N (A_{282} \times A_{304}) - (A_{304} \times A_{282})}{(A_{282} \times A_{304}) - (A_{304} \times A_{282})} W_U$$

where $W_N$ is the weight, in mg, of nystatin RS, $A_{282}$ and $A_{304}$ are the specific absorbances of amphotericin B RS at about 282 nm and 304 nm, respectively, $A_{282}$ and $A_{304}$ are the specific absorbances of nystatin RS at about 282 nm and 304 nm respectively, and $W_U$ is the weight in mg of the sample taken.

Sulphated ash (2.3.18). Not more than 3.0 per cent; for parenteral use, not more than 0.5 per cent.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 60°C at a pressure not exceeding 0.7 kPa.

Assay. Determine by the microbiological assay of antibiotics, Method A (2.2.10) on a solution prepared in the following manner. Weigh accurately about 60 mg, triturate with dimethylformamide and add, with shaking, sufficient dimethylformamide to produce 100.0 ml. Dilute 10.0 ml to 100.0 ml with dimethylformamide. Express the result in Units per mg.

Amphotericin B intended for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 1.0 Endotoxin Unit per mg, using the supernatant liquid obtained after shaking 50 mg with 25 ml of water BET and centrifuging.

Amphotericin B intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility, using 50 mg from each container.

Storage. Store protected from light in a refrigerator (2°C to 8°C). Do not freeze.

Labelling. The label states (1) the number of Units per mg; (2) whether the material is intended for use in the manufacture of parenteral preparations.

Amphotericin B Injection

Amphotericin B Injection is a sterile freeze dried mixture of Amphotericin B and deoxycholate sodium with one or more buffering agents. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate Matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case within the period recommended by the manufacturer.

Amphotericin B Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of amphotericin B, C_{57}H_{77}NO_{17}. 

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The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Tests

**pH** (2.4.24). 7.2 to 8.0 determined in a solution containing 50 mg per ml of Amphotericin B.

**Bacterial Endotoxins** (2.2.3). Not more than 5.0 Endotoxin unit per mg of amphotericin B. For products used or labelled for intrathecal injection, not more than 0.9 Endotoxin unit per mg.

**Loss on drying** (2.4.19). Not more than 8.0 per cent, determined on 0.1 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa.

**Assay**. Determine by the microbiological assay of antibiotics, Method A (2.2.10) on a solution prepared in the following manner.

Mix the contents of 10 containers, dissolve in *dimethylformamide*. Express the results in mg per vial, taking each 1000 units found to be equivalent to 1 mg of amphotericin B.

**Storage**. Store in tightly closed containers between 2° to 8°, protected from light.

**Labeling**. Label it to state that it is intended for use by intravenous infusion to hospitalised patients only, and that the solution should be protected from light during administration.

Ampicillin

\[
\begin{align*}
\text{C}_{16}\text{H}_{19}\text{N}_{3}\text{O}_{4}\text{S} & \quad \text{Mol. Wt. 349.4} \\
\text{Ampicillin is } (6R)-6-(\alpha\text{-phenyl-D-glycylamino})\text{penicillanic acid.} \\
\text{Ampicillin contains not less than 96.0 per cent and not more than 100.5 per cent of } C_{16}H_{19}N_{3}O_{4}S, \text{ calculated on the anhydrous basis.} \\
\text{Description} & \quad \text{A white, crystalline powder.} \\
\text{Identification} & \quad \text{A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ampicillin RS or with the reference spectrum of ampicillin.}
\end{align*}
\]

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

**Tests**

**Appearance of solution**. Dissolve 1.0 g in 10 ml of *1 M hydrochloric acid* and a further 1.0 g in a mixture of 3 ml of *dilute ammonia solution* and 7 ml of *water*. Both solutions when freshly prepared are not more opalescent than opalescence standard OS2 (2.4.1).

**pH** (2.4.24). 3.5 to 5.5, determined in a 0.25 per cent w/v solution.

**Specific optical rotation** (2.4.22). +280° to +305°, determined in a 0.25 per cent w/v solution.

**N, N-Dimethylaniline** (2.3.21). Not more than 20 ppm. determined by Method B.

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.5 per cent.

**Water** (2.3.43). Not more than 2.0 per cent, determined on 0.3 g.

**Assay**. Determine by liquid chromatography (2.4.14).

Solvent mixture. Mix 10 ml of *1 M monobasic potassium phosphate* and 1 ml of *1 M acetic acid* and dilute to 1000 ml with water.

**Test solution**. Transfer an accurately weighed quantity containing about 100 mg of ampicillin to a 100-ml volumetric flask, add about 80 ml of the solvent mixture, shake and mix with the aid of ultrasound if necessary to achieve complete dissolution and dilute to 100.0 ml with the solvent mixture. Use this solution promptly after preparation.

**Reference solution (a)**. Weigh accurately a suitable quantity of ampicillin RS, dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound to obtain a solution having a known concentration of about 1 mg per ml. Use this solution promptly after preparation.

**Reference solution (b)**. Dissolve caffeine in reference solution (a) to obtain a solution containing about 0.12 mg per ml.

Chromatographic system

- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 900 volumes of water, 80 volumes of acetonitrile, 10 volumes of 1 M monobasic potassium phosphate, and 1 ml of 1 M acetic acid,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.
Inject reference solution (b). The resolution between the caffeine and ampicillin peaks is not less than 2.0. The relative retention times are about 0.5 for ampicillin and 1.0 for caffeine.

Inject reference solution (a). The test is valid unless the capacity factor is not more than 2.5, the tailing factor is not more than 1.4 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and reference solution (a). Calculate the percentage content of \( \text{C}_{16}\text{H}_{19}\text{N}_{3}\text{O}_{4}\text{S} \).

**Storage.** Store protected from moisture at a temperature not exceeding 30°.

**Ampicillin Capsules**

Ampicillin Capsules contain Ampicillin or Ampicillin Trihydrate equivalent to not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of ampicillin, \( \text{C}_{16}\text{H}_{19}\text{N}_{3}\text{O}_{4}\text{S} \).

**Identification**

The contents of the capsules comply with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ampicillin RS* or with the reference spectrum of ampicillin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

**Tests**

**Dissolution** (2.5.2).

Apparatus. No 1

Medium. 900 ml of *water*.

Speed and time. 100 rpm and 45 minutes.

Use one capsule in the vessel for each test.

Withdraw a suitable volume of the medium and filter promptly. Dilute the filtrate, if necessary, with the same solvent. Measure the absorbance (2.4.7) of the resulting solution at the maximum at about 272 nm. Calculate the content of \( \text{C}_{16}\text{H}_{19}\text{N}_{3}\text{O}_{4}\text{S} \) in the medium from the absorbance obtained from a solution of known concentration of *ampicillin RS*.

D: Not less than 80 per cent of the stated amount of \( \text{C}_{16}\text{H}_{19}\text{N}_{3}\text{O}_{4}\text{S} \).

**Other tests.** Comply with the tests stated under Capsules.

**Assay.** Determine by liquid chromatography (2.4.14).

*Solvent mixture.* Mix 10 ml of 1 M monobasic potassium phosphate and 1 ml of 1 M acetic acid and dilute to 1000 ml with *water*.

**Test solution.** Weigh accurately a quantity of the mixed contents of 20 capsules containing about 100 mg of ampicillin, add about 80 ml of the solvent mixture and dissolve by shaking for 15 minutes and mixing if necessary, with the aid of ultrasound. Dilute to 100.0 ml with the solvent mixture and filter. Use this solution promptly after preparation.

**Reference solution (a).** Weigh accurately a suitable quantity of *ampicillin RS*, dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound to obtain a solution having a known concentration of about 1 mg per ml. Use this solution promptly after preparation.

**Reference solution (b).** Dissolve caffeine in reference solution (a) to obtain a solution containing about 0.12 mg per ml.

**Chromatographic system**

- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic micro particles (5 µm),
- mobile phase: a mixture of 900 volumes of *water*, 80 volumes of *acetonitrile*, 10 volumes of 1 M monobasic potassium phosphate, and 1 ml of 1 M acetic acid,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject reference solution (b). The resolution between the caffeine and ampicillin peaks is not less than 2.0. The relative retention times are about 0.5 for ampicillin and 1.0 for caffeine.

Inject reference solution (a). The capacity factor is not more than 2.5 and the tailing factor is not more than 1.4. The test is not valid unless the relative standard deviation for replicate injections is at most 2.0 per cent.

Inject alternately the test solution and reference solution (a). Calculate the content of \( \text{C}_{16}\text{H}_{19}\text{N}_{3}\text{O}_{4}\text{S} \) in the capsules.

**Storage.** Store protected from moisture at a temperature not exceeding 30°.

**Labelling.** The label states the strength in terms of the equivalent amount of ampicillin (when Ampicillin Trihydrate is used).

**Ampicillin Sodium**

\[
\text{C}_{16}\text{H}_{18}\text{N}_{3}\text{NaO}_{4}\text{S}, \quad \text{Mol. Wt. 371.4}
\]
Ampicillin Sodium is sodium (6R)-6-(α-phenyl-D-glycyl-amino)penicillinate

Ampicillin Sodium contains not less than 92.5 per cent and not more than 100.5 per cent of C\textsubscript{16}H\textsubscript{18}N\textsubscript{3}NaO\textsubscript{4}S, calculated on the anhydrous basis.

**Description.** A white, crystalline powder; hygroscopic.

**Identification.**
A. Determine by infrared absorption spectophotometry (2.4.6). Compare the spectrum with that obtained with ampicillin sodium RS or with the reference spectrum of ampicillin sodium.
B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).
C. A 5 per cent w/v solution gives the reactions of sodium salts (2.3.1).

**Tests.**

**Appearance of solution.** A 10.0 per cent w/v solution is clear, when examined immediately after preparation (2.4.1), and the absorbance of the solution at about 430 nm is not more than 0.15 (2.4.7).

**pH** (2.4.24). 8.0 to 10.0, determined 10 minutes after dissolution in a 10.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +258° to +287°, determined in a 0.25 per cent w/v solution of potassium hydrogen phthalate.

**N,N-Dimethylaniline** (2.3.21). Not more than 20 ppm, determined by Method B.

**Dichloromethane.** Not more than 0.2 per cent w/w, determined in the following manner.

Determine by gas chromatography (2.4.13).

*Internal standard solution.* A 0.02 per cent v/v solution of 1,2-dichloroethane in water.

**Test solution.** Dissolve 1.0 g of the substance under examination in sufficient water to produce 10 ml.

**Reference solution (a).** Dissolve 1.0 g of the substance under examination in 10 ml of the internal standard solution.

**Reference solution (b).** Mix equal volumes of the internal standard solution and a 0.02 per cent v/v solution of dichloromethane in water.

Chromatographic system
- a glass column 1.5 m x 5 mm, packed with acid-washed silanised diatomaceous support (100 to 120 mesh) coated with 10 per cent w/w of polyethylene glycol 1000,
- temperature: column, 60°,
- inlet port and detector, 150°,
- flow rate, 40 ml per minute of the carrier gas.

Calculate the percentage w/w of dichloromethane, assuming its relative density (2.4.29) to be 1.325 g.

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Water** (2.3.43). Not more than 2.0 per cent, determined on 0.3 g.

**Assay.** Determine by liquid chromatography (2.4.14).

*Solvent mixture.* Mix 10 ml of 1 M monobasic potassium phosphate and 1 ml of 1 M acetic acid and dilute to 1000 ml with water.

**Test solution.** Transfer an accurately weighed quantity containing about 100 mg of ampicillin to a 100-ml volumetric flask and dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound and dilute to 100.0 ml with the solvent mixture. Use this solution promptly after preparation.

**Reference solution (a).** Weigh accurately a suitable quantity of ampicillin RS, dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound to obtain a solution having a known concentration of about 1 mg per ml. Use this solution promptly after preparation.

**Reference solution (b).** Dissolve caffeine in reference solution (a) to obtain a solution containing about 0.12 mg per ml.

Chromatographic system
- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 900 volumes of water, 80 volumes of acetonitrile, 10 volumes of 1 M monobasic potassium phosphate, and 1 ml of 1 M acetic acid,
- flow rate, 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject reference solution (b). The resolution between the caffeine and ampicillin peaks is not less than 2.0. The relative retention times are about 0.5 for ampicillin and 1.0 for caffeine. Inject reference solution (a). The test is not valid unless the capacity factor is not more than 2.5, the tailing factor is not more than 1.4 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and reference solution (a). Calculate the percentage content of C\textsubscript{16}H\textsubscript{19}N\textsubscript{3}O\textsubscript{4}S.

*Ampicillin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*
Bacterial endotoxins (2.2.3). Not more than 0.15 Endotoxin Unit per mg.

*Amipicillin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate sterilization procedure complies with the following additional requirement.*

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture at a temperature not exceeding 30°. If it is intended for use in the manufacture of parenteral preparations, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

Labelling. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

**Ampicillin Injection**

Ampicillin Sodium Injection

Ampicillin Injection is a sterile material consisting of Ampicillin Sodium with or without buffering agents and other excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Ampicillin Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of ampicillin, C_{16}H_{19}N_{3}O_{4}S.

Description. A white or almost white powder; hygroscopic.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injections) and with the following requirements.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ampicillin sodium RS or with the reference spectrum of ampicillin sodium.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

C. A 5 per cent w/v solution gives the reactions of sodium salts (2.3.1).

**Tests**

Appearance of solution. A 10 per cent w/v solution is clear, when examined immediately after preparation (2.4.1), and the absorbance of the solution at about 430 nm is not more than 0.15.

pH (2.4.24). 8.0 to 10.0, determined 10 minutes after dissolution in a 10 per cent w/v solution.

Specific optical rotation (2.4.22). +258° to +287°, determined in a 0.25 per cent w/v solution in a 0.4 per cent w/v solution of potassium hydrogen phthalate.

N,N-Dimethylaniline (2.3.21). Not more than 20 ppm, determined by Method B.

Dichloromethane. Not more than 0.2 per cent w/w, determined in the following manner.

Determine by gas chromatography (2.4.13).

Internal standard solution. A 0.02 per cent v/v solution of 1,2-dichloroethane in water.

Test solution. Dissolve 1.0 g of the substance under examination in sufficient water to produce 10 ml.

Reference solution (a). Dissolve 1.0 g of the substance under examination in 10 ml of the internal standard solution.

Reference solution (b). Mix equal volumes of the internal standard solution and a 0.02 per cent v/v solution of dichloromethane in water.

Chromatographic system

– a glass column 1.5 m x 5 mm, packed with acid-washed silanised diatomaceous support (100 to 120 mesh) coated with 10 per cent w/w of polyethylene glycol 1000,
– temperature: column. 60°,
– inlet port and detector. 150°,
– flow rate. 40 ml per minute of the carrier gas.

Calculate the percentage w/w of dichloromethane, assuming its relative density (2.4.29) to be 1.325 g.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Bacterial endotoxins (2.2.3). Not more than 0.15 Endotoxin Unit per mg of ampicillin.

Sterility (2.2.11). Complies with the test for sterility.

Water (2.3.43). Not more than 2.0 per cent, determined on 0.3 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Mix 10 ml of 1 M monobasic potassium phosphate and 1 ml of 1 M acetic acid and dilute to 1000 ml with water.
**Test solution.** Determine the weight of the contents of 10 containers. Transfer an accurately weighed quantity of the mixed contents of the 10 containers containing about 100 mg of ampicillin to a 100-ml volumetric flask, add about 80 ml of the solvent mixture and dissolve by shaking and mixing if necessary, with the aid of ultrasound. Dilute to 100.0 ml with the solvent mixture and filter. Use this solution promptly after preparation.

**Reference solution (a).** Weigh accurately a suitable quantity of ampicillin RS, dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound to obtain a solution having a known concentration of about 1 mg per ml. Use this solution promptly after preparation.

**Reference solution (b).** Dissolve caffeine in reference solution (a) to obtain a solution containing about 0.12 mg per ml.

**Chromatographic system**
- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 900 volumes of water, 80 volumes of acetonitrile, 10 volumes of 1 M monobasic potassium phosphate, and 1 ml of 1 M acetic acid,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject reference solution (b). The resolution between the caffeine and ampicillin peaks is not less than 2.0. The relative retention times are about 0.5 for ampicillin and 1.0 for caffeine.

Inject reference solution (a). The test is not valid unless the capacity factor is not more than 2.5, the tailing factor is not more than 1.4 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and reference solution (a). Calculate the content of C₁₆H₁₉N₃O₄S in the injection.

**Storage.** Store protected from moisture, in a sterile, tamper-evident container sealed so as to exclude micro-organisms, at a temperature not exceeding 30°.

**Labelling.** The label states the quantity of Ampicillin Sodium contained in the sealed container in terms of the equivalent amount of anhydrous ampicillin.

**Ampicillin Oral Suspension**

Ampicillin Oral Suspension is a mixture consisting of Ampicillin or Ampicillin Trihydrate with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of Water just before issue.

Ampicillin oral suspension contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of ampicillin, C₁₆H₁₉N₃O₄S.

The constituted suspension, when stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use, contains not less than 80.0 per cent of the stated amount of ampicillin, C₁₆H₁₉N₃O₄S.

**Storage.** Store protected from moisture at a temperature not exceeding 30°.

**Identification**

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

The constituted suspension complies with the tests stated under Oral liquids and with the following tests.

**pH (2.4.24).** 4.0 to 7.0.

**Assay.** Determine by liquid chromatography (2.4.14).

**Solvent mixture.** Mix 10 ml of 1 M monobasic potassium phosphate and 1 ml of 1 M acetic acid and dilute to 1000 ml with water.

**Test solution.** Transfer an accurately weighed quantity containing about 100 mg of ampicillin to a 100-ml volumetric flask and dilute to 100.0 ml with the solvent mixture and filter. Use this solution promptly after preparation.

**Reference solution (a).** Weigh accurately a suitable quantity of ampicillin RS, dissolve in the solvent mixture by shaking and mixing with the aid of ultrasound if necessary, to obtain a solution having a known concentration of about 1 mg per ml. Use this solution promptly after preparation.

**Reference solution (b).** Dissolve caffeine in reference solution (a) to obtain a solution containing about 0.12 mg per ml.

**Chromatographic system**
- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 900 volumes of water, 80 volumes of acetonitrile, 10 volumes of 1 M monobasic potassium phosphate, and 1 ml of 1 M acetic acid,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject reference solution (b). The resolution between the caffeine and ampicillin peaks is not less than 2.0. The relative retention times are about 0.5 for ampicillin and 1.0 for caffeine.
Inject reference solution (a). The test is not valid unless the capacity factor is not more than 2.5, the tailing factor is not more than 1.4 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and reference solution (a).

Determine the weight per ml (2.4.29) of the suspension and calculate the content of C₁₆H₁₉N₃O₄S, weight in volume.

Repeat the procedure using a portion of the constituted suspension that has been stored at the temperature and for the period stated on the label.

**Labelling.** The label states (1) the quantity of active ingredient in terms of the equivalent amount of ampicillin when the active ingredient is Ampicillin Trihydrate; (b) the temperature of storage and the period during which the constituted suspension may be expected to be satisfactory for use.

---

**Ampicillin Dispersible Tablets**

Dispersible Ampicillin Tablets

Ampicillin Dispersible Tablets contain Ampicillin or Ampicillin Trihydrate in a suitable dispersible base.

Ampicillin Dispersible Tablets contain Ampicillin or Ampicillin Trihydrate equivalent to not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of ampicillin, C₁₆H₁₉N₃O₄S.

**Identification**

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

**Tests**

**Uniformity of dispersion.** Place 2 tablets in 100 ml of water and stir until completely dispersed. A smooth dispersion is produced, which passes through a sieve screen with a nominal mesh aperture of 710 µm.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets.

Determine by liquid chromatography (2.4.14).

**Solvent mixture.** Mix 10 ml of 1 M monobasic potassium phosphate and 1 ml of 1 M acetic acid and dilute to 1000 ml with water.

**Test solution.** Transfer an accurately weighed quantity of the powdered tablets containing about 100 mg of ampicillin to a 100-ml volumetric flask, add about 80 ml of the solvent mixture, shake for 15 minutes and mix with the aid of ultrasound to achieve complete dissolution. Dilute to 100.0 ml with the solvent mixture and filter. Use this solution promptly after preparation.

**Reference solution (a).** Weigh accurately a suitable quantity of ampicillin RS, dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound to obtain a solution having a known concentration of about 1 mg per ml. Use this solution promptly after preparation.

**Reference solution (b).** Dissolve caffeine in reference solution (a) to obtain a solution containing about 0.12 mg per ml.

**Chromatographic system**

- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 900 volumes of water, 80 volumes of acetonitrile, 10 volumes of 1 M monobasic potassium phosphate, and 1 ml of 1 M acetic acid,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject reference solution (b). The resolution between the caffeine and ampicillin peaks is not less than 2.0. The relative retention times are about 0.5 for ampicillin and 1.0 for caffeine.

Inject reference solution (a). The test is not valid unless the capacity factor is not more than 2.5, the tailing factor is not more than 1.4 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and reference solution (a). Calculate the percentage content of C₁₆H₁₉N₃O₄S in the tablets.

**Storage.** Store protected from moisture at a temperature not exceeding 30°.

**Labelling.** The label states (1) the strength in terms of the equivalent amount of ampicillin (when Ampicillin Trihydrate is used); (2) that the tablets should be dispersed in water immediately before use.

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**Ampicillin Trihydrate**

\[
\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_4\text{S},3\text{H}_2\text{O}
\]

Mol. Wt. 403.5

Ampicillin Trihydrate is \((6R)-6-(\alpha\text{-phenyl}-\text{D}-\text{glycyl}-\text{amino})\text{penicillanic acid trihydrate.}\)
Ampicillin Trihydrate contains not less than 96.0 per cent and not more than 100.5 per cent of \(\text{C}_{16}\text{H}_{19}\text{N}_{3}\text{O}_{4}\text{S}\), calculated on the anhydrous basis.

**Description.** A white, crystalline powder.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ampicillin trihydrate RS* or with the reference spectrum of ampicillin trihydrate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

**Tests**

**Appearance of solution.** Dissolve 1.0 g in 10 ml of \(1 \text{ M hydrochloric acid}\) and a further 1.0 g in a mixture of 3 ml of \(\text{dilute ammonia solution}\) and 7 ml of \(\text{water}\). Both solutions when freshly prepared are not more opalescent than opalescence standard OS2 (2.4.1).

**pH** (2.4.24). 3.5 to 5.5, determined in a 0.25 per cent w/v solution.

**Specific optical rotation** (2.4.22). +280° to +305°, determined in a 0.25 per cent w/v solution.

**N,N-Dimethylaniline** (2.3.21). Not more than 20 ppm, determined by Method B.

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.5 per cent.

**Water** (2.3.43). 12.0 per cent to 15.0 per cent, determined on 0.1 g.

**Assay.** Determine by liquid chromatography (2.4.14).

Solvent mixture. Mix 10 ml of \(1 \text{ M monobasic potassium phosphate}\) and 1 ml of \(1 \text{ M acetic acid}\) and dilute to 1000 ml with \(\text{water}\).

Test solution. Transfer an accurately weighed quantity containing about 100 mg of ampicillin to a 100-ml volumetric flask and dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound and dilute to 100.0 ml with the solvent mixture. Use this solution promptly after preparation.

Reference solution (a). Weigh accurately a suitable quantity of *ampicillin RS*, dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound to obtain a solution having a known concentration of about 1 mg per ml. Use this solution promptly after preparation.

Reference solution (b). Dissolve caffeine in reference solution (a) to obtain a solution containing about 0.12 mg per ml.

Chromatographic system

- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 900 volumes of \(\text{water}\), 80 volumes of \(\text{acetonitrile}\), 10 volumes of \(1 \text{ M monobasic potassium phosphate}\), and 1 ml of \(1 \text{ M acetic acid}\),
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject reference solution (b). The resolution between the caffeine and ampicillin peaks is not less than 2.0. The relative retention times are about 0.5 for ampicillin and 1.0 for caffeine.

Inject reference solution (a). The test is not valid unless the capacity factor is not more than 2.5, the tailing factor is not more than 1.4 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and reference solution (a). Calculate the percentage content of \(\text{C}_{16}\text{H}_{19}\text{N}_{3}\text{O}_{4}\text{S}\).

**Storage.** Store at a temperature not exceeding 30°.

**Alpha Amylase**

**Diastase**

Alpha Amylase is an amylolytic enzyme or a mixture of enzymes obtained from fungi such as *Aspergillus oryzae* or from a non-pathogenic variant of bacteria such as *Bacillus subtilis* and with the specific activity for converting starch into dextrin and maltose. It may contain suitable harmless diluents such as Lactose or Dibasic Calcium Phosphate.

Alpha Amylase has amylase activity of not less than 800 Units which represents the number of grams of dry, soluble maize or corn starch digested by 1.0 g of Alpha Amylase under the conditions of the Assay.

**Description.** A cream to light brown-coloured powder; almost odourless or with faint characteristic odour; hygroscopic.

**Tests**

**Loss on drying** (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 105° for 1 hour.

**Assay.** Weigh accurately a quantity containing 100 Units of amylase activity and triturate with 200 ml of \(\text{buffer solution pH 6.0 (for bacterial amylase)}\) or of \(\text{acetate buffer pH 5.0 (for fungal amylase)}\) and add sufficient \(\text{buffer solution pH 6.0 or acetate buffer pH 5.0, as appropriate, to produce 1000.0 ml}\). Dilute 10.0 ml to 100.0 ml with \(\text{buffer solution pH 6.0 or acetate buffer pH 5.0, as appropriate, to give the test solution; filter if necessary (1 ml of the test solution should be capable of}
digesting about 10 mg of dry soluble maize or corn starch. Into each of six stoppered test-tubes add 5.0 ml of starch substrate without touching the sides of the test-tube. Place the test-tubes in a water-bath maintained at 40° ± 0.1°. When the temperature of the solution in the tubes has reached 40°, add 0.35 ml, 0.4 ml, 0.45 ml, 0.5 ml, 0.55 ml and 0.6 ml of the test solution to each of the test-tubes marked 1 to 6 respectively and record the time of addition. Mix thoroughly and replace the tubes in the water-bath. After exactly 60 minutes remove the tubes and cool rapidly in cold water. Add to each tube 0.05 ml of 0.02 M iodine and mix well. Note the tube containing the lowest volume of test solution that does not show a bluish or violet tinge (if there is doubt, warm the solution slightly, when the colour distinction is prominent). From this volume calculate the number of grams of dry soluble maize or corn starch digested by 1.0 g of the substance under examination. This represents the number of Units of amylase activity per g.

**Tests**

**Appearance of solution.** A 5.0 per cent w/v solution in water is clear (2.4.1).

**Acidity or alkalinity.** Dissolve 0.1 g in 10 ml of freshly boiled and cooled water and add a few drops of bromothymol blue solution. Not more than 0.05 ml of 0.01 M hydrochloric acid or 0.01 M sodium hydroxide is required to change the colour of the solution.

**Aminoantipyrine.** Wet about 0.2 g with a few drops of water in a test-tube and add 3 ml of ethanol (95 per cent); shake until dissolved and add successively with shaking, 2 drops of dilute ammonia solution, 5 drops of potassium ferricyanide solution, 2 drops of liquified phenol and 5 ml of water. The solution acquires a green colour gradually but not an orange or pink colour.

**Arsenic** (2.3.10). To 1.0 g in a long-necked, round-bottomed flask add 5 ml of sulphuric acid and bring to the boil gently and continue boiling on a low flame until the solution acquires a light brown colour. Cool, add dropwise about 5 ml of hydrogen peroxide solution (100 vol) and heat gently until the solution just boils and continue the heating until the solution becomes colourless. Cool and add cautiously about 20 ml of water and mix. The resulting solution complies with the limit test for arsenic, but using 0.1 ml of arsenic standard solution (10 ppm As) and dipping the stained mercuric chloride papers from the test and standard solutions in a 10 per cent w/v solution of potassium iodide before comparison of the stains (1 ppm).

**Heavy metals** (2.3.13). Ignite 1.0 g until completely ashed. Dissolve the residue in a mixture of 23 ml of water and 2 ml of dilute acetic acid. The solution complies with the limit test for heavy metals, Method A (20 ppm).

**Loss on drying** (2.4.19). Not more than 5.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.4 g, dissolve in a mixture of 40 ml of ethanol (95 per cent) and 10 ml of 0.01 M hydrochloric acid and titrate with 0.05 M iodine until a yellow colour stable for 30 seconds is produced. 1 ml of 0.05 M iodine is equivalent to 0.01667 g of C_{13}H_{16}N_{3}NaO_{4}S.

**Storage.** Store protected from light.

**Analgin Tablets**

**Metamizol Tablets**

Analgin Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of analgin, C_{13}H_{16}N_{3}NaO_{4}S.
Identification

Powder a few tablets and shake a quantity of the powder containing about 0.5 g of Analgin with 10 ml of water and filter. The filtrate complies with the following tests.

A. To 2 ml of the filtrate, add 5 ml of ethanol (95 per cent) and 0.5 ml of dilute hydrochloric acid. To the solution add 5 ml of potassium iodate solution; a crimson colour is produced which deepens on further addition of potassium iodate solution.

B. Heat 4 ml of the filtrate with 2 ml of dilute hydrochloric acid; the characteristic odour of sulphur dioxide is produced followed by that of formaldehyde.

Tests

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.5 g of Analgin and transfer to a 50-ml volumetric flask. Add 10 ml of water and shake for 1 minute. Dilute to volume with ethanol (95 per cent), shake well and filter. Titrate 25.0 ml of the filtrate with 0.05 M iodine until a yellow colour stable for 30 seconds is produced.

1 ml of 0.05 M iodine is equivalent to 0.01757 g of C_{13}H_{16}N_{3}NaO_{4}S\cdot H_{2}O.

Storage. Store protected from light and moisture.

Anticoagulant Citrate Dextrose Solution

ACD Solution

Anticoagulant Citrate Dextrose Solution is a sterile solution of Sodium Citrate, Citric Acid and Dextrose in Water for Injections.

Anticoagulant Citrate Dextrose Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of Sodium Citrate, C_{6}H_{5}Na_{3}O_{7}\cdot 2H_{2}O, Citric Acid monohydrate, C_{6}H_{8}O_{7}\cdot H_{2}O (or Anhydrous Citric Acid, C_{6}H_{8}O_{7}), and Dextrose, C_{6}H_{12}O_{6}\cdot H_{2}O. It contains no antimicrobial agent. It is usually of two strengths as indicated below.

<table>
<thead>
<tr>
<th></th>
<th>Solution A</th>
<th>Solution B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Citrate</td>
<td>2.20 g</td>
<td>1.32 g</td>
</tr>
<tr>
<td>Citric Acid (Anhydrous)</td>
<td>0.73 g</td>
<td>0.44 g</td>
</tr>
<tr>
<td>or Citric Acid (Monohydrate)</td>
<td>0.80 g</td>
<td>0.48 g</td>
</tr>
<tr>
<td>Dextrose (Monohydrate)</td>
<td>2.45 g</td>
<td>1.47 g</td>
</tr>
<tr>
<td>Water for Injection</td>
<td>100 ml</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

NOTE — 15 ml of solution A or 25 ml of solution B are to be used for 100 ml of whole blood.

Description. A clear, colourless or faintly straw-coloured liquid; odourless.

Identification

A. To 1 ml add 0.05 ml of potassium cupri-tartrate solution; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. Gives the reactions of sodium salts (2.3.1).

C. To 2 ml (for Solution A) add 3 ml of water or to 4 ml (for Solution B) add 1 ml of water. The resulting solution gives reaction A of citrates (2.3.1).

Tests

pH (2.4.24). 4.5 to 5.5.

Bacterial endotoxins (2.2.3). Not more than 5.56 Endotoxin Units per ml.

Sterility (2.2.11). Complies with the test for sterility.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. For sodium citrate — Pipette 50.0 ml into a beaker and titrate with 1.3 M hydrochloric acid to a pH of 1.98 ± 0.02, determining the end-point potentiometrically (2.4.25). Carry out a blank titration with 50 ml of water.

1 ml of 1.3 M hydrochloric acid is equivalent to 0.1274 g of C_{6}H_{5}Na_{3}O_{7}\cdot 2H_{2}O.

For free citric acid — Pipette 20.0 ml into a conical flask and titrate with 0.1 M sodium hydroxide using phenolphthalein solution as indicator.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.006404 g of C_{6}H_{8}O_{7} or 0.007005 g of C_{6}H_{8}O_{7}\cdot H_{2}O.

For dextrose — Determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation multiplied by 1.0425, represents the weight of C_{6}H_{12}O_{6}\cdot H_{2}O in 100 ml of the solution.

Storage. Store protected from light in a single dose, tamper-evident container of colourless, transparent glass or of a suitable plastic material.

Labelling. The label states (1) whether the contents are Solution A or Solution B; (2) volume of the solution required per 100 ml of whole blood or the volume of the solution required per volume of whole blood to be collected; (3) where applicable, the maximum amount of blood to be collected in the container.
Anticoagulant Citrate Phosphate Dextrose Solution

CPD Solution

Anticoagulant Citrate Phosphate Dextrose Solution is a sterile solution of Sodium Citrate, Citric Acid, Sodium Dihydrogen Phosphate Dihydrate and Dextrose in Water for Injection.

Anticoagulant Citrate Phosphate Dextrose Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of Sodium Citrate, C₆H₅Na₃O₇·2H₂O, Citric Acid, C₆H₈O₇·H₂O, Sodium Dihydrogen Phosphate Dihydrate, NaH₂PO₄·2H₂O and Dextrose, C₆H₁₂O₆·H₂O. It contains no antimicrobial agent. It usually has the following composition:

- Sodium Citrate: 2.630 g
- Citric Acid (Monohydrate): 0.327 g
- Dextrose (Monohydrate): 2.550 g
- Sodium Dihydrogen Phosphate (Dihydrate): 0.251 g
- Water for Injection: 100 ml

NOTE – 14 ml are to be used for 100 ml of whole blood.

Description. A clear, colourless or faintly straw-coloured liquid; odourless.

Identification

A. To 1 ml add 0.05 ml of potassium cupri-tartrate solution; the solution remains blue and clear. Heat to boiling, a copious precipitate is formed.

B. Gives the reactions of sodium salts and reaction B of phosphates (2.3.1).

C. To 2 ml add 3 ml of water. The resulting solution gives reaction A of citrates (2.3.1).

Tests

pH (2.4.24). 5.0 to 6.0.

Bacterial endotoxins (2.2.3). Not more than 5.56 Endotoxin Units per ml.

Sterility (2.2.11). Complies with the test for sterility.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. For sodium citrate — Dilute 25.0 ml to 100.0 ml with water and mix. Dilute 5.0 ml of the resulting solution to 100.0 ml with water and mix. Transfer 1.0 ml of this solution to a test-tube, add 1.3 ml of pyridine, swirl to mix, add 5.7 ml of acetic anhydride, mix and immediately place in a water-bath at 31° ± 0.5°. Allow the colour to develop for 35 minutes and measure the absorbance of the resulting solution at about 425 nm (2.4.7) using as the blank solution 1 ml of water treated in the same manner. Prepare a calibration curve by measuring the absorbance of solutions prepared by treating in the same manner 1 ml quantities of suitable dilutions of a solution in water containing 2.5 mg per ml of C₆H₈O₇, prepared by using anhydrous citric acid, previously dried for 3 hours at 90°. Calculate the total citrate content, as C₆H₈O₇, in mg per ml of the solution under examination from the expression 0.2 C, where C is the concentration in µg per ml of C₆H₈O₇, read from the curve.

Calculate the quantity, in mg, of C₆H₅Na₃O₇·2H₂O in 1 ml of the solution under examination from the expression 1.53 (A – B), where A is the concentration in mg per ml of total citrate as C₆H₅O₇, and B is the concentration in mg per ml of free citric acid in the solution.

For free citric acid — Pipette 20.0 ml into a conical flask and titrate with 0.1 M sodium hydroxide using phenolphthalein solution as indicator.

From the volume of 0.1 M sodium hydroxide required subtract a volume, in ml, equal to 1.28 times the number of mg of NaH₂PO₄·2H₂O present, as determined in the Assay for sodium acid phosphate.

1 ml of the remainder is equivalent to 0.007005 g of C₆H₅O₇·H₂O.

For sodium dihydrogen phosphate dihydrate — Dilute 5.0 ml to 100.0 ml with water. Transfer 5.0 ml to a 25-ml graduated flask and add 10.0 ml of a 2.8 per cent w/v solution of sulphuric acid followed by 2.0 ml of a 2.5 per cent w/v solution of ammonium molybdate, mixing after each addition. Add 1.0 ml of aminohydroxynaphthalenesulphonic acid solution and sufficient water to produce 25.0 ml, mix and keep aside at 25° for 10 minutes. Measure the absorbance (A₁) of the resulting solution at the maximum at about 660 nm (2.4.7) using as the blank 5 ml of water treated in the same manner. Calculate the content of NaH₂PO₄·2H₂O in each ml of the solution under examination from the absorbance (A₂) obtained by simultaneously carrying out the operation using 5.0 ml of a solution of potassium dihydrogen phosphate containing 0.11 mg of KH₂PO₄ per ml (C) and from the expression

\[ 22.92 C \left( A_1/A_2 \right). \]

For dextrose — Weigh a clean, medium-porosity sintered-glass crucible containing a few glass beads. To 50 ml of potassium cupri-tartrate solution add the glass beads from the weighed crucible, 45 ml of water and 5.0 ml of the solution under examination. Heat the solution at such a rate that it begins to boil in 3.5 to 4 minutes, boil the solution for exactly 2 minutes and filter immediately through the weighed crucible, taking care to transfer all the glass beads to the crucible, along with the precipitate. Wash the precipitate with hot water and then with 10 ml of ethanol (95 per cent) and dry it to constant weight at 110°. Carry out a blank determination.
1 mg of the precipitate is equivalent to 0.000496 g of C₆H₁₂O₆·H₂O.

**Storage.** Store in a single dose, tamper-evident container of colourless, transparent glass or of a suitable plastic material, protected from light.

**Labelling.** The label states (1) the composition and volume of the solution; (2) volume of the solution required per 100 ml of whole blood or the volume of the solution required per volume of whole blood to be collected; (3) where applicable, the maximum amount of blood to be collected in the container.

### Anticoagulant Citrate Phosphate Dextrose Adenine Solution

**CPDA Solution**

Anticoagulant Citrate Phosphate Dextrose Adenine Solution is a sterile solution of Citric Acid, Sodium Citrate, Sodium Dihydrogen Phosphate Dihydrate, Dextrose and Adenine in Water for Injection. Anticoagulant Citrate Phosphate Dextrose Adenine Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of total Sodium, Na, total Citrate, C₆H₈O₇, Sodium Dihydrogen Phosphate Dihydrate, NaH₂PO₄·2H₂O, Adenine, C₅H₅N₅ and Dextrose Monohydrate, C₆H₁₂O₆·H₂O. It contains no antimicrobial agent. It usually has the following composition:

- Citric Acid (Anhydrous) 0.2990 g
- Sodium Citrate (Dihydrate) 2.6300 g
- Sodium Dihydrogen Phosphate (Dihydrate) 0.2510 g
- Adenine 0.0275 g
- Dextrose (Monohydrate) 3.1900 g
- Water for Injection to 100 ml

**NOTE — 14 ml are to be used for 100 ml of whole blood.**

**Description.** A clear, colourless or faintly straw-coloured liquid; odourless.

**Identification**

- A. To 1 ml add 0.05 ml of potassium cupri-tartrate solution; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.
- B. Gives the reaction B of phosphates and the reactions of sodium salts (2.3.1).
- C. To 2 ml add 3 ml of water. The resulting solution gives reaction A of citrates (2.3.1).

D. In the test for adenine in the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (c).

**Tests**

- **pH** (2.4.24). 5.0 to 6.0.
- **Bacterial endotoxins** (2.2.3). Not more than 5.56 Endotoxin Units per ml.
- **Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** *For total sodium* — Dilute suitably with water and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.3), measuring at 589 nm and using sodium solution FP or sodium solution AAS respectively, suitably diluted with water for the standard solutions.

*For total citrate* — Dilute 5.0 ml of the solution under examination to 1000.0 ml with water and mix. Transfer 1.0 ml of this solution to a test-tube, add 1.3 ml of pyridine, swirl to mix, add 5.7 ml of acetic anhydride, mix and immediately place in a water-bath at 31° ± 1°. Allow the colour to develop for 33 ± 1 minutes and measure the absorbance of the resulting solution at about 425 nm (2.4.7), using as the blank 1 ml of water treated in the same manner. Prepare a calibration curve by measuring the absorbance of the solutions prepared by treating in the same manner 1 ml quantities of suitable dilutions of a solution in water containing 1.0 mg per ml of C₆H₈O₇, prepared by using anhydrous citric acid, previously dried for 3 hours at 90°. Calculate the total citrate content, as C₆H₈O₇, in mg per ml of the solution under examination from the expression 0.2 C, where C is the concentration in µg per ml of C₆H₈O₇, read from the curve.

*For sodium dihydrogen phosphate dihydrate* — Dilute 5.0 ml to 100.0 ml with water. Transfer 5.0 ml of this solution to a 25-ml volumetric flask and add 10.0 ml of a 2.8 per cent w/v solution of sulphuric acid followed by 2.0 ml of a 2.5 per cent w/v solution of ammonium molybdate, mixing after each addition. Add 1.0 ml of aminohydroxynaphthalenesulphonic acid solution and sufficient water to produce 25.0 ml. Mix and keep aside at 25° for 10 minutes. Measure the absorbance (A1) of the resulting solution at about 660 nm (2.4.7), using as the blank 5 ml of water treated in the same manner. Calculate the content of NaH₂PO₄·2H₂O in each ml of the solution under examination from the absorbance (A2) obtained by simultaneously carrying out the operation using 5.0 ml of a solution of potassium dihydrogen phosphate containing 0.11 mg of KH₂PO₄ per ml (C) using the expression

\[ 25 \times A1 / A2 \times C \]
For adenine — Determine by liquid chromatography (2.4.14).

Test solution. Substance under examination.

Reference solutions (a), (b) and (c) are prepared by dissolving accurately weighed quantities of adenine RS in dilute hydrochloric acid in three separate volumetric flasks, diluting with the same solvent to volume and mixing to obtain reference solutions having known concentrations of about 0.25 mg, 0.275 mg and 0.30 mg of adenine per ml respectively.

Reference solution (d). A solution containing 0.0275 per cent w/v each of adenine RS and purine in dilute hydrochloric acid.

Chromatographic system
- a stainless steel column 30 cm x 4 mm, packed with irregular or spherical, totally porous silica gel (10 µm) having a chemically bonded strongly acidic cation-exchange coating,
- mobile phase: dissolve 3.45 g of ammonium dihydrogen phosphate in 950 ml of water in a 1000-ml volumetric flask, add 10 ml of glacial acetic acid, dilute to volume with water and mix,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject solution (d) at least four times and record the chromatograms. The test is not valid unless the relative standard deviation of the peak response of adenine is not more than 2.5 per cent, the relative standard deviation of the retention time of adenine is not more than 2.0 per cent and the resolution factor of adenine and purine is not less than 3.0.

Inject separately the test solution and reference solutions (a), (b) and (c). Record the chromatograms and measure the responses for the major peaks. Plot the responses against the concentrations in mg of adenine per ml of reference solutions (a), (b) and (c).

Calculate the quantity, in mg, of C₅H₇N₅ in each ml of the solution under examination as the value read directly from the standard curve corresponding to the response obtained with the test solution.

For dextrose — Weigh a clean, medium porosity sintered-glass crucible containing a few glass beads. To 50 ml of potassium cupri-tartrate solution add the glass beads from the weighed crucible, 45 ml of water and 5.0 ml of the solution under examination. Heat the solution at such a rate that it begins to boil in 3.5 to 4 minutes, boil the solution for exactly 2 minutes and filter immediately through the weighed crucible, taking care to transfer all the glass beads with the precipitate to the crucible. Wash the precipitate with hot water and then with 10 ml of ethanol (95 per cent) and dry it to constant weight at 110°. Carry out a blank determination.

1 mg of the precipitate is equivalent to 0.000496 g of C₆H₁₂O₆.

Storage. Store protected from light, in single dose, tamper-evident containers made of a suitable plastic material in a cool place.

Labelling. The label states (1) the composition and volume of the solution; (2) volume of the solution required per 100 ml of whole blood or the volume of the solution required per volume of whole blood to be collected; (3) where applicable, the maximum amount of blood to be collected in the container.

Arteether

C₁₇H₂₈O₅ Mol. Wt. 312.4

Arteether is dihydroartemisinin ethyl ether.

Arteether contains β-isomer not less than 25.0 per cent and not more than 35.0 per cent and β-isomer not less than 65.0 per cent and not more than 75.0 per cent and total arteether is not less than 95.0 per cent and not more than 105.0 per cent of C₁₇H₂₈O₅, calculated on the dried basis.

Description. A light yellow coloured semi-solid, lipophylic powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with arteether RS or with the reference spectrum of arteether.

B. In the Assay, the principal peak in the chromatogram obtained with test solution corresponds to the peak obtained in the chromatogram with reference solution.

C. Determine by thin layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 10 volumes of ethyl acetate and 90 volumes of hexane.

Test solution. Dissolve 20 mg of the substance under examination in 10 ml of ethyl acetate.

Reference solution. A solution containing 0.2 per cent w/v each of α-artether RS and β-artether RS in ethyl acetate.
Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 8 cm. Dry the plate in air, spray with a mixture of 50 volumes of glacial acetic acid, 1 volume of sulphuric acid and 0.5 volume of anisaldehyde and heat at 100° for 15 minutes. The principal spots in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution.

**Tests**

**Appearance of solution.** A 40.0 per cent w/v solution in methanol is clear (2.4.1).

**Specific optical rotation** (2.4.22). +92.9° to +93.7°, determined in a 1.8 per cent w/v solution in chloroform.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 100 mg of the substance under examination in 100 ml of acetonitrile.

**Reference solution (a).** A solution containing 0.1 per cent w/v each of Ï„–arteether RS and Ï–arteether RS in acetonitrile.

**Reference solution (b).** Dilute 1 ml of reference solution (a) to 100 ml with acetonitrile.

Chromatographic system as described under Assay.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0 for both component.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peaks in the chromatogram obtained with the reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peaks in the chromatogram obtained with the reference solution (b) (2.0 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 4 per cent, determined on 1.0 g by drying in an oven at 80°.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 100 mg of the substance under examination in 100 ml of acetonitrile.

**Reference solution.** A solution containing 0.1 per cent w/v each of Ï„–arteether RS and Ï–arteether RS in acetonitrile.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octadecysilane bonded to porous silica (5 μm),
- mobile phase: a mixture of 65 volumes of acetonitrile, 32 volumes of water and 3 volumes of 1,4-dioxane
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 235 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5 for both component. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

**Storage.** Store protected from light and moisture.

**Artemether**

C_{16}H_{26}O_{5} \hspace{1cm} \text{Mol. Wt. 298.4}

Arteether is dihydroartemisinin methyl ether.

Artemether contains not less than 98.0 per cent and not more than 102.0 per cent of C_{16}H_{26}O_{5}, calculated on the dried basis.

**Description.** A white crystalline, odourless, slightly bitter, lipophylic substance.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with artemether RS or with the reference spectrum of arteether.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. Determine by thin layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 10 volumes of ethyl acetate and 90 volumes of hexane.

**Test solution.** Dissolve 20 mg of the substance under examination in 10 ml of ethyl acetate.

**Reference solution.** A 0.2 per cent w/v solution of artemether RS in ethyl acetate.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 8 cm. Dry the plate in air, spray with a mixture of
50 volumes of glacial acetic acid, 1 volume of sulphuric acid and 0.5 volume of anisaldehyde and heat at 100° for 15 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution.

D. Dissolve 5 mg in 1 ml of ethanol anhydrous and add 20 mg of potassium iodide. Heat the mixture on a water-bath. A yellow colour is produced.

E. Dissolve 5 mg in 1 ml of ethanol anhydrous. Add a few drops on a white porcelain dish and add 1 drop of vanillin sulphuric acid TS. A pink colour is produced.

Tests

Appearance of solution. Dissolve 0.5 g in 0.5 ml of methanol, this solution is clear (2.4.1) and colourless (2.4.1).

Specific optical rotation (2.4.22). +159.3° to +160.2° at 34°, determined in a 1.0 per cent w/v solution in chloroform.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in 100 ml of acetonitrile.

Reference solution (a). A 0.1 per cent w/v solution of artemether RS in acetonitrile.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with acetonitrile.

Chromatographic system as described under Assay.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with the reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over phosphorous pentaoxide under vacuum at 2.67 kPa.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in 100.0 ml in acetonitrile.

Reference solution. A 0.1 per cent w/v solution of artemether RS in acetonitrile.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of acetonitrile, 32 volumes of water and 3 volumes of 1,4-dioxane,
- flow rate 1.2 ml per minute,
- spectrophotometer set at 235 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of C_{15}H_{22}O_{5}.

Storage. Store protected from light and moisture.

Artemisinin

C_{15}H_{22}O_{5} \quad \text{Mol.Wt. 282.3}

Artemesine is (3R,5aS,6R,8aS,12S,12aR)-octahydro-3,6,9-trimethyl-3,12-epoxy-12H-pyran[4,3-\gamma]-1,2-benzodioxepin-10(3H)-one.

Artemisinin contains not less than 98.0 per cent and not more than 103.0 per cent of artemisinin, C_{15}H_{22}O_{5} calculated on the dried basis.

Description. A white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with artemisinin RS or with the reference spectrum of artemisinin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. Determine by thin layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 15 volumes of ethyl acetate and 85 volumes of hexane.

Test solution. Dissolve 2 mg of the substance under examination in 1 ml of ethyl acetate.
Reference solution. A 0.2 per cent w/v solution of artemisinin RS in ethyl acetate.

Apply to the plate 5 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air, spray with a mixture of 50 volumes of glacial acetic acid, 1 volume of sulphuric acid and 0.5 volume of anisaldehyde and heat at 100° for 15 minutes. The chromatogram obtained with test solution shows pink band corresponding to the band in the chromatogram obtained with reference solution.

Tests

Appearance of solution. Dissolve 0.5 g in 0.5 ml of chloroform, the solution is clear (2.4.1) and colourless (2.4.1).

Specific optical rotation (2.4.22). +64.7° to +65.4°, determined in a 1.0 per cent w/v solution in chloroform.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in 100 ml of acetonitrile.

Reference solution (a). A 0.1 per cent w/v solution of artemisinin RS in acetonitrile.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with acetonitrile.

Chromatographic system as described in the Assay.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with the reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent w/w.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 80°.

Assay. Determine liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in 100 ml of acetonitrile.

Reference solution. A 0.1 per cent w/v solution of artemisinin RS in acetonitrile.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of acetonitrile, 32 volumes of water and 3 volumes of 1,4-dioxane,
- flow rate 1.2 ml per minute,
- spectrophotometer set at 235 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution. Calculate the content of C15H22O5.

Storage. Store protected from light and moisture.

Ascorbic Acid

Vitamin C; L-Ascorbic Acid

\[
\text{CH}_2\text{OH} \\
\text{HO OH} \\
\text{COH} \\
\text{O} \\
\text{CH}_2\text{OH} \\
\text{H} \\
\text{C}_6\text{H}_8\text{O}_6 \text{ Mol. Wt. 176.1}
\]

Ascorbic Acid is (R)-5-[(S)-1,2-dihydroxyethyl]-3,4-dihydroxy-5(H)-furan-2-one.

Ascorbic Acid contains not less than 99.0 per cent and not more than 100.5 per cent of C6H8O6.

Description. A white to very pale yellow crystalline powder or colourless crystals; odourless. On exposure to light it gradually darkens.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ascorbic acid RS or with the reference spectrum of ascorbic acid.

B. Add 2 ml of a 2 per cent w/v solution to a few ml of 2,6-dichlorophenolindophenol solution; the solution is decolourised.

C. Add 1 drop of a freshly prepared 5 per cent w/v solution of sodium nitroprusside and 2 ml of dilute sodium hydroxide solution. Add 0.6 ml of hydrochloric acid dropwise and stir; the yellow colour turns blue.

D. To 2 ml of a 2 per cent w/v solution add 2 ml of water, 0.1 g of sodium bicarbonate and about 20 mg of ferrous sulphate,
shake and allow to stand; a deep violet colour is produced. Add 5 ml of 1 M sulphuric acid; the colour disappears.

**Tests**

**Appearance of solution.** A 5.0 per cent w/v solution in water is clear (2.4.1), and not more intensely coloured than reference solution BY7 (2.4.1).

**pH** (2.24.4). 2.2 to 2.5, determined in a 5.0 per cent w/v solution.

**Specific optical rotation** (2.24.2). +20.5° to +21.5°, determined in a 10.0 per cent w/v solution.

**Light absorption.** Absorbance (2.4.7) of a 0.001 per cent w/v solution in 0.01 M hydrochloric acid at the maximum about 244 nm, about 0.56.

**Oxalic acid.** Dissolve 0.25 g in 5 ml of water and neutralise to litmus paper with 2 M sodium hydroxide. Add 1 ml of 2 M acetic acid and 0.5 ml of 0.5 M calcium chloride. Any opalescence, after 60 minutes, is not more intense than that produced by treating 5 ml of a solution prepared by dissolving 70 mg of oxalic acid in 500 ml of water in a similar manner (0.3 per cent).

**Heavy metals** (2.3.13). 1.0 g dissolved in 25 ml of water complies with the limit test for heavy metals, Method A (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Assay.** Weigh accurately about 0.1 g and dissolve in a mixture of 100 ml of freshly boiled and cooled water and 25 ml of 1 M sulphuric acid. Immediately titrate with 0.05 M iodine, using starch solution as indicator until a persistent blue-violet colour is obtained.

1 ml of 0.05 M iodine is equivalent to 0.008806 g of C₆H₈O₆.

**Storage.** Store protected from light and moisture avoiding contact with metals. It undergoes rapid decomposition in solutions in contact with air.

### Ascorbic Acid Injection

Vitamin C Injection; L-Ascorbic Acid Injection

Ascorbic Acid Injection is a sterile solution of Sodium Ascorbate or of Ascorbic Acid prepared with the aid of Sodium Hydroxide or Sodium Carbonate or Sodium Bicarbonate in Water for Injections.

Ascorbic Acid Injection contains not less than 95.0 per cent and not more than 115.0 per cent of the stated amount of ascorbic acid, C₆H₈O₆.

**Description.** A clear, colourless liquid.

**Identification**

A. To a volume containing 5 mg of Ascorbic Acid add 0.5 ml of 0.1 M hydrochloric acid and 3 drops of sodium nitroprusside solution followed immediately by 1 ml of 0.1 M sodium hydroxide; a transient blue colour is produced.

B. To a volume containing 40 mg of Ascorbic Acid add 4 ml of 0.1 M hydrochloric acid and 4 drops of methylene blue solution and warm to 40°; the deep blue colour becomes appreciably lighter or is completely discharged within 3 minutes.

C. The solution responds to the flame test for sodium salts (2.3.1).

**Tests**

**pH** (2.24.4). 5.5 to 7.0.

**Oxalic acid.** Dilute a volume containing 0.25 g of Ascorbic Acid in 5 ml of water and neutralise to litmus paper with 2 M sodium hydroxide. Add 1 ml of 2 M acetic acid and 0.5 ml of 0.5 M calcium chloride. Any opalescence, after 60 minutes, is not more intense than that produced by treating 5 ml of a solution prepared by dissolving 70 mg of oxalic acid in 500 ml of water in a similar manner (0.3 per cent).

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** Measure accurately a volume containing about 50 mg of Ascorbic Acid and transfer to a 250-ml volumetric flask. Add 20 ml of metaphosphoric-acetic acids solution, dilute with water to 250.0 ml and mix. Pipette 10.0 ml into a 50-ml Erlenmeyer flask, add 5 ml of metaphosphoric-acetic acids solution and titrate with standard 2,6-dichlorophenolindophenol solution, until the pink colour persists for at least 10 seconds, the titration occupying not more than 2 minutes. Repeat the operation with a mixture of 5.5 ml of metaphosphoric-acetic acids solution and 15 ml of water omitting the preparation being examined. From the difference calculate the ascorbic acid in each ml of the injection from the ascorbic acid equivalent of the standard 2,6-dichlorophenolindophenol solution.

**Storage.** Store protected from light, in a single dose container.

### Ascorbic Acid Tablets

Vitamin C Tablets; L-Ascorbic Acid Tablets

Ascorbic Acid Tablets contain not less than 95.0 per cent and not more than 115.0 per cent of the stated amount of ascorbic acid, C₆H₈O₆. The tablets may contain permitted flavouring agents.

**Identification**

A. Shake a quantity of the powdered tablets with sufficient water to make approximately the equivalent of a 2 per cent
w/v solution of Ascorbic Acid and filter. The filtrate (solution A) is acid to litmus solution.

B. To solution A add a few ml of 2,6-dichlorophenolindophenol solution; the solution is decolorised.

C. To 1 ml of solution A, add about 0.1 ml of 2 M nitric acid and 0.05 ml of silver nitrate solution; a grey precipitate is produced.

Tests

Disintegration. The test does not apply to Ascorbic Acid Tablets containing 500 mg or more of Ascorbic Acid.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.15 g of Ascorbic Acid and dissolve as completely as possible in a mixture of 30 ml of water and 20 ml of 1 M sulphuric acid. Titrate with 0.1 M ceric ammonium sulphate using ferroin sulphate solution as indicator.

1 ml of 0.1 M ceric ammonium sulphate is equivalent to 0.008806 g of C6H8O6.

Storage. Store protected from light and moisture avoiding contact with metals.

Labelling. For tablets containing 500 mg or more of Ascorbic Acid the label states, where applicable, that the tablets should be chewed before swallowing.

Aspartame

\[ \text{H}_3\text{C}-\text{O} \quad \text{N} \quad \text{H} \quad \text{NH}_2 \quad \text{COOH} \]

\[ \text{C}_{14}\text{H}_{18}\text{N}_2\text{O}_3 \quad \text{Mol. Wt. 294.3} \]

Aspartame is \( \text{N-L-\alpha-aspartyl-L-phenylalanine} \) 1-methyl ester.

Aspartame contains not less than 98.0 per cent and not more than 102.0 per cent of \( \text{C}_{14}\text{H}_{18}\text{N}_2\text{O}_3 \), calculated on the dried basis.

Description. A white, crystalline powder; odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with aspartame RS.

B. When examined in the range 230 nm to 300 nm (2.4.7), a 0.1 per cent w/v solution in ethanol (95 per cent) shows absorption maxima at about 247 nm, 252 nm, 258 nm and 264 nm.

Tests

pH (2.4.24). About 5.0, determined in a 0.8 per cent w/v solution.

Specific optical rotation (2.4.22). +14.5° to +16.5°, determined at 20° in a 4.0 per cent w/v solution in 15 M formic acid within 30 minutes of preparing the solution.

Light absorption (2.4.7). Absorbance of a 1.0 per cent w/v solution in 2 M hydrochloric acid, prepared with the aid of ultrasound, at the maximum at about 430 nm, not more than 0.022.

5-Benzyl-3,6-dioxo-2-piperazineacetic acid. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.5 g of the substance under examination in 100 ml of a mixture of 10 volumes of methanol and 90 volumes of water.

Reference solution. A 0.0075 per cent w/v solution of 5-benzyl-3,6-dioxo-2-piperazine- acetic acid RS in a mixture of 10 volumes of methanol and 90 volumes of water.

Chromatographic system
– a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (3 to 10 µm),
– mobile phase: dissolve 5.6 g of potassium dihydrogen phosphate in 820 ml of water, adjust to pH 4.3 with phosphoric acid and dilute to 1000 ml with methanol, flow rate. 2 ml per minute,
– spectrophotometer set at 210 nm,
– a 20 µl loop injector.

Inject the test solution and reference solution. Record the chromatograms. The test is not valid if the relative standard deviations for replicate injections is more than 4.0 per cent and the symmetry factor of the principle peak in the chromatogram obtained with the reference solution is more than 2.0.

In the chromatogram obtained with the test solution the response obtained for any peak at a retention time corresponding to that of 5-benzyl-3,6-dioxo-2-piperazineacetic acid RS is not greater than the response obtained for the peak in the chromatogram of the reference solution corresponding to not more than 1.5 per cent of 5-benzyl-3,6-dioxo-2-piperazineacetic acid.

Other Related substances. Carry out the test for 5-Benzyl-3,6-dioxo-2-piperazineacetic acid, using reference solution (b) prepared by diluting 2.0 ml of the test solution to 100 ml with a mixture of 10 volumes of methanol and 90 volumes of water.
Inject 20 µl of the test solution and reference solution (b), record the chromatograms and measure the peak responses. Continue elution of the test solution for twice the retention time of the aspartame peak. The sum of the areas of any peaks observed in the chromatogram obtained with the test solution, other than the peaks for aspartame and 5-benzyl-3,6-dioxo-2-piperazinacetic acid, is not more than the area of the aspartame peak obtained with reference solution (b)(2.0 per cent).

Arsenic (2.3.10). Mix 3.3 g with 3 g of anhydrous sodium carbonate, add 10 ml of bromine solution and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite, dissolve the cooled residue in 16 ml of brominated hydrochloric acid AsT and add 45 ml of water. Remove the excess of bromine with 2 ml of stannous chloride AsT. The resulting solution complies with the limit test for arsenic (3 ppm).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent, determined on 2.0 g.

Loss on drying (2.4.19). Not more than 4.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

Assay. Weigh accurately about 0.3 g, dissolve in 1.5 ml of anhydrous formic acid, add 60 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, using crystal violet solution as indicator. Carry out a blank titration. A blank titration of more than 0.1 ml may be indicative of excessive water content. In such a case, repeat the test after taking precautions to maintain anhydrous conditions throughout.

1 ml of 0.1 M perchloric acid is equivalent to 0.02943 g of C₁₃H₁₈N₂O₅.

Storage. Store protected from light and moisture.

Aspirin
Acetylsalicylic Acid

COOH
O
\[\text{C₆H₃O₄} \quad \text{Mol. Wt. 180.2}\]

Aspirin is 2-acetoxybenzoic acid.

Aspirin contains not less than 99.5 per cent and not more than 100.5 per cent of C₆H₃O₄, calculated on the dried basis.

Description. Colourless crystals or a white, crystalline powder; odourless or almost odourless.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6) Compare the spectrum with that obtained with aspirin RS or with the reference spectrum of aspirin.

B. Boil about 0.5 g with 10 ml of sodium hydroxide solution for 3 minutes, cool and add 10 ml of dilute sulphuric acid; a white, crystalline precipitate is produced and the odour of acetic acid is perceptible. Filter, dissolve the precipitate in about 2 ml of water and add ferric chloride test solution; a deep violet colour is produced.

C. To the filtrate obtained in test B add 3 ml of ethanol (95 per cent) and 3 ml of sulphuric acid and warm; the odour of ethyl acetate is perceptible.

Tests

Appearance of solution. A 1.0 per cent w/v solution in ethanol (95 per cent) is clear (2.4.1) and not more intensely coloured than reference solution BS8 (2.4.1).

Clarity of solution in alkali. A 5.0 per cent w/v solution in a warm 5 per cent w/v solution of sodium carbonate is clear (2.4.1).

Arsenic (2.3.10). Mix 5.0 g with 3 g of anhydrous sodium carbonate, add 10 ml of bromine solution and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite, and dissolve the cooled residue in 16 ml of brominated hydrochloric acid and 45 ml of water. Remove the excess of bromine with 2 ml of stannous chloride AsT. The resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals. Not more than 10 ppm, determined by the following method. Dissolve 2.0 g in 25 ml of acetone, add 1 ml of water and 10 ml of hydrogen sulphide solution; any colour produced is not more intense than that produced by mixing 25 ml of acetone, 1.0 ml of lead standard solution (20 ppm Pb) and 10 ml of hydrogen sulphide solution.

Chlorides (2.3.12). Boil 1.75 g with 75 ml of water for 5 minutes, cool, add sufficient water to restore the original volume and filter. 25 ml of the filtrate complies with the limit test for chlorides (430 ppm).

Sulphates (2.3.17). 10 ml of the filtrate obtained in the test for Chlorides complies with the limit test for sulphates (600 ppm).

Readily carbonisable substances. Dissolve 0.5 g in 5 ml of sulphuric acid (containing 94.5 per cent to 95.5 per cent w/w of H₂SO₄); any colour produced is not more intense than that of reference solution BYS4 (2.4.1).

Salicylic acid. Dissolve 2.5 g in sufficient ethanol (95 per cent) to produce 25.0 ml (test solution). To each of two matched
Nessler cylinders add 48 ml of water and 1 ml of a freshly prepared acid ferric ammonium sulphate solution. Into one cylinder add 1.0 ml of a freshly prepared 0.01 per cent w/v solution of salicylic acid and into the other pipette 1.0 ml of the test solution. Mix the contents of the cylinders; after 30 seconds, the colour in the cylinder containing the test solution is not more intense than that in the cylinder containing the standard solution (0.1 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over phosphorus pentoxide at a pressure of 1.5 to 2.5 kPa.

Assay. Weigh accurately about 1.5 g, dissolve in 15 ml of ethanol (95 per cent), add 50.0 ml of 0.5 M sodium hydroxide, boil gently for 10 minutes, cool and titrate the excess of alkali with 0.5 M hydrochloric acid using phenol red solution as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of sodium hydroxide required.

1 ml of 0.5 M sodium hydroxide is equivalent to 0.04504 g of C9H8O4.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Aspirin Tablets

Acetylsalicylic Acid Tablets

Aspirin Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of aspirin, C9H8O4.

Identification

Boil a quantity of the powdered tablets containing 0.5 g of Aspirin with 10 ml of sodium hydroxide solution for 3 minutes, cool and add 10 ml of dilute sulphuric acid; a white, crystalline precipitate is produced and the odour of acetic acid is perceptible. Filter, dissolve the precipitate in about 2 ml of water and add ferric chloride test solution; a deep violet colour is produced.

Tests

Salicylic acid. Shake a quantity of the powdered tablets containing 0.2 g of Aspirin with 4 ml of ethanol (95 per cent), dilute to 100.0 ml with water, filter immediately, transfer 50 ml of the filtrate to a Nessler cylinder, add 1.0 ml of freshly prepared acid ferric ammonium sulphate solution, mix and allow to stand for 1 minute; the violet colour produced is not more intense than that produced by adding 1 ml of freshly prepared acid ferric ammonium sulphate solution to a mixture of 3.0 ml of a freshly prepared 0.01 per cent w/v solution of salicylic acid, 2 ml of ethanol (95 per cent) and sufficient water to produce 50 ml contained in a second Nessler cylinder (0.3 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.5 g of Aspirin, add 30.0 ml of 0.5 M sodium hydroxide, boil gently for 10 minutes, cool and titrate the excess of alkali with 0.5 M hydrochloric acid using phenol red solution as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of sodium hydroxide required.

1 ml of 0.5 M sodium hydroxide is equivalent to 0.04504 g of C9H8O4.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Soluble Aspirin Tablets

Dispersible Aspirin Tablets; Calcium Aspirin Tablets

Soluble Aspirin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of aspirin, C9H8O4.

Identification

A. The tablets effervesce on the addition of water.
B. Boil 0.1 g of the powdered tablets with 10 ml of water and 0.5 ml of ferric chloride test solution; a violet-red colour is produced.

Tests

Salicylic acid. To a quantity of the powdered tablets containing 0.5 g of Aspirin add 25.0 ml of chloroform, shake vigorously for 2 minutes and filter through a dry filter paper. Evaporate 5.0 ml of the filtrate rapidly to dryness in a dish in a current of dry air at room temperature. Dissolve the residue in 2 ml of ethanol (95 per cent), transfer to a Nessler cylinder, using a further 1 ml of ethanol (95 per cent) to rinse the dish, dilute to 50 ml with water, add 1 ml of acid ferric ammonium sulphate solution, mix, and allow to stand for 1 minute; the violet colour produced is not more intense than that produced by adding 1 ml of acid ferric ammonium sulphate solution to a mixture of 2.0 ml of a freshly prepared 0.15 per cent w/v solution of salicylic acid, 3 ml of ethanol (95 per cent) and sufficient water to produce 50 ml contained in a second Nessler cylinder (3 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.3 g of Aspirin,
dissolve in 10 ml of 0.5 M sulphuric acid and boil under a reflux condenser for 1 hour. Cool, transfer to a separating funnel with the aid of small quantities of water, and extract the liberated salicylic acid with four quantities, each of 20 ml, of ether. Wash the combined ether extracts with two quantities, each of 5 ml, of water, remove the ether in a current of air at a temperature not exceeding 30°, dissolve the residue in 20 ml of 0.5 M sodium hydroxide, and dilute to 200.0 ml with water. Transfer 50.0 ml to a stoppered flask, add 50.0 ml of 0.05 M bromine and 5 ml of hydrochloric acid, protect the mixture from light and shake repeatedly during 25 minutes. Add 20 ml of potassium iodide solution, shake thoroughly and titrate with 0.1 M sodium iodide solution to produce a white precipitate. Dissolve the precipitate with 4 ml of 5 M sodium hydroxide and dilute to 250.0 ml with water. Measure the absorbance at 273 nm (2.4.7).

C. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution of the residue reserved in Test B shows an absorption maximum at about 273 nm.

**Tests**

**Salicylic acid.** Shake a quantity of the powdered tablets containing 0.5 g of Aspirin with 50.0 ml of chloroform and 10 ml of water and allow to separate. Filter the chloroform layer through a dry filter paper and evaporate 10 ml of the filtrate to dryness at room temperature using a rotary evaporator. To the residue add 4 ml of ethanol (95 per cent), stir well, dilute to 100 ml with water at a temperature not exceeding 10°, filter immediately, rapidly transfer 50 ml to a Nessler cylinder, add 1 ml of freshly prepared acid ferric ammonium sulphate solution, mix and allow to stand for 1 minute; the violet colour produced is not more intense than that produced by adding 1 ml of acid ammonium sulphate solution to a mixture of 3.0 ml of a freshly prepared 0.01 per cent w/v solution of salicylic acid, 2 ml of ethanol (95 per cent) and sufficient water to produce 50 ml contained in a second Nessler cylinder (0.6 per cent).

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets.

For aspirin — Weigh accurately a quantity of the powder containing about 0.7 g of Aspirin, add 20 ml of water and 2 g of sodium citrate and heat under a reflux condenser for 30 minutes. Cool, wash the condenser with 30 ml of warm water and titrate with 0.5 M sodium hydroxide using phenolphthalein solution as indicator.

1 ml of 0.5 M sodium hydroxide is equivalent to 0.04504 g of C_{9}H_{8}O_{4}.

For caffeine — Weigh accurately a quantity of the powder containing about 30 mg of Caffeine and heat under a reflux condenser for 30 minutes. Cool, add sufficient water to produce 250.0 ml and filter. To 10.0 ml of the filtrate add 10 ml of 1 M sodium hydroxide and extract immediately with five quantities, each of 30 ml, of chloroform, washing each extract with the same 10 ml of water. Filter the combined chloroform extracts, if necessary, through absorbent cotton previously moistened with chloroform. Evaporate the solution to dryness and the residue as completely as possible in water, warming gently if necessary. Cool, add sufficient water to produce 100.0 ml, mix and filter if necessary. Measure the absorbance of the resulting solution at the maximum at about 273 nm (2.4.7).

Calculate the content of C_{14}H_{22}N_{2}O_{3} taking 504 as the specific absorbance at 273 nm.

**Storage.** Store protected from moisture.

**Aspirin And Caffeine Tablets**

Acetylsalicylic Acid and Caffeine Tablets

Aspirin and Caffeine Tablets contain not less than 330 mg and not more than 370 mg of aspirin, C_{9}H_{8}O_{4}, and not less than 27.5 mg and not more 32.5 mg of caffeine, C_{8}H_{10}N_{4}O_{2}.

**Identification**

A. Boil 1 g of the powdered tablets with 10 ml of 1 M sodium hydroxide, cool and filter. Acidify the filtrate with 1 M sulphuric acid; a white precipitate is produced. Dissolve the precipitate in about 2 ml of water and add ferric chloride test solution; a deep violet colour is produced.

B. Shake 0.5 g of the powdered tablets with 10 ml of water for 5 minutes, filter and add 10 ml of 1 M sodium hydroxide. Extract with three quantities, each of 30 ml, of chloroform, washing each extract with the same 10 ml of water. Filter the combined extracts through absorbent cotton and evaporate the filtrate to dryness. Reserve a quantity of the residue for test C. Dissolve 10 mg of the residue in 1 ml of hydrochloric acid, add 0.1 g of potassium chloride and evaporate to dryness in a porcelain dish; a reddish residue remains which becomes purple on exposure to ammonia vapour.

C. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution of the residue reserved in Test B shows an absorption maximum at about 273 nm.

**Tests**

**Salicylic acid.** Shake a quantity of the powdered tablets containing 0.5 g of Aspirin with 50.0 ml of chloroform and 10 ml of water and allow to separate. Filter the chloroform layer through a dry filter paper and evaporate 10 ml of the filtrate to dryness at room temperature using a rotary evaporator. To the residue add 4 ml of ethanol (95 per cent), stir well, dilute to 100 ml with water at a temperature not exceeding 10°, filter immediately, rapidly transfer 50 ml to a Nessler cylinder, add 1 ml of freshly prepared acid ferric ammonium sulphate solution, mix and allow to stand for 1 minute; the violet colour produced is not more intense than that produced by adding 1 ml of acid ammonium sulphate solution to a mixture of 3.0 ml of a freshly prepared 0.01 per cent w/v solution of salicylic acid, 2 ml of ethanol (95 per cent) and sufficient water to produce 50 ml contained in a second Nessler cylinder (0.6 per cent).

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets.

For aspirin — Weigh accurately a quantity of the powder containing about 0.7 g of Aspirin, add 20 ml of water and 2 g of sodium citrate and heat under a reflux condenser for 30 minutes. Cool, wash the condenser with 30 ml of warm water and titrate with 0.5 M sodium hydroxide using phenolphthalein solution as indicator.

1 ml of 0.5 M sodium hydroxide is equivalent to 0.04504 g of C_{9}H_{8}O_{4}.

For caffeine — Weigh accurately a quantity of the powder containing about 30 mg of Caffeine and heat under a reflux condenser for 30 minutes. Cool, add sufficient water to produce 250.0 ml and filter. To 10.0 ml of the filtrate add 10 ml of 1 M sodium hydroxide and extract immediately with five quantities, each of 30 ml, of chloroform, washing each extract with the same 10 ml of water. Filter the combined chloroform extracts, if necessary, through absorbent cotton previously moistened with chloroform. Evaporate the solution to dryness and the residue as completely as possible in water, warming gently if necessary. Cool, add sufficient water to produce 100.0 ml, mix and filter if necessary. Measure the absorbance of the resulting solution at the maximum at about 273 nm (2.4.7).

Calculate the content of C_{14}H_{22}N_{2}O_{3} taking 504 as the specific absorbance at 273 nm.

**Storage.** Store protected from moisture.
Atenolol is (RS)-4-(2-hydroxy-3-isopropylaminopropoxy)phenylacetamide.

Atenolol contains not less than 99.0 per cent and not more than 101.0 per cent of C_{14}H_{22}N_{2}O_{3}, calculated on the dried basis.

**Description.** A white or almost white powder.

**Identification**

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with atenolol RS or with the reference spectrum of atenolol.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.01 per cent w/v solution in methanol shows absorption maxima at about 275 nm and 282 nm. The ratio of the absorbance at the maximum at about 275 nm to that at the maximum at about 282 nm is 1.15 to 1.20.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 99 volumes of methanol and 1 volume of strong ammonia solution.

**Test solution.** Dissolve 1.0 g of the substance under examination in sufficient methanol to produce 100 ml.

**Reference solution.** A 1.0 per cent w/v solution of atenolol RS in methanol.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

**Tests**

**Appearance of solution.** A 1.0 per cent w/v solution is clear (2.4.1), and not more intensely coloured than degree 6 of the appropriate range of reference solutions.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution (a).** Dissolve 50 mg of the substance under examination in 20 ml of the mobile phase and dilute to 25 ml with the mobile phase.

**Test solution (b).** Dissolve 50 mg of the substance under examination in 0.1 ml of dimethyl sulphoxide, if necessary heating the mixture by placing the container in a water-bath for a few seconds and dilute to 25 ml with the mobile phase.

**Reference solution (a).** Dilute 0.5 ml of the test solution (a) to 100 ml with the mobile phase.

**Reference solution (b).** Dissolve 50 mg of atenolol impurity standard RS in 0.1 ml of dimethyl sulphoxide, if necessary heating the mixture by placing the container in a water-bath for a few seconds and dilute to 25 ml with the mobile phase.

**Chromatographic system**

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: dissolve 1.0 g of sodium octanesulphonate and 0.4 gm of tetrabutyl ammonium hydrogen sulphate in 1000 ml of a mixture of 20 volumes of tetrahydrofuran, 180 volumes of methanol and 800 volumes of a 0.34 per cent w/v solution of potassium dihydrogen phosphate and adjust the pH to 3.0 with phosphoric acid,
- flow rate. 1 ml per minute,
- spectrophotometer set at 226 nm,
- a 10 µl loop injector.

Equilibrate the column with the mobile phase for about 30 minutes and adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (a) is at least 50 per cent of the full scale of the recorder.

Inject reference solution (b). The resulting chromatogram is similar to that of the specimen chromatogram provided with atenolol impurity standard RS in that the peak due to bis-ether precedes and is separated from that due to tertiary amine, which normally appears as a doublet. If necessary, adjust the concentration of sodium octanesulphonate; if its concentration is increased, the retention time of the tertiary amine is prolonged.

Inject separately test solution (a) and reference solution (a). Continue the chromatography for four times the retention time of the principal peak. The area of any secondary peak in the chromatogram obtained with test solution (a) is not greater than half the area of the principal peak obtained with reference solution (a) (0.25 per cent); the sum of the areas of any secondary peaks is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 10 per cent of that of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

If the substance under examination is found to contain more than 0.15 per cent of bis-ether, its compliance is confirmed by repeating the chromatography using 10 µl of test solution (b).

**Chlorides** (2.3.12). Dissolve 0.25 g in a mixture of 1 ml of 2 M nitric acid and 15 ml of water. The solution complies with the limit test for chlorides without further addition of 2 M nitric acid (0.1 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.
Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°C.

Assay. Weigh accurately about 0.2 g and dissolve in 80 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02663 g of C14H22N2O3.

Atenolol Tablets

Atenolol Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of atenolol, C14H22N2O3. The tablets may be coated.

Identification

A. Heat a quantity of the powdered tablets containing about 0.1 g of Atenolol with 15 ml of methanol to 50°C, shake for 5 minutes, filter (Whatman No. 42 paper is suitable) and evaporate the filtrate to dryness on a water-bath. Warm the residue with 10 ml of 0.1 M hydrochloric acid, shake and filter. Add to the filtrate sufficient 1 M sodium hydroxide to make it alkaline, extract with 10 ml of chloroform, dry by shaking with anhydrous sodium sulphate, filter, evaporate the filtrate to dryness on a water-bath and dry the residue at 105°C for 1 hour. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with atenolol RS or with the reference spectrum of atenolol.

B. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows absorption maxima at about 275 nm and 282 nm.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the powdered tablets containing 25 mg of Atenolol with 25 ml of the mobile phase and mix with the aid of ultrasound for 20 minutes, filter (such as Whatman GF/C filter) and use the filtrate.

Reference solution (a). Dilute 1 volume of the test solution to 200 volumes with the mobile phase.

Reference solution (b). Dissolve 10 mg of atenolol impurity standard RS in 0.1 ml of dimethyl sulphoxide with the aid of gentle heat, dilute to 10 ml with the mobile phase and mix.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: dissolve 0.8 g of sodium octanesulphonate and 0.4 gm of tetrabutyl-ammonium hydrogen sulphate in 1000 ml of a mixture of 20 volumes of tetrahydrofuran, 180 volumes of methanol and 800 volumes of a 0.34 per cent w/v solution of potassium dihydrogen phosphate and adjust the pH to 3.0 with phosphoric acid,
- flow rate. 1 ml per minute,
- spectrophotometer set at 226 nm,
- a 20 µl loop injector.

Inject each solution. The test is not valid unless the chromatogram obtained with reference solution (b) resembles the reference chromatogram supplied with the atenolol impurity standard RS in that the peak due to bis-ether precedes and is separated from that due to tertiary amine, which is normally a doublet. If necessary, adjust the concentration of sodium octanesulphonate in the mobile phase; if its concentration is increased, the retention time of the tertiary amine is prolonged.

In the chromatogram obtained with the test solution, the area of any peak corresponding to 4-(2-hydroxy-3-isopropylamino-propoxy)phenylacetic acid (blocker acid) is not greater than half the area of the peak in the chromatogram obtained with reference solution (a) (0.25 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.2 g of Atenolol, transfer to a 500-ml volumetric flask using 300 ml of methanol, heat the resulting suspension to 60°C and shake for 15 minutes. Cool, dilute to 500.0 ml with methanol, filter through a sintered-glass funnel (Porosil G3) and dilute a suitable volume of the filtrate with sufficient methanol to produce a solution containing 0.01 per cent w/v of Atenolol. Measure the absorbance of the resulting solution at the maximum at about 275 nm (2.4.7). Calculate the content of C14H22N2O3 taking 53.7 as the value of the specific absorbance at 275 nm.

Atorvastatin Calcium

\[
\begin{align*}
\text{C}_{66}\text{H}_{68}\text{CaF}_{2}\text{N}_{4}\text{O}_{10} & \quad \text{Mol. Wt. 1155.36} \\
\text{Ca}^{++} & \quad \text{OH} \quad \text{OH} \\
\text{H} & \quad \text{CH}_3 \\
\text{H}_3\text{C} & \quad \text{OH} \\
\text{O} & \quad \text{COO}^- \\
\end{align*}
\]
Atorvastatin Calcium is calcium salt of \((\beta R,\beta R)-2-(4\text{-}fluorophenyl)\alpha,\delta\text{-dihydroxy}-5\text{-}(1\text{-}methyl ethyl)-3\text{-}phenyl-4\text{-[}(\text{phenylamino})\text{carbonyl}]\text{-}H\text{-pyrrole-1-heptanoic acid trihydrate.}

Atorvastatin Calcium contains not less than 98.0 per cent and not more than 102.0 per cent of \(C_{66}H_{68}\text{CaF}_2\text{N}_4\text{O}_{10}\), calculated on the anhydrous basis.

**Description.** A white to off-white, crystalline powder.

**Identification**

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with atorvastatin calcium RS or with the reference spectrum of atorvastatin calcium.

**Tests**

**Specific optical rotation** (2.4.22). -6.0° to -12.0°, determined in a 1.0 per cent w/v solution in dimethylsulphoxide.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Solvent mixture.** A mixture of 40 volumes of acetonitrile and 60 volumes of water.

**Test solution.** Dissolve 50 mg of the substance under examination in 10 ml of methanol and dilute to 100 ml with the solvent mixture.

**Reference solution (a).** A 0.5 per cent w/v solution of atorvastatin calcium RS in methanol. Dilute 5 ml of the solution to 50 ml with the solvent mixture.

**Reference solution (b).** Dilute 1 ml of reference solution (a) to 100 ml with the solvent mixture.

**Chromatographic system**

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 92.5 volumes of acetonitrile and 7.5 volumes tetrahydrofuran,
  - B. a mixture of 58 volumes of a buffer solution prepared by dissolving 5.75 g of ammonium dihydrogen orthophosphate in 1000 ml of water and 42 volumes of mobile phase A,
  - C. a mixture of 20 volumes of the buffer solution, 20 volumes of mobile phase A and 60 volumes of methanol,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 246 nm,
- 20 µl loop injector,
- injection delay 10 minutes,

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<th>Time (in min.)</th>
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<th>Mobile phase C (per cent v/v)</th>
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<td>1.8</td>
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Inject reference solution (a). The test is not valid unless the column efficiency is not less than 10000 theoretical plates and the tailing factor is not more than 1.5.

Inject alternatively the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any individual secondary peak is not more than half the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than 2 times the area of the peak in the chromatogram obtained with reference solution (b) (2.0 per cent). Ignore any peak with an area less than 0.05 times the area of the peak obtained in the chromatogram obtained in the chromatogram obtained with reference solution (b) (0.05 per cent)

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

**Water** (2.3.43). 3.0 per cent to 7.0 per cent, determined on 0.3 g.

**Assay.** Determine by liquid chromatography (2.4.14).

**Solvent mixture.** A mixture of 40 volumes of acetonitrile and 60 volumes of water.

**Test solution.** Dissolve 80 mg of the substance under examination in 20 ml of methanol and dilute to 200 ml with the solvent mixture. Dilute this solution with the solvent mixture to produce a solution containing 0.008 per cent w/v of Atorvastatin Calcium.

**Reference solution.** Dissolve 20 mg of atorvastatin calcium RS in 5 ml of methanol and dilute to 50 ml with the solvent mixture. Dilute the solution with the solvent mixture to produce a solution containing 0.008 per cent w/v of Atorvastatin Calcium.

**Chromatographic system**

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 58 volumes of a buffer solution prepared by dissolving 5.75 g of ammonium dihydrogen orthophosphate in 1000 ml of water and 42 volumes of mobile phase A,
– flow rate. 1.8 ml per minute,
– spectrophotometer set at 246 nm,
– a 20 µl loop injector.
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent,
Inject alternatively the test solution and the reference solution.
Calculate the content of C₆₆H₆₈F₂N₄O₁₀.

Storage. Store protected from light at a temperature not exceeding 30º.

Atorvastatin Tablets

Atorvastatin Calcium Tablets

Atorvastatin Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of atorvastatin, C₆₆H₆₈F₂N₄O₁₀.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1

Medium. 900 ml of phosphate buffer pH 6.8

Speed and time. 75 rpm for 30 minutes.

Withdraw a suitable volume of the medium and filter. Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, diluted if necessary, with the dissolution medium.

Reference solution. Weigh a suitable quantity of atorvastatin calcium RS and dissolve in sufficient methanol to produce a solution containing 0.088 per cent of atorvastatin. Dilute 10.0 ml of the resulting solution to 100.0 ml with the medium.

Use the chromatographic system described under the Assay,

Inject the reference solution. The test is not valid unless the column efficiency is not less than 7000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent,
Calculate the content of C₆₆H₆₈F₂N₄O₁₀.

D. Not less than 70 per cent of the stated amount of C₆₆H₆₈F₂N₄O₁₀.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of 40 volumes of acetonitrile and 60 volumes of the buffer solution (see below).

Test solution. Weigh accurately a quantity of the powdered tablets containing 50 mg of atorvastatin, disperse in 10 ml of methanol, add 20 ml of the solvent mixture, disperse with the aid of ultrasound, if required, and dilute to 100 ml with the solvent mixture and filter.

Reference solution (a). Weigh accurately a suitable quantity of atorvastatin calcium RS, dissolve in 5 ml of methanol and dilute to 50 ml with the solvent mixture, to produce 0.05 percent of atorvastatin

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with the solvent mixture

Chromatographic system

– a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
– mobile phase: A. a mixture of 92.5 volumes of acetonitrile and 7.5 volumes tetrahydrofuran,
  B. a mixture of 58 volumes of a buffer solution prepared by dissolving 5.75 g of ammonium dihydrogen orthophosphate in 1000 ml of water and 42 volumes of mobile phase A,
  C. a mixture of 20 volumes of the buffer solution, 20 volumes of mobile phase A and 60 volumes of methanol,
– a linear gradient programme using the conditions given below,
– spectrophotometer set at 246 nm,
– 20 µl loop injector.
– injection delay 10 minutes.

<table>
<thead>
<tr>
<th>Time (in min.)</th>
<th>Flow rate (ml per minute)</th>
<th>Mobile phase B (per cent v/v)</th>
<th>Mobile phase C (per cent v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.8</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>1.8</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>35</td>
<td>1.5</td>
<td>25</td>
<td>75</td>
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<tr>
<td>40</td>
<td>1.5</td>
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<td>75</td>
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<tr>
<td>55</td>
<td>1.5</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>60</td>
<td>1.8</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 10000 theoretical plates and the tailing factor is not more than 1.5,

Inject alternatively the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the
peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than 4 times the area of the peak in the chromatogram obtained with reference solution (b) (4.0 per cent). Ignore any peak with an area less than 0.05 times the area of the peak obtained with reference solution (b) (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. A solution prepared by dissolving 6.8 g of potassium dihydrogen orthophosphate and 0.9 g of sodium hydroxide in 1000 ml of water and adjusting the pH to 6.8 with phosphoric acid or sodium hydroxide.

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablets containing about 80 mg of atorvastatin and disperse in sufficient methanol to produce a solution containing 0.08 per cent w/v of atorvastatin. Disperse with the aid of ultrasound, if required, and filter. Dilute the filtrate with sufficient of the solvent mixture to produce a solution containing 0.008 per cent w/v of atorvastatin.

Reference solution. Weigh accurately a suitable quantity of atorvastatin calcium RS and dissolve in sufficient methanol to produce a solution containing 0.016 per cent w/v of atorvastatin. To 5 ml of this solution, add 20 ml of methanol and dilute to 50 ml with the solvent mixture to produce a solution containing 0.008 per cent w/v of atorvastatin.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of a buffer solution prepared by dissolving 1.54 g of ammonium acetate in 1000 ml of water and adjusting the pH to 4.0 with glacial acetic acid, and 50 volumes of a mixture of 92.5 volumes of acetonitrile and 7.5 volumes of tetrahydrofuran,
- flow rate. 2 ml per minute,
- spectrophotometer set at 246 nm,
- a 20 µl loop injector,

Inject the reference solution. The test is not valid unless the column efficiency is not less than 7000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject alternatively the test solution and the reference solution.

Calculate the content of C_{68}H_{68}F_{2}N_{4}O_{10}, in the tablets.

Storage. Store protected from moisture at a temperature not exceeding 30º.

Labelling. The label states the strength in terms of the equivalent amount of atorvastatin.

### Atropine Methonitrate

**Methylation Nitrate**

![Atropine Methonitrate](image)

C_{18}H_{32}N_{2}O_{6}  
Mol. Wt. 366.4

Atropine Methonitrate is \((RS)-(1R,3r,5S)-8\)-methyl-3-tropoyloxytropanium nitrate.

Atropine Methonitrate contains not less than 99.0 per cent and not more than 101.0 per cent of C_{18}H_{26}N_{2}O_{6}, calculated on the dried basis.

**Description.** Colourless crystals or a white, crystalline powder.

**Identification**

Test A may be omitted if tests B, C, and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with atropine methonitrate RS.

B. To 0.05 ml of a 1 per cent w/v solution add 0.05 ml of a 0.1 per cent w/v solution of diphenylamine in nitrogen-free sulphuric acid; an intense blue colour is produced.

C. To 2.5 ml of a 10 per cent w/v solution add 2.5 ml of water and 2 ml of dilute sodium hydroxide solution; no precipitate is produced.

D. Add about 1 mg to 4 drops of fuming nitric acid and evaporate to dryness on a water-bath; a yellow residue is obtained. To the cooled residue add 2 ml of acetone and 4 drops of a 3 per cent w/v solution of potassium hydroxide in methanol; a violet colour is produced.

**Tests**

**Appearance of solution.** A 5.0 per cent w/v solution in carbon dioxide-free water is clear (2.4.1) and not more intensely coloured than reference solution BS8 (2.4.1).

**pH** (2.4.24). 6.0 to 7.5, determined in a 5.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). –0.25° to +0.05°, determined in a 10.0 per cent w/v solution, using a 2-dm tube (distinction from hyoscyamine).
Silver. To 10 ml of a 10.0 per cent w/v solution add 0.1 ml of sodium sulphide solution. The solution is not more intensely coloured than reference solution BS8 (2.4.1).

Halides (2.3.12). 15 ml of a 5.0 per cent w/v solution in carbon dioxide-free water complies with the limit test for chlorides, using 0.3 ml of chloride standard solution (25 ppm Cl) for preparing the standard.

Apoamylatropine. A 0.1 per cent w/v solution in 0.01 M hydrochloric acid shows absorption maxima at about 252 nm and 257 nm (2.4.7). The ratio of the absorbance at about 257 nm to that at about 252 nm is not less than 1.17.

Related substances. Determine by thin-layer chromatography (2.4.17) coating the plate with silica gel G.

Mobile phase. A mixture of 60 volumes of ethyl acetate, 15 volumes of anhydrous formic acid, 15 volumes of water and 10 volumes of methanol.

Test solution. A 4.0 per cent w/v solution of the substance under examination in methanol (90 per cent).

Reference solution. Dilute 5 ml of the test solution to 100 ml with methanol (90 per cent), mix and dilute 10 ml of the resulting solution to 100 ml with methanol (90 per cent).

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate at 105° until the odour of the solvent is not detectable. Allow it to cool to room temperature and spray with dilute potassium iodobismuthate solution until spots appear. Any secondary spot in the chromatogram obtained with test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.3 g and dissolve in 50 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.03664 g of C18H26N2O6.

Storage. Store protected from light.

Atropine Sulphate

\[
\begin{align*}
\text{N}^-\text{CH}_3 & \\
\text{H} & \\
\text{O} & \\
\text{O} & \\
\text{H}_2\text{SO}_4, \text{H}_2\text{O} & \\
\end{align*}
\]

(C17H23NO3)2.H2SO4, H2O

Mol. Wt. 694.8

Atropine Sulphate is (RS)-(1R,3r,5S)-3-tropoyloxytropanium sulphate monohydrate.

Atropine Sulphate contains not less than 99.0 per cent and not more than 101.0 per cent of atropine sulphate, (C17H23NO3)2.H2SO4, calculated on the anhydrous basis.

Description. Colourless crystals or a white, crystalline powder; odourless.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with atropine sulphate RS or with the reference spectrum of atropine sulphate.

B. To a 2 per cent w/v solution add sodium hydroxide solution, filter and transfer the precipitate with water. Dry the precipitate at 60°. To 5 mg of the residue add 5 drops of fuming nitric acid and evaporate to dryness on a water-bath. Cool the faintly yellow coloured residue and add 2 ml of acetone and 4 drops of a 3 per cent w/v solution of potassium hydroxide in methanol; a violet colour is produced.

C. A 5 per cent w/v solution gives the reactions of sulphates (2.3.1).

Tests

pH (2.4.24). 4.5 to 6.2, determined in a 2.0 per cent w/v solution.

Specific optical rotation (2.4.22). –0.50° to +0.05°, determined in a 10.0 per cent w/v solution, using a 2-dm tube (distinction from hyoscyamine).

Apoatropine. Absorbance of a 0.1 per cent w/v solution in 0.01 M hydrochloric acid at about 245 nm, not more than 0.4 (2.4.7).
Foreign alkaloids and decomposition products. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 90 volumes of acetone, 7 volumes of water and 3 volumes of strong ammonia solution.

Test solution. A 2.0 per cent w/v solution of the substance under examination in methanol.

Reference solution (a). Dilute 1 ml of the test solution to 100 ml with methanol.

Reference solution (b). Dilute 25 ml of reference solution (a) to 50 ml with methanol.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate at 105° for 15 minutes. Allow it to cool to room temperature and spray with dilute potassium iodobismuthate solution. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 4.0 per cent, determined on 0.5 g.

Assay. Weigh accurately about 0.5 g, dissolve in 30 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.06768 g of (C₁₇H₂₃NO₃)₂H₂SO₄.

Storage. Store protected from light.

Atropine Injection

Atropine Sulphate Injection

Atropine Injection is a sterile solution of Atropine Sulphate in water for Injections.

Atropine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of atropine sulphate, (C₁₇H₂₃NO₃)₂H₂SO₄·H₂O.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 50 volumes of chloroform, 40 volumes of acetone and 10 volumes of diethylamine.

Test solution. Evaporate a volume of the injection containing 5 mg of Atropine Sulphate to dryness on a water-bath, triturate the residue with 1 ml of ethanol (95 per cent), allow to stand and use the supernatant liquid.

Reference solution. A 0.5 per cent w/v solution of atropine sulphate RS in ethanol (95 per cent).

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate at 105° for 20 minutes, allow it to cool to room temperature and spray with potassium iodobismuthate solution. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with reference solution (a) corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

pH (2.4.24). 3.0 to 5.5.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by gas chromatography (2.4.13).

Test solution. Add 1.0 ml of a 0.25 per cent w/v solution of homatropine hydrobromide RS (internal standard) in methanol (solution A), 1 ml of 5 M ammonia to a volume of the injection containing 2.5 mg of Atropine Sulphate, diluted if necessary to 5 ml with water and extract with two quantities, each of 10 ml, of 0.1 M hydrochloric acid. Wash the combined extracts with 5 ml of chloroform, shake the combined extracts with 1 g of anhydrous sodium sulphate, filter and evaporate the filtrate to dryness. Dissolve the residue in 5.0 ml of dichloromethane. To 1.0 ml of this solution add 0.2 ml of a mixture of 4 volumes of N,O-bis(trimethylsilyl)acetamide and 1 volume of trimethylchlorosilane, mix and allow to stand for 30 minutes.

Reference solution (a). Prepare in the same manner as the test solution but omitting the addition of solution A.

Reference solution (b). Add 1 ml of solution A and 1 ml of 5 M ammonia to 5.0 ml of a 0.05 per cent w/v solution of atropine sulphate RS.

Chromatographic system

- a glass column 1.5 m x 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) (such as OV-17),
- temperature: column, 220°,
  inlet port and detector, 260°,
- flow rate: 30 ml per minute of the carrier gas.

Calculate the content of (C₁₇H₂₃NO₃)₂H₂SO₄·H₂O in the injection.
Storage. Store protected from light.

Atropine Eye Ointment

Atropine Sulphate Eye Ointment

Atropine Eye Ointment is a sterile preparation of Atropine Sulphate in an eye ointment base.

Atropine Eye Ointment contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of atropine sulphate, \((C_{17}H_{23}NO_3)_{2}\cdot H_2SO_4\cdot H_2O\).

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 50 volumes of chloroform, 40 volumes of acetone and 10 volumes of diethylamine.

Test solution. Dissolve a quantity of the ointment containing 10 mg of Atropine Sulphate as completely as possible in 10 ml of light petroleum (40° to 60°) and extract with two quantities, each of 15 ml, of 0.05 M sulphuric acid, washing each acid solution with the same 5 ml of light petroleum (40° to 60°). Mix the acid solutions, make alkaline with dilute ammonia solution, and extract with two quantities, each of 15 ml, of chloroform. Remove the chloroform and dissolve the residue in 2 ml of ethanol (95 per cent).

Reference solution. A 0.5 per cent w/v solution of atropine sulphate RS in ethanol (95 per cent).

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate at 105° for 20 minutes, allow it to cool to room temperature and spray with potassium iodobismuthate solution. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Other tests. Complies with the tests stated under Eye Ointments.

Assay. Determine by gas chromatography (2.4.13).

Test solution (a). Dissolve a quantity of the ointment containing about 10 mg of Atropine Sulphate in 15 ml of chloroform, add 2 ml of 0.5 per cent w/v solution of homatropine hydrobromide RS (internal standard) in methanol (solution A) and extract with two quantities, each of 10.0 ml, of 0.1 M hydrochloric acid. Wash the combined extracts with 10 ml of chloroform, add 2 ml of 5 M ammonia. Extract with two quantities, each of 10 ml, of chloroform, shake the combined extracts with 2 g of anhydrous sodium sulphate, filter and evaporate the filtrate to dryness. Dissolve the residue in 5.0 ml of dichloromethane. To 1.0 ml of this solution add 0.2 ml of a mixture of 4 volumes of \(N,O\)-bis (trimethylsilyl) acetamide and 1 volume of trimethylchlorosilane, mix and allow to stand for 30 minutes.

Reference solution (a). Prepare in the same manner as the test solution but omitting the addition of solution A.

Reference solution (b). Add 2.0 ml of solution A and 2.0 ml of 5 M ammonia to 20.0 ml of a 0.05 per cent w/v solution of atropine sulphate RS and complete the procedure described under the test solution beginning at the words “Extract with two quantities, each of 10.0 ml, of chloroform.”

Chromatographic system

- a glass column 1.5 m x 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) (such as OV-17),
- temperature: column, 220°,
  inlet port and detector, 260°,
- flow rate, 30 ml per minute of the carrier gas.

Calculate the content of \((C_{17}H_{23}NO_3)_{2}\cdot H_2SO_4\cdot H_2O\) in the ointment.

Storage. Store at a temperature not exceeding 30°.

Atropine Tablets

Atropine Sulphate Tablets

Atropine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of atropine sulphate, \((C_{17}H_{23}NO_3)_{2}\cdot H_2SO_4\cdot H_2O\).

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 50 volumes of chloroform, 40 volumes of acetone and 10 volumes of diethylamine.

Test solution. Shake a quantity of the powdered tablets containing 5 mg of Atropine Sulphate with 1 ml of ethanol (95 per cent), centrifuge and use the supernatant liquid.

Reference solution. A 0.5 per cent w/v solution of atropine sulphate RS in ethanol (95 per cent).

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate at 105° for 20 minutes, allow it to cool to room temperature and spray with potassium iodobismuthate solution. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.
B. The powdered tablets give the reactions of sulphates (2.3.1).

**Tests**

**Uniformity of content.** Comply with the test stated under Tablets.

Determine by gas chromatography (2.4.13).

**Test solution.** Powder one tablet and shake in a centrifuge tube with 5 ml of 0.1 M hydrochloric acid. Add 1 ml of a 0.06 per cent w/v solution of homatropine hydrobromide RS (internal standard) in methanol (solution A), extract with two quantities, each of 5 ml, of chloroform and discard the chloroform extracts. Add 1 ml of 5 M ammonium. Extract with two quantities, each of 5 ml, of chloroform, shake the combined extracts with 1 g of anhydrous sodium sulphate, filter and evaporate the filtrate to dryness. Dissolve the residue in 0.5 ml of a mixture of 20 volumes of dichloromethane, 4 volumes of N,O-
bis (trimethylsilyl)-acetamide and 1 volume of trimethylchlorosilane, mix and allow to stand for 30 minutes.

**Reference solution (a).** Add 1 ml of solution A and 1 ml of 5 M ammonium to 5.0 ml of a 0.012 per cent w/v solution of atropine sulphate RS. Extract with two quantities, each of 5 ml, of chloroform and complete the procedure described under solution (1) beginning at the words “shake the combined extracts with 1 g of anhydrous sodium sulphate,......”.

**Reference solution (b).** Prepare in the same manner as reference solution (a) but omitting the addition of solution A.

Carry out the chromatographic procedure described under Assay.

Calculate the content of \((C_{17}H_{23}NO_3)_2\cdot H_2SO_4\cdot H_2O\) in the tablets.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by gas chromatography (2.4.13).

**Test solution.** Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing about 5 mg of Atropine Sulphate with 10 ml of 0.1 M hydrochloric acid, add 1 ml of a 0.5 per cent w/v solution of homatropine hydrobromide RS (internal standard) in methanol (solution A), extract with two quantities, each of 10 ml, of chloroform and discard the chloroform extracts. Add 1 ml of 5 M ammonium. Extract with two quantities, each of 10 ml, of chloroform, shake the combined extracts with 2 g of anhydrous sodium sulphate, filter and evaporate the filtrate to dryness. Dissolve the residue in 2.0 ml of dichloromethane. To 1.0 ml of this solution, add 0.2 ml of a mixture of 4 volumes of N,O-
bis (trimethylsilyl)-acetamide and 1 volume of trimethylchlorosilane, mix and allow to stand for 30 minutes.

**Reference solution (a).** Add 1 ml of solution A and 1 ml of 5 M ammonium to 10 ml of a 0.05 per cent w/v solution of atropine sulphate RS. Extract with two quantities, each of 10 ml, of chloroform and complete the procedure described under the test solution beginning at the words “shake the combined extracts with 2 g of....”.

**Reference solution (b).** Prepare in the same manner as reference solution (a) but omitting the addition of solution A.

Chromatographic system
- a glass column 1.5 m x 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) (such as OV-17),
- temperature: column, 220°C, inlet port and detector, 260°C,
- flow rate, 30 ml per minute of the carrier gas.

Calculate the content of \((C_{17}H_{23}NO_3)_2\cdot H_2SO_4\cdot H_2O\) in the tablets.

### Azathioprine

\[
\begin{align*}
C_9H_7N_7O_2S & \quad \text{Mol. Wt. 277.3} \\
\end{align*}
\]

Azathioprine is 6-[(1-methyl-4-nitro-1H-imidazol-5-yl) sulphanilyl]-7H-purine.

Azathioprine contains not less than 98.5 per cent and not more than 101.0 per cent of \(C_9H_7N_7O_2S\), calculated on the dried basis.

**Description.** A pale-yellow powder.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6).

Compare the spectrum with that obtained with azathioprine RS.

B. Dissolve 0.15 g in 30 ml of dimethyl sulfoxide and dilute to 500 ml with 0.1 M hydrochloric acid. Dilute 25 ml of this solution to 1000 ml with 0.1 M hydrochloric acid.

When examined in the range 230 nm to 350 nm (2.4.7), the resulting solution shows an absorption maximum at about 280 nm; absorbance at 280 nm, about 0.600 to 0.660.

C. To about 20 mg add 100 ml of water, heat and filter. To 5 ml of the filtrate add 1 ml of hydrochloric acid and about 10 mg of zinc powder, stand for 5 minutes. The solution becomes yellow. Filter, cool in iced water, add 0.1 ml of sodium nitrite solution and 0.1 g of sulphamic acid and shake until the

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bubbles disappear. Add 1 ml of á-naphthol solution. A pale-pink precipitate is formed.

**Tests**

**Acidity or alkalinity.** To 0.5 g add 25 ml of carbon dioxide free water, shake for 15 minutes and filter. To 20 ml of the filtrate add 0.1 ml of methyl red solution. Not more than 0.2 ml of 0.01 M hydrochloric acid or 0.01 M sodium hydroxide is required to change the colour of the indicator.

**Chloromethyl nitroimidazole and mercaptopurine.** Determine by thin-layer chromatography (2.4.17), coating the plate with cellulose GF254.

**Mobile phase.** Butanol saturated with dilute ammonia solution.

**NOTE – Prepare the following solutions immediately before use.**

**Test solution.** Dissolve 0.2 g of the substance under examination in dilute ammonia solution and add sufficient dilute ammonia solution to produce 10 ml.

**Reference solution (a).** A 0.02 per cent w/v solution of chloromethyl nitroimidazole RS in dilute ammonia solution.

**Reference solution (b).** A 0.02 per cent w/v solution of mercaptopurine in dilute ammonia solution.

Apply to the plate 5 µl of each solution. After development, dry the plate at 50° and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the test solution, any spots corresponding to chloromethyl nitroimidazole and mercaptopurine are not more intense than the spots in the chromatograms obtained with reference solution (a) (1.0 per cent) and reference solution (b) (1.0 per cent).

**Sulphated ash (2.3.18).** Not more than 0.1 per cent.

**Loss on drying (2.4.19).** Not more than 1.0 per cent, determined on 0.5 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.25 g and dissolve in 25 ml of dimethylformamide. Titrate with 0.1 M tetrabutylammonium hydroxide, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M tetrabutylammonium hydroxide is equivalent to 0.02773 g of C₉H₇N₇O₂S.

**Storage.** Store protected from light.

**Azathioprine Tablets**

Azathioprine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of azathioprine, C₉H₇O₂S.

**Identification**

A. Determine by thin-layer chromatography (2.4.17), coating the plate with cellulose F 254.

**Mobile phase.** A mixture of butan-1-ol saturated with 6 M ammonia.

**Test solution.** Shake a quantity of the powdered tablets containing 0.2 g of Azathioprine with 50 ml of 6 M ammonia, filter through a glass micro fibre filter paper (such as Whatman GF/C) and use the filtrate.

**Reference solution.** A 0.4 per cent w/v solution of azathioprine RS in 6 M ammonia.

Apply to the plate 5 µl of each solution. After removal of the plate, dry the plate at 50° and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Heat a quantity of the powdered tablets containing 20 mg of Azathioprine with 100 ml of water and filter. To 5 ml of the filtrate add 1 ml of hydrochloric acid and 10 mg of zinc powder and allow to stand for 5 minutes; a yellow colour is produced. Filter, cool in ice, add 0.1 ml of a 10 per cent w/v solution of sodium nitrite and 0.1 g of sulphamic acid and shake until the bubbles disappear. Add 1 ml of 2-naphthol solution; a pale pink precipitate is produced.

**Tests**

**5-Chloro-1-methyl-4-nitroimidazole and 6-mercaptopurine.** Determine by thin-layer chromatography (2.4.17), coating the plate with cellulose F 254.

**Mobile phase.** A mixture of butan-1-ol saturated with 6 M ammonia.

**Test solution.** Shake a quantity of the powdered tablets containing 0.2 g of Azathioprine with 10 ml of 6 M ammonia and filter through a glass micro fibre filter paper (such as Whatman GF/C).

**Reference solution (a).** A solution containing 2.0 per cent w/v of azathioprine RS and 0.02 per cent w/v of 6-mercaptopurine in 6 M ammonia.

**Reference solution (b).** A 0.02 per cent w/v solution of 6-mercaptopurine in 6 M ammonia.

**Reference solution (c).** A 0.02 per cent w/v solution of chloromethyl nitroimidazole RS in 6 M ammonia.

Apply to the plate 5 µl of each solution. After removal of the plate, dry the plate at 50° and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution corresponding to 6-mercaptopurine in the chromatogram obtained with reference solution (a) is not more
intense than the spot in the chromatogram obtained with reference solution (b). Any spot corresponding to 5-chloro-1-methyl-4-nitroimidazole in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (c).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Shake a quantity of the powder containing about 0.15 g of Azathioprine with 20 ml of dimethyl sulphoxide for 15 minutes and dilute to 500.0 ml with 0.1 M hydrochloric acid, filter. Dilute 25.0 ml of the filtrate to 1000.0 ml with 0.1 M hydrochloric acid. Measure the absorbance of the resulting solution at the maximum at about 280 nm (2.4.7) using 0.1 M hydrochloric acid as the blank. Calculate the content of C$_9$H$_7$N$_7$O$_2$S using a solution of azathioprine RS of the same concentration in 0.1 M hydrochloric acid.

Storage. Store protected from light.

Azithromycin

![Azithromycin structure](image)

C$_{38}$H$_{72}$N$_2$O$_{12}$ Mol. Wt. 749.0


Azithromycin contains not less than 94.0 per cent and not more than 102.0 per cent of C$_{38}$H$_{72}$N$_2$O$_{12}$, calculated on the anhydrous basis.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with azithromycin RS.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Appearance of solution. Dissolve 0.5 g in anhydrous ethanol and dilute to 50.0 ml with the same solvent (solution A). Solution A is clear (2.4.1) and colourless (2.4.1).

pH (2.4.24). 9.0 to 11.0 determined in a solution prepared by dissolving 0.1 g in 25.0 ml of methanol and further diluting to 50.0 ml with carbon dioxide-free water.

Specific optical rotation (2.4.22). –45.0º to –49.0º, determined in solution A.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 40 volumes acetonitrile and 60 volumes water.

Test solution (a). Dilute 0.1 g of the substance under examination in the solvent mixture and dilute to 25.0 ml with the solvent mixture.

Test solution (b). Dilute 5.0 ml of test solution (a) to 20.0 ml with the solvent mixture.

Reference solution (a). A 0.1 per cent w/v solution of azithromycin RS in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of test solution (a) to 100.0 ml with the solvent mixture.

Reference solution (c). A solution containing 0.01 per cent w/v of azithromycin RS and 6-demethyl-azithromycin RS (azithromycin impurity A) in the solvent mixture.

Reference solution (d). Dissolve the contents of a vial of 3-deoxyazithromycin RS (azithromycin impurity B) in 1.0 ml of the solvent mixture. Use this solution for identification of the peak due to impurity B.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- column temperature 70º,
- mobile phase: a mixture of 10 volumes of a 3.484 per cent w/v solution of dipotassium hydrogen phosphate previously adjusted to pH 6.5 with phosphoric acid, 35 volumes of acetonitrile and 55 volumes of water,
- flow rate. 1 ml per minute,
- spectrophotometer set at 215 nm,
- a 100 µl loop injector.

The relative retention times with reference to azithromycin: impurity A, about 0.42; impurity B, about 1.7.
Inject reference solution (c). The chromatogram obtained shows peaks corresponding to azithromycin and azithromycin impurity A. The test is not valid unless the resolution between these peaks is at least 7.0.

Inject test solution (a) and reference solutions (b) and (d). Record the chromatogram of the test solution for 4.5 times the retention time of azithromycin.

In the chromatogram obtained with test solution (a) the area of any peak corresponding to impurity B is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent). The area of any other secondary peak is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5.0 per cent). Ignore any peak with an area 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Heavy metals (2.3.13). 0.8 g complies with the limit test for heavy metals, Method B (25 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2,3,43). 1.8 per cent to 6.5 per cent, determined on 0.2 g.

Assay. Determine by liquid chromatography (2.4.14) as described under Related Substances.

Inject alternately test solution (b) and reference solution (a).

Calculate the content of C_{38}H_{72}N_{2}O_{12}.

Storage. Store protected from moisture.

Azithromycin Capsules

Azithromycin Capsules contain not less than 90.0 percent and not more than 110.0 percent of the stated amount of azithromycin, (C_{38}H_{72}N_{2}O_{12}).

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 40 volumes acetonitrile and 60 volumes water.

Test solution (a). Dissolve a suitable quantity of the mixed contents of 20 capsules containing about 0.1 g of Azithromycin in the solvent mixture by shaking mechanically, dilute to 25.0 ml with the solvent mixture and filter.

Test solution (b). Dilute 5.0 ml of test solution (a) to 20.0 ml with the solvent mixture.

Reference solution (a). A 0.1 per cent w/v solution of azithromycin RS in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of test solution (a) to 100.0 ml with the solvent mixture.

Reference solution (c). A solution containing 0.01 per cent w/v of azithromycin RS and 6-demethyl-azithromycin RS (azithromycin impurity A) in the solvent mixture.

Reference solution (d). Dissolve the contents of a vial of 3-deoxyazithromycin RS (azithromycin impurity B) in 1.0 ml of the solvent mixture. Use this solution for identification of the peak due to impurity B.

Chromatographic system

– a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
– column temperature 70º,
– mobile phase: a mixture of 10 volumes of a 3.484 per cent w/v solution of dipotassium hydrogen phosphate with the pH previously adjusted to 6.5 with phosphoric acid, 35 volumes of acetonitrile and 55 volumes of water,
– flow rate. 1 ml per minute,
– spectrophotometer set at 215 nm,
– a 100 µl loop injector.

The relative retention times with reference to azithromycin: impurity A, about 0.42; impurity B, about 1.7.

Inject reference solution (c). The chromatogram obtained shows peaks corresponding to azithromycin and azithromycin impurity A. The test is not valid unless the resolution between these peaks is at least 7.0.

Inject test solution (a) and reference solutions (b) and (d). Record the chromatogram of the test solution for 4.5 times the retention time of azithromycin.

In the chromatogram obtained with test solution (a) the area of any peak corresponding to impurity B is not more than 2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent). The area of any other impurity peak is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5.0 per cent). Ignore any peak with an area 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).
Dissolution (2.5.2).

Apparatus No. 1
Medium. 900 ml of a buffer solution prepared by adding to 6 litres of 0.1 M dibasic sodium phosphate about 40 ml of hydrochloric acid to adjust the pH to 6.0, adding 600 mg of trypsin, and mixing.
Speed and time. 100 rpm and 45 minutes.
Withdraw a suitable volume of the medium and filter through a filter having a porosity of 0.5 µm or less.
Determine by liquid chromatography (2.4.14) as described under Related substances using the following solutions.

Test solution. The filtrate from the dissolution vessel suitably diluted with the mobile phase if necessary.

Test solution. A solution of azithromycin RS in the dissolution medium suitably diluted with the mobile phase to obtain a solution having the same concentration as that of the test solution.

Calculate the content of C_{38}H_{72}N_{2}O_{12} in the medium.

D. Not less than 75 per cent of the stated amount of C_{38}H_{72}N_{2}O_{12}.

Water (2.3.43). Not more than 5.0 per cent determined on 0.2 g of the contents of the capsules.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14) as described under Related Substances.

Inject alternately test solution (b) and reference solution (a).

Calculate the content of C_{38}H_{72}N_{2}O_{12} in the capsules.

Storage. Store protected from moisture.

The constituted suspension complies with the tests stated under Oral liquids and with the following tests.

Identification

In the Assay, the retention time of the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 8.5 to 11.0

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 40 volumes acetonitrile and 60 volumes water.

Test solution (a). Transfer an accurately weighed quantity of the oral suspension containing about 0.1 g of Azithromycin to a 25-ml volumetric flask, dissolve in the solvent mixture, dilute to 25.0 ml with the solvent mixture and filter.

Test solution (b). Dilute 5.0 ml of test solution (a) to 20.0 ml with the solvent mixture.

Reference solution (a). A 0.1 per cent w/v solution of azithromycin RS in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of test solution (a) to 100.0 ml with the solvent mixture.

Reference solution (c). A solution containing 0.01 per cent w/v of azithromycin RS and 6-demethyl-azithromycin RS (azithromycin impurity A) in the solvent mixture.

Reference solution (d). Dissolve the contents of a vial of 3-deoxyazithromycin RS (azithromycin impurity B) in 1.0 ml of the solvent mixture. Use this solution for identification of the peak due to impurity B.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- column temperature 70º,
- mobile phase: a mixture of 10 volumes of a 3.484 per cent w/v solution of dipotassium hydrogen phosphate with

Azithromycin Oral Suspension

Azithromycin Oral Suspension is a dry mixture of Azithromycin with buffering agents and other excipients. It contains a suitable flavouring agent.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of Water just before use.

Azithromycin Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of azithromycin, (C_{38}H_{72}N_{2}O_{12}).

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of azithromycin, (C_{38}H_{72}N_{2}O_{12}).

The contents of the sealed container comply with the following test.

Water (2.3.43). Not more than 1.5 per cent, determined on 0.5 g.

Storage. Store protected from moisture.

The constituted suspension complies with the tests stated under Oral liquids and with the following tests.

Identification

In the Assay, the retention time of the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 8.5 to 11.0

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 40 volumes acetonitrile and 60 volumes water.

Test solution (a). Transfer an accurately weighed quantity of the oral suspension containing about 0.1 g of Azithromycin to a 25-ml volumetric flask, dissolve in the solvent mixture, dilute to 25.0 ml with the solvent mixture and filter.

Test solution (b). Dilute 5.0 ml of test solution (a) to 20.0 ml with the solvent mixture.

Reference solution (a). A 0.1 per cent w/v solution of azithromycin RS in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of test solution (a) to 100.0 ml with the solvent mixture.

Reference solution (c). A solution containing 0.01 per cent w/v of azithromycin RS and 6-demethyl-azithromycin RS (azithromycin impurity A) in the solvent mixture.

Reference solution (d). Dissolve the contents of a vial of 3-deoxyazithromycin RS (azithromycin impurity B) in 1.0 ml of the solvent mixture. Use this solution for identification of the peak due to impurity B.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- column temperature 70º,
- mobile phase: a mixture of 10 volumes of a 3.484 per cent w/v solution of dipotassium hydrogen phosphate with
the pH previously adjusted to 6.5 with phosphoric acid, 35 volumes of acetonitrile and 55 volumes of water,
  – flow rate. 1 ml per minute,
  – spectrophotometer set at 215 nm,
  – a 100 µl loop injector.

The relative retention times with reference to azithromycin:
impurity A, about 0.42; impurity B, about 1.7.

Inject reference solution (c). The chromatogram obtained shows peaks corresponding to azithromycin and azithromycin
impurity A. The test is not valid unless the resolution between these peaks is at least 7.0.

Inject test solution (a) and reference solutions (b) and (d). Record the chromatogram of the test solution for 4.5 times the
retention time of azithromycin.

In the chromatogram obtained with test solution (a) the area of any peak corresponding to impurity B is not more than
twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent). The area of
any other impurity peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Assay. Determine by liquid chromatography (2.4.14) as
described under Related Substances.

Inject alternately test solution (b) and reference solution (a).

Calculate the content of C38H72N2O12 in the suspension.

Azithromycin Tablets

Azithromycin Tablets contain not less than 90.0 percent and
not more than 110.0 percent of the stated amount of
azithromycin, (C38H72N2O12).

Identification

In the Assay, the retention time of the principal peak in the
chromatogram obtained with the test solution corresponds to
the peak in the chromatogram obtained with the reference
solution.

Tests

Related substances. Determine by liquid chromatography
(2.4.14).

Solvent mixture. 40 volumes acetonitrile and 60 volumes
water.

Test solution (a). Weigh and powder 20 tablets. Dissolve a
quantity of the powder containing about 0.1 g of Azithromycin
in the solvent mixture by shaking mechanically, dilute to 25.0
ml with the solvent mixture and filter.

Test solution (b). Dilute 5.0 ml of test solution (a) to 20.0 ml
with the solvent mixture.

Reference solution (a). A 0.1 per cent w/v solution of
azithromycin RS in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of test solution (a) to
100.0 ml with the solvent mixture.

Reference solution (c). A solution containing 0.01 per cent w/ v of azithromycin RS and 6-demethyl-azithromycin RS
(azithromycin impurity A) in the solvent mixture.

Reference solution (d). Dissolve the contents of a vial of 3-
deoxyazithromycin RS (azithromycin impurity B) in 1.0 ml of
the solvent mixture. Use this solution for identification of the
peak due to impurity B.

Chromatographic system
  – a stainless steel column 15 cm x 4.6 mm, packed with
    octadecylsilane chemically bonded to porous silica or
    ceramic microparticles (5 µm),
  – column temperature 70º,
  – mobile phase: a mixture of 10 volumes of a 3.484 per cent
    w/v solution of dipotassium hydrogen phosphate with
    the pH previously adjusted to 6.5 with phosphoric acid,
    35 volumes of acetonitrile and 55 volumes of water,
  – flow rate. 1 ml per minute,
  – spectrophotometer set at 215 nm,
  – a 100 µl loop injector.

The relative retention times with reference to azithromycin:
impurity A, about 0.42; impurity B, about 1.7.

Inject reference solution (c). The chromatogram obtained
shows peaks corresponding to azithromycin and azithromycin
impurity A. The test is not valid unless the resolution between
these peaks is at least 7.0.

Inject test solution (a) and reference solutions (b) and (d).
Record the chromatogram of the test solution for 4.5 times the
retention time of azithromycin.

In the chromatogram obtained with test solution (a) the area
of any peak corresponding to impurity B is not more than
twice the area of the principal peak in the chromatogram
obtained with reference solution (b) (2.0 per cent). The area of
any other impurity peak is not more than the area of the
principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

The sum of the areas of all the
impurity peaks is not more than 5 times the area of the principal
peak in the chromatogram obtained with reference solution (b) (5.0 per cent). Ignore any peak with an area 0.1 times the
area of the principal peak in the chromatogram obtained with
reference solution (b) (0.1 per cent).
**Dissolution (2.5.2).**

Apparatus No. 1

Medium. 900 ml of a buffer solution prepared by adding to 6 litres of 0.1 M dibasic sodium phosphate about 40 ml of hydrochloric acid to adjust the pH to 6.0, adding 600 mg of trypsin, and mixing.

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a filter having a porosity of 0.5 μm or less.

Determine by liquid chromatography (2.4.14) as described under Related substances using the following solutions.

*Test solution.* The filtrate from the dissolution vessel suitably diluted with the mobile phase if necessary.

*Reference solution.* A solution of azithromycin RS in the dissolution medium suitably diluted with the mobile phase to obtain a solution having the same concentration as that of the test solution.

Calculate the content of C_{38}H_{72}N_{2}O_{12} in the medium.

D. Not less than 75 per cent of the stated amount of C_{38}H_{72}N_{2}O_{12}.

*Water (2.3.43).* Not more than 6.0 per cent determined on 0.2 g of the powdered tablets.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14) as described under Related Substances.

Inject alternately test solution (b) and reference solution (a).

Calculate the content of C_{38}H_{72}N_{2}O_{12} in the tablets.
B

Bacitracin
Bacitracin Zinc
Baclofen
Baclofen Oral Suspension
Baclofen Tablets
Barium Sulphate
Barium Sulphate For Suspension
Beclomethasone Dipropionate
Beclomethasone Inhalation
White Beeswax
Yellow Beeswax
Bentonite
Benzalkonium Chloride
Benzalkonium Chloride Solution
Benzathine Penicillin
Benzathine Penicillin Injection
Fortified Benzathine Penicillin Injection
Benzathine Penicillin Tablets
Benzhexol Hydrochloride
Benzhexol Tablets
Benzocaine
Benzoic Acid
Compound Benzoic Acid Ointment
Benzoin
Compound Benzoin Tincture
Benzyl Alcohol
Benzyl Benzoate
Benzyl Benzoate Application
Benzylpenicillin Potassium
Benzylpenicillin Sodium
Benzylpenicillin Injection
Betahistine Dihydrochloride
Betahistine Tablets
Betamethasone
Betamethasone Tablets
Betamethasone Sodium Phosphate
Betamethasone Eye Drops
Betamethasone Injection
Betamethasone Sodium Phosphate Tablets
Betamethasone Valerate
Betamethasone Valerate Ointment
Biperiden Hydrochloride
Biperiden Tablets
Bisacodyl
Bisacodyl Suppositories
Bisacodyl Tablets
Bismuth Subcarbonate
Bleomycin Sulphate
Bleomycin Injection
Boric Acid
Bromhexine Hydrochloride
Bromhexine Tablets
Bromocriptine Mesylate
Bromocriptine Capsules
Bromocriptine Tablets
Bronopol
Budesonide
Bupivacaine Hydrochloride
Bupivacaine Injection
Buprenorphine Hydrochloride
Buprenorphine Injection
Buprenorphine Tablets
Busulphan

Busulphan Tablets

Butylated Hydroxyanisole

Butylated Hydroxytoluene
Bacitracin

Bacitracin is a mixture of polypeptides produced by the growth of certain strains of *Bacillus licheniformis* and *B. subtilis* (Fam. Bacillaceae). Its main components are Bacitracin A1, B1 and B2.

Bacitracin has a potency of not less than 60 Units of bacitracin activity per mg, calculated on the dried basis.

**Description.** A white or almost white powder; odourless or with a faint odour; hygroscopic.

**Identification**

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 75 parts of phenol and 25 parts of water.

**Test solution.** Dissolve 5 mg of the substance under examination in a mixture of 0.5 ml of hydrochloric acid and 0.5 ml of water, heat in a sealed tube at 135° for 5 hours, evaporate to dryness on a water-bath, continue to heat until the odour of hydrogen chloride is no longer detectable and dissolve the residue in 0.5 ml of water.

**Reference solution.** Prepare in the same manner as the test solution but using bacitracin zinc RS in place of the substance under examination.

Apply to the plate 5 µl of each solution as bands 10 mm wide. Place the plate in the tank so that it is not in contact with the mobile phase and allow to stand for at least 12 hours before development. Allow the mobile phase to rise 10 cm. Dry the plate at 105°, spray with ethanolic ninhydrin solution and heat at 110° for 5 minutes. The bands in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

B. Shake 5 mg with 1 ml of water, add 1 ml of a 0.2 per cent w/v solution of ninhydrin in *J*-butanol and 0.5 ml of pyridine and heat at 100° for 5 minutes; a deep purple colour is produced.

**Tests**

**Appearance of solution.** A 1.0 per cent w/v solution in carbon dioxide-free water is clear, (2.4.1), and not more intensely coloured than reference solution BYS6 (2.4.1).

**pH** (2.4.24), 5.5 to 7.5, determined in a 1.0 per cent w/v solution.

**Bacitracin F and related substances.** The ratio of the absorbance (2.4.7) at the maximum at about 290 nm to that at the maximum at about 252 nm of a 0.03 per cent w/v solution in 0.05 M sulphuric acid is not more than 0.20.

**Sulphated ash** (2.3.18). Not more than 3.0 per cent.

**Loss on drying** (2.4.19). Not more than 5.0 per cent, determined on 0.5 g by drying in an oven over phosphorus pentoxide at 60° at a pressure not exceeding 0.1 kPa for 3 hours.

**Assay.** Determine by the microbiological assay of antibiotics, Method A (2.2.10), and express the results in Units per mg.

*Bacitracin intended for administration as a spray in internal body cavities without a further appropriate procedure for removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 0.01 Endotoxin Unit per unit.

*Bacitracin intended for use in the manufacture of parenteral preparations without a further appropriate sterilization procedure complies with the following additional requirement.*

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage.** Store protected from moisture at a temperature not exceeding 30°. If it is intended for use in the manufacture of ophthalmic preparations, the container should be sterile and sealed so as to exclude micro-organisms.

**Labelling.** The label states (1) the number of Units per mg; (2) whether or not the contents are intended for use in the manufacture of ophthalmic preparations.

Bacitracin Zinc

Bacitracin Zinc is the zinc complex of Bacitracin.

Bacitracin Zinc has a potency of not less than 60 Units of bacitracin activity per mg, calculated on the dried basis.

**Description.** A white or light yellowish-grey powder; odourless or with a faint odour; hygroscopic.

**Identification**

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 75 volumes of phenol and 25 volumes of water.

**Test solution.** Dissolve 5 mg of the substance under examination in a mixture of 0.5 ml of hydrochloric acid and 0.5 ml of water, heat in a sealed tube at 135° for 5 hours, evaporate to dryness on a water-bath, continue to heat until the odour of hydrogen chloride is no longer detectable and dissolve the residue in 0.5 ml of water.

**Reference solution.** Prepare in the same manner as the test solution but using bacitracin zinc RS in place of the substance under examination.
Apply to the plate 5 µl of each solution as bands 10 mm wide. Place the plate in the tank so that it is not in contact with the mobile phase and allow to stand for at least 12 hours before development. Allow the mobile phase to rise to 10 cm. Dry the plate at 105°, spray with ethanolic ninhydrin solution and heat at 110° for 5 minutes. The bands in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

B. Shake 5 mg with 1 ml of water, add 1 ml of a 0.2 per cent w/v solution of ninhydrin in 1-butanol and 0.5 ml of pyridine and heat at 100° for 5 minutes; a deep purple colour is produced.

C. Ignite 0.15 g and allow to cool. The residue on dissolving in 1 ml of 2 M hydrochloric acid and diluting with 4 ml of water gives the reactions of zinc salts (2.3.1).

Tests

pH (2.4.24). 6.0 to 7.5, determined on the filtrate obtained by shaking 1.0 g with 10 ml of carbon dioxide-free water.

Bacitracin F and related substances. The ratio of the absorbance (2.4.7) at the maximum at about 290 nm to that at the maximum at about 252 nm of a 0.03 per cent w/v solution in 0.05 M sulphuric acid is not more than 0.15.

Zinc content. 4.0 per cent to 8.0 per cent, calculated on the dried basis, determined by the following method. Weigh accurately about 0.2 g and dissolve in 20 ml of water and 3 ml of strong ammonia-ammonium chloride solution and titrate with 0.01 M disodium edetate using mordant black 11 mixture as indicator.

1 ml of 0.01 M disodium edetate is equivalent to 0.000654 g of Zn.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 0.5 g by drying in an oven over phosphorus pentoxide at 60° at a pressure not exceeding 0.1 kPa for 3 hours.

Assay. Weigh accurately about 0.1 g, suspend in 10 ml of water and 0.5 ml of 2 M hydrochloric acid and add sufficient water to produce 200.0 ml. Allow to stand at room temperature for 30 minutes. Determine by the microbiological assay of antibiotics, Method A (2.2.10), and express the results in Units per mg.

Bacitracin Zinc intended for administration as a spray in internal body cavities without a further appropriate procedure for removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.01 Endotoxin Unit per unit.

Bacitracin Zinc intended for administration as a spray in internal body cavities without a further appropriate sterilization procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture at a temperature not exceeding 30°. If it is intended for administration as a spray in internal body cavities, the container should be sterile and sealed so as to exclude micro-organisms.

Labelling. The label states (1) the number of Units per mg; (2) whether or not the contents are intended for administration as a spray in internal body cavities.

Baclofen

C_{10}H_{12}ClNO_{2}  Mol. Wt. 213.7

Baclofen is (3RS)-4-amino-3-(4-chlorophenyl)butanoic acid.

Baclofen contains not less than 98.0 per cent and not more than 101.0 per cent of C_{10}H_{12}ClNO_{2}, calculated on the anhydrous basis.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with baclofen RS.

B. When examined in the range 220 nm to 320 nm (2.4.7), a 0.07 per cent w/v solution shows three absorption maxima, at 259 nm, 266 nm and 275 nm. The specific absorbances at these maxima are 9.8 to 10.8, 11.5 to 12.7 and 8.4 to 9.3, respectively.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 5 volumes of anhydrous formic acid, 5 volumes of water, 20 volumes of methanol and 40 volumes of ethyl acetate.

Test solution. Dissolve 10 mg of the substance under examination in the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution. A 0.1 per cent w/v solution of baclofen RS in the mobile phase.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate and spray with ninhydrin solution until the plate is slightly wet. Place the plate in an
oven maintained at 100º for 10 minutes. Examine in daylight. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Appearance of solution. Dissolve 0.5 g in 1 M sodium hydroxide and dilute to 25 ml with the same solvent. The freshly prepared solution is not more intensely coloured than reference solution BY5 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (a). A 0.25 per cent w/v solution of (4RS)-4-(4-chlorophenyl)pyrrolidin-2-one RS (baclofen impurity A) in the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

Reference solution (c). Dilute 2 ml of the test solution to 100 ml with the mobile phase.

Reference solution (d). Dilute 2 ml of the test solution and 2 ml of reference solution (a) to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilil silica gel (10 µm),
- mobile phase: a solution of 1.822 g of sodium hexanesulphonate in 1000 ml of a mixture of 560 volumes of water, 440 volumes of methanol and 5 volumes of glacial acetic acid,
- flow rate. 2 ml per minute,
- spectrophotometer set at 266 nm,
- a 20 µl loop injector.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (c) is at least 50 per cent of the full scale of the recorder. Inject reference solution (d). The test is not valid unless the resolution between the peaks corresponding to baclofen and impurity A is at least 2.0.

Inject the test solution, reference solution (b) and reference solution (c). Continue the chromatography for 5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of the peak corresponding to baclofen impurity A is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of the areas of all such peaks is not greater than the area of the principal peak in the chromatogram obtained with reference solution (c) (2.0 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 1.0 per cent, determined on 1.0 g.

Assay. Weigh accurately about 0.15 g and dissolve in 50 ml of anhydrous acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration. 1 ml of 0.1 M perchloric acid is equivalent to 0.02137 g of C_{10}H_{12}ClNO_2.

Storage. Store protected from moisture.

Baclofen Oral Solution

Baclofen Oral Solution is a solution of Baclofen in a suitable aqueous vehicle.

Baclofen Oral Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of baclofen, C_{10}H_{12}ClNO_2.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Solvent mixture. 35 volumes of acetonitrile and 65 volumes of water.

Mobile phase. A mixture of 20 volumes of glacial acetic acid, 20 volumes of water and 80 volumes of butan-1-ol.

Test solution. Dilute a volume of the oral solution containing 5 mg of Baclofen to 100 ml with the solvent mixture.

Reference solution. A 0.005 per cent w/v solution of baclofen RS in the solvent mixture.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air. Place an evaporating dish containing a mixture of 4 ml of water, 1 ml of 7 M hydrochloric acid and 0.5 g of potassium permanganate in a chromatography tank, close the tank and allow to stand for 2 minutes. Place the plate in the tank, close the tank and leave the plate in contact with the vapour for 1 minute. After removal of the plate, place it in a current of cold air until an area of coating below the line of application shows only a faint blue colour on the addition of 0.05 ml of potassium iodide and starch solution. Spray the plate with potassium iodide and starch solution and examine in daylight. The chromatogram obtained with the test solution exhibits a spot that corresponds to the spot in the chromatogram obtained with the reference solution.
B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Lactam. Determine by liquid chromatography (2.4.14) as described under Assay using the following solutions.

Test solution. Use the test solution prepared for the Assay.

Reference solution (a). A solution containing 0.0002 per cent w/v of (4RS)-4-(4-chlorophenyl)pyrrolidin-2-one RS (baclofen impurity A) in the mobile phase.

Reference solution (b). A solution containing 0.01 per cent w/v of baclofen RS, 0.0003 per cent w/v of propyl 4-hydroxybenzoate, 0.0003 per cent w/v of methyl 4-hydroxybenzoate and 0.0002 per cent w/v of baclofen impurity A in the mobile phase.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to baclofen impurity A and propyl-4-hydroxybenzoate is at least 5.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution the area of any peak corresponding to baclofen impurity A (lactam) is not greater than the area of the peak in the chromatogram obtained with reference solution (a) (2.0 per cent).

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a weighed quantity of the oral solution containing about 5 mg of Baclofen to 50.0 ml with the mobile phase.

Reference solution (a). A 0.01 per cent w/v solution of baclofen RS in the mobile phase.

Reference solution (b). A solution containing 0.01 per cent w/v of baclofen RS, 0.0003 per cent w/v of propyl 4-hydroxybenzoate and 0.0002 per cent w/v of baclofen impurity A RS in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilyl silica gel (10 µm) (such as Nucleosil C18),
- mobile phase: a solution prepared by dissolving 5 g of sodium dodecyl sulphate in a mixture of 5 ml of orthophosphoric acid and 650 ml of water and diluting to 1000 ml with acetonitrile,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 218 nm,
- a 20 µl loop injector.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to baclofen impurity A and propyl-4-hydroxybenzoate is at least 5.0.

Inject alternately the test solution and reference solution (a).

Determine the weight per ml (2.4.29) of the oral solution and calculate the content of C_{10}H_{12}ClNO_{2}, weight in volume.

Storage. Store protected from light in a refrigerator (2º and 8º). Do not freeze.

Baclofen Tablets

Baclofen tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of baclofen, C_{10}H_{12}ClNO_{2}.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Solvent mixture. 4 volumes of absolute ethanol and 1 volume of glacial acetic acid.

Mobile phase. A mixture of 80 volumes of butan-1-ol, 20 volumes of glacial acetic acid and 20 volumes of water.

Test solution. Shake a quantity of the powdered tablets containing 20 mg of Baclofen with 20 ml of the solvent mixture for 30 minutes and filter.

Reference solution. A 0.1 per cent w/v solution of baclofen RS in the solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in air, spray with ninhydrin solution and heat at 100º for 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Lactam. Determine by liquid chromatography (2.4.14).

Test solution. Mix with the aid of ultrasound a quantity of the powdered tablets containing 0.1 g of Baclofen with 50 ml of the mobile phase for 30 minutes, shaking occasionally to disperse the sample, and filter through a glass-fibre filter (such as Whatman GF/C).

Reference solution (a). A solution containing 0.004 per cent w/v of (4RS)-4-(4-chlorophenyl)pyrrolidin-2-one RS (baclofen impurity A) in the mobile phase.
Reference solution (b). A solution containing 0.2 per cent w/v of baclofen RS and 0.004 per cent w/v of (4RS)-4-(4-chlorophenyl)pyrrolidin-2-one RS (baclofen impurity A) in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilyl silica gel (10 µm) (such as Spherisorb ODS 1),
- mobile phase: a mixture of 5 volumes of glacial acetic acid, 440 volumes of methanol and 560 volumes of water containing 1.822 g per litre of sodium hexanesulphonate,
- flow rate. 2 ml per minute,
- spectrophotometer set at 265 nm,
- a 20 µl loop injector.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to baclofen and baclofen impurity A is at least 2.0.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution the area of any peak corresponding to baclofen impurity A (lactam) is not greater than the area of the peak in the chromatogram obtained with the reference solution (2.0 per cent).

Dissolution (2.5.2)
Apparatus No 1
Medium. 900 ml of 0.1 M hydrochloric acid
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14) as described under Assay using the following solutions.

Test solution. Use the filtrate as given above.

Reference solution. A 0.001 per cent w/v solution of baclofen RS in the dissolution medium.

Calculate the content of C₁₀H₁₂ClNO₂ in the tablets.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Add a quantity of whole tablets containing 0.1 g of Baclofen to 25 ml of a mixture of 100 volumes of water and 1 volume of glacial acetic acid and disperse with the aid of ultrasound. Dilute to 50.0 ml with methanol, filter and use the filtrate.

Reference solution. A solution containing 0.2 per cent w/v of baclofen RS in a mixture of 100 volumes of methanol, 100 volumes of water and 1 volume of glacial acetic acid.

Chromatographic system
- a stainless steel column 20 cm × 4.6 mm, packed with octadecylsilyl silica gel (10 µm) (such as Nucleosil C18),
- mobile phase: 0.01 M sodium hexanesulphonate in a mixture of 100 volumes of methanol, 100 volumes of water and 1 volume of glacial acetic acid,
- flow rate. 2 ml per minute,
- spectrophotometer set at 265 nm,
- a 20 µl loop injector.

Inject alternately the test solution and the reference solution. Calculate the content of C₁₀H₁₂ClNO₂ in the tablets.

Barium Sulphate
BaSO₄, Mol. Wt. 233.4

Barium Sulphate contains not less than 97.5 per cent and not more than 100.5 per cent of BaSO₄.

Description. A fine, heavy, white powder, free from gritty particles; odourless.

Identification
A. Boil 0.2 g with 5 ml of a 50 per cent w/v solution of sodium carbonate for 5 minutes, add 10 ml of water and filter. Reserve the residue for test B. Acidify the filtrate with dilute hydrochloric acid; the solution gives the reactions of sulphates (2.3.1).

B. Wash the residue obtained in test A three times with successive small quantities of water. To the residue add 5 ml of dilute hydrochloric acid, filter and add to the filtrate 0.3 ml of dilute sulphuric acid; a white precipitate is formed which is insoluble in dilute sodium hydroxide solution.

Tests
Acidity or alkalinity. Heat 5.0 g with 20 ml of carbon dioxide-free water on a water-bath for 5 minutes and filter. To 10 ml of the filtrate add 1 drop of bromthymol blue solution. Not more than 0.5 ml of 0.01 M hydrochloric acid or 0.01 M sodium hydroxide is required to change the colour of the solution.

Arsenic (2.3.10). Disperse 5.0 g in 50 ml of water and add 10 ml of stannated hydrochloric acid AsT. The resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals (2.3.13). Boil 4.0 g with a mixture of 2 ml of glacial acetic acid and 48 ml of water for 10 minutes. Add water to make up to 50 ml, filter and reject the first 5 ml of the filtrate. 25 ml of the filtrate complies with the limit test for heavy metals, Method A (10 ppm).

Phosphate. Boil 1 g with a mixture of 3 ml of nitric acid and 5 ml of water for 5 minutes and add water to restore the original volume. Filter through a filter paper previously washed with dilute nitric acid. Add to the warm filtrate an equal volume of ammonium molybdate solution; no yellow precipitate is formed.
**Sulphide.** Boil 10 g with a mixture of 10 ml of *dilute hydrochloric acid* and 90 ml of *water* for 10 minutes. Expose a *lead acetate paper* to the vapours; the paper does not darken.

**Acid-soluble substances.** Cool the mixture obtained in the test for Sulphide, add *water* to restore the original volume and filter through a filter paper previously washed with a mixture of 10 ml of *dilute hydrochloric acid* and 90 ml of *water*, returning the first portions, if necessary, to obtain a clear filtrate. Evaporate 50 ml of the filtrate to dryness on a water-bath and add 2 drops of *hydrochloric acid* and 10 ml of *hot water*. Filter again through acid-washed paper, prepared as directed above, wash the filter paper with 10 ml of *hot water* and evaporate the combined filtrate and washings. Dry the residue at 105°C, cool and weigh (0.3 per cent).

**Soluble barium salts.** Digest the residue obtained in the test for Acid-soluble substances with 10 ml of *water* and filter through a filter paper previously washed with a mixture of 10 ml of *dilute hydrochloric acid* and 90 ml of *water*. Add 0.5 ml of *dilute sulphuric acid* to the clear filtrate and set aside for 30 minutes; no turbidity is produced.

**Bulkiness.** Place 5.0 g in a glass-stoppered 50-ml graduated cylinder having the 50-ml graduation mark 14 cm from the base. Add *water* to 50 ml, shake the mixture for 5 minutes and allow to stand for 15 minutes; it does not settle below the 15 ml mark.

**Loss on ignition.** Not more than 2.5 per cent, determined on 1.0 g at 600°C.

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**Barium Sulphate Suspension**

**Barium Meal**

Barium Sulphate Suspension is a dry mixture of Barium Sulphate with suitable flavours, colours, preservatives and suspending/dispersing agents.

Barium Sulphate Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of barium sulphate, BaSO₄.

**Description.** A white or coloured, fine powder or granules.

**Identification.**

A. Ignite 1 g to constant weight. Cool, boil 0.2 g of the residue with 5 ml of a 50 per cent w/v solution of *sodium carbonate* for 5 minutes, add 10 ml of *water* and filter. Reserve the residue for test B. Acidify the filtrate with *dilute hydrochloric acid*; the solution gives the reactions of sulphates (2.3.1).

B. Wash the residue obtained in test A three times with successive small quantities of *water*. To the residue add 5 ml of *dilute hydrochloric acid*, filter and add to the filtrate 0.3 ml of *dilute sulphuric acid*; a white precipitate is formed which is insoluble in *dilute sodium hydroxide solution*.

**Tests**

**pH (2.4.24).** 4.0 to 8.0, determined in a 75.0 per cent w/v suspension in *water*.

**Loss on drying (2.4.19).** Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°C for 4 hours.

**Assay.** Weigh accurately about 0.6 g in a platinum crucible, add 5 g of *sodium carbonate* and 5 g of *potassium carbonate* and mix. Heat to 1000°C and maintain at this temperature for 15 minutes. Allow to cool and suspend the residue in 150 ml of *water*. Wash the crucible with 2 ml of *acetic acid* and add to the suspension. Cool in ice and filter by decantation, transferring as little of the solid matter as possible to the filter. Wash the residue with successive quantities of a 2 per cent w/v solution of *sodium carbonate* until the washings are free from sulphate and discard the washings. Add 5 ml of *dilute hydrochloric acid* to the filter and wash through into the vessel containing the bulk of the solid matter with *water*. Add 5 ml of *hydrochloric acid* and dilute to 100 ml with *water*. Add 10 ml of a 40 per cent w/v solution of *ammonium acetate*, 25 ml of a 10 per cent w/v solution of *potassium dichromate* and 10 g of *urea*. Cover, digest in an oven at 80°C to 85°C for 16 hours and filter while still hot through a sintered-glass filter (porosity No. 4), washing the precipitate initially with a 0.5 per cent w/v solution of *potassium dichromate* and finally with 2 ml of *water*. Dry to constant weight at 105°C. 1 g of the residue is equivalent to 0.9213 g of BaSO₄.

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**Beclomethasone Dipropionate**

Beclomethasone Dipropionate is 9α-chloro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-diene-17,21-diyldipropionate.

Beclomethasone Dipropionate contains not less than 96.0 per cent and not more than 103.0 per cent of C₂₈H₃₇ClO₇, calculated on the dried basis.

**Description.** A white to creamy-white, crystalline powder; odourless.
**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with beclomethasone dipropionate RS or with the reference spectrum of beclomethasone dipropionate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Solvent mixture.** A mixture of 90 volumes of acetone and 10 volumes of 1,2-propanediol.

**Mobile phase.** A mixture of 40 volumes of cyclohexane and 10 volumes of toluene.

**Test solution.** Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

**Reference solution (a).** Dissolve 25 mg of beclomethasone dipropionate RS in 10 ml of the solvent mixture.

**Reference solution (b).** Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with ethanolic sulphuric acid (20 per cent v/v). Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. Determine by the oxygen flask method (2.3.34), on 25 mg and use a mixture of 20 ml of water and 1 ml of 1 M sodium hydroxide as the absorbing liquid. The liquid gives reaction A of chlorides (2.3.1).

D. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

**Tests**

**Specific optical rotation** (2.4.22). +88.0° to +94.0°, determined in a 1.0 per cent w/v solution in dioxan.

**Light absorption.** Dissolve 50.0 mg in sufficient ethanol (95 per cent) to produce 100.0 ml and dilute 2.0 ml of this solution to 50.0 ml with the same solvent. Absorbance of the resulting solution at the maximum at about 238 nm, 0.57 to 0.60 (2.4.7).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh accurately about 70 mg of the substance under examination, dissolve in methanol and dilute to 50.0 ml with same solvent. To 4.0 ml of this solution add 4.0 ml of a 0.12 per cent w/v solution of testosterone propionate RS (internal standard).

**Reference solution.** Dissolve an accurately weighed quantity of beclomethasone dipropionate RS in methanol and dilute to obtain a solution having a known concentration of about 1.4 mg per ml. To 4.0 ml of this solution add 4.0 ml of a 0.12 per cent w/v solution of testosterone propionate RS (internal standard).

**Chromatographic system**
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilyl silica gel (3 to 10 µm),
- mobile phase: a mixture of 3 volumes of acetonitrile and 2 volumes of water, or such that the retention time of beclomethasone dipropionate is approximately 6 minutes and that of testosterone propionate is approximately 10 minutes,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 3.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the percentage content of C28H37ClO7.

**Storage.** Store protected from light.

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**Beclomethasone Inhalation**

Beclomethasone Dipropionate Inhalation; Beclomethasone Inhalation Aerosol

Beclomethasone Inhalation is a suspension of Beclomethasone Dipropionate in a suitable liquid in a suitable pressurised container.

Beclomethasone Inhalation delivers not less than 80.0 per cent and not more than 120.0 per cent of the stated amount per inhalation of beclomethasone dipropionate, C28H37ClO7, by actuation of the valve.
Identification

A. Discharge the container a sufficient number of times at low relative humidity into a mortar to obtain about 2 mg of anhydrous Beclomethasone Dipropionate. Heat at 110º for 2 hours at a pressure of 2kPa, cool, grind the residue thoroughly with 0.1 g of potassium bromide, add a further 0.2 g of potassium bromide and mix thoroughly.

On the resultant dispersion determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with beclomethasone dipropionate RS or with the reference spectrum of beclometasone dipropionate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to beclometasone dipropionate in the reference solution.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 3 volumes of methanol and 97 volumes of dichloroethane.

Test solution. Discharge from the container into a small, dry flask a sufficient number of times to obtain 0.5 g of Beclometasone Dipropionate and dissolve the residue in 2 ml of acetone. Evaporate the solution to a volume such that the whole solution can be applied to the plate.

Reference solution (a). A 0.1 per cent w/v solution of beclomethasone dipropionate RS in acetone.

Reference solution (b). Dilute 5 ml of reference solution (a) to 10 ml with acetone.

Reference solution (c). Dilute 5 ml of reference solution (a) to 20 ml with acetone.

Test solution. Discharge from the container into a small, dry flask a sufficient number of times to obtain 0.5 g of Beclometasone Dipropionate and dissolve the residue in 2 ml of acetone. Evaporate the solution to a volume such that the whole solution can be applied to the plate.

Reference solution (a). A 0.1 per cent w/v solution of beclomethasone dipropionate RS in acetone.

Reference solution (b). Dilute 5 ml of reference solution (a) to 10 ml with acetone.

Reference solution (c). Dilute 5 ml of reference solution (a) to 20 ml with acetone.

Other tests. Complies with the tests stated under Inhalation Preparations (Pressurised metered-dose Preparations).

Follow the procedure described under Assay wherever the amount of active substance is to be determined in any test.

Assay. Carry out the test for Content of active ingredient delivered per actuation stated under Inhalation Preparations (Pressurised metered-dose Preparations).

Use 40 ml of dehydrated methanol as the solvent. Discharge the number of deliveries that constitute the minimum recommended dose, keep the solution on a water-bath for 5 minutes to expel the propellants. Transfer the solution and washings to a flask containing sufficient testosterone propionate RS (internal standard) in methanol that, on dilution to a suitable volume with appropriate amounts of water and methanol, the final solution contains 0.00015 per cent w/v each of testosterone propionate and beclomethasone dipropionate in the methanol-water mixture in the proportions 70:30 by volume.

Determine by liquid chromatography (2.4.14).

Test solution. The diluted solution obtained as given above.

Reference solution. A solution containing 0.00015 per cent w/v each of the internal standard and beclomethasone dipropionate RS in the mobile phase.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 50º
- mobile phase: a mixture of 70 volumes of methanol and 30 volumes of water, adjusted if necessary so that the resolution between the peaks due to beclomethasone dipropionate and the internal standard is not less than 2.0,
- flow rate. 2 ml per minute,
- spectrophotometer set at 239 nm,
- a 20 µl loop injector.

Inject the test solution and the reference solution. The test is not valid unless the resolution between the two principal peaks in the chromatogram obtained with the reference solution is at least 2.0.

Calculate the amount of C_{28}H_{37}ClO_{7} delivered per actuation of the valve.

Determine the content of active ingredient a second and third time by repeating the procedure on the middle ten and on the last ten successive combined actuations of the valve. For each of the three determinations the average content of C_{28}H_{37}ClO_{7} delivered per actuation of the valve meets the requirements.

Storage. Store protected from moisture at a temperature not exceeding 30º.

Labelling. The label states the amount of active ingredient delivered per inhalation.
White Beeswax

White Beeswax is obtained by bleaching Yellow Beeswax.

Description. Yellowish-white pieces or plates, translucent when thin, with a fine-grained, matt, non-crystalline fracture; becomes soft and pliable when warmed by hand. Odour, faint and characteristic and similar to that of yellow beeswax.

Tests

Melting range (2.4.21). 61°C to 65°C, determined by Method IV.

Acid value (2.3.23). 5 to 15, determined by the following method. Weigh accurately about 5.0 g in a 250-ml conical flask fitted with a reflux condenser, add 40 ml of xylene and a few glass beads, heat until dissolved, add 20 ml of ethanol (95 per cent) and 0.5 ml of phenolphthalein solution and titrate the hot solution with 0.5 M ethanolic potassium hydroxide until a red colour persists for at least 10 seconds (n₁, ml). Repeat the procedure omitting the substance under examination (n₂, ml). Calculate the Acid value from the expression 28.05(n₁ - n₂)/w, where w is the weight, in g, of the substance taken.

Ester value (2.3.26). 75 to 95, determined by subtracting the Acid value from the Saponification value.

Ratio number. The Ester value divided by the Acid value is between 5 and 19.

Saponification value (2.3.37). 87 to 104, determined by the following method. Weigh accurately about 2.0 g, add 30 ml of a mixture of equal volumes of xylene and ethanol (95 per cent) and a few glass beads, heat until dissolved, add 25.0 ml of 0.5 M ethanolic potassium hydroxide and heat under a reflux condenser for 3 hours. Titrate the hot solution immediately with 0.5 M hydrochloric acid using 1 ml of phenolphthalein solution as indicator, bringing the solution back to boil several times during the titration (n₁, ml). Repeat the procedure omitting the substance under examination (n₂, ml). Calculate the Saponification value from the expression 28.05(n₁ - n₂)/w, where w is the weight, in g, of the substance taken.

Fats, fatty acids, Japan wax and resin. Boil 5.0 g for 10 minutes with 80 ml of a 10 per cent w/v solution of sodium hydroxide, replace the water lost by evaporation, cool, filter the solution through a plug of glass wool and acidify with hydrochloric acid; no precipitate is produced.

Ceresin, paraffin and other waxes. To 3.0 g in a 100-ml round-bottomed flask add 30 ml of a 4 per cent w/v solution of potassium hydroxide in aldehyde-free ethanol (95 per cent) and boil gently under a reflux condenser for 2 hours. Remove the condenser and immediately insert a thermometer, place the flask in a water-bath at 80°C and allow to cool with continuous swirling. The solution may be opalescent, but no precipitate is formed before the temperature reaches 65°C.

Glycerin and other polyhydric alcohols. To 0.2 g add 10 ml of ethanolic potassium hydroxide solution, heat under a reflux condenser in a water-bath for 30 minutes, add 50 ml of 1 M sulphuric acid, cool and filter. Rinse the flask and filter with 1 M sulphuric acid, combine the filtrate and washings and dilute to 100 ml with 1 M sulphuric acid (solution A). Into two matched test-tubes introduce, respectively, 1 ml of solution A and 1 ml of a 0.001 per cent w/v solution of glycerin in 1 M sulphuric acid (solution B). Add 0.5 ml of a 1.07 per cent w/v solution of sodium periodate to each tube, mix, allow to stand for 5 minutes, add to each tube 1 ml of decolorised fuchsin solution and mix; any precipitate disappears. Place the tubes in a beaker containing water at 40°C and observe for 10 to 15 minutes during cooling. Any bluish violet colour in the tube containing solution A is not more intense than that in the tube containing solution B (0.5 per cent w/w, calculated as glycerin).

Yellow Beeswax

Yellow beeswax is the wax obtained by melting the walls of the honeycomb of the bee, Apis mellifera Linn. with hot water and removing the foreign matter.

Description. Yellow or light brown pieces or plates, with a fine-grained, matt, non-crystalline fracture; becomes soft and pliable when warmed by hand. Odour, faint and characteristic. It is tasteless and does not stick to the teeth.

Tests

Melting range (2.4.21). 61°C to 65°C, determined by Method IV.

Acid value (2.3.23). 5 to 15, determined by the following method. Weigh accurately about 5.0 g in a 250-ml conical flask fitted with a reflux condenser, add 40 ml of xylene and a few glass beads, heat until dissolved, add 20 ml of ethanol (95 per cent) and 0.5 ml of phenolphthalein solution and titrate the hot solution with 0.5 M ethanolic potassium hydroxide until a red colour persists for at least 10 seconds (n₁, ml). Repeat the procedure omitting the substance under examination (n₂, ml). Calculate the Acid value from the expression 28.05(n₁ - n₂)/w, where w is the weight, in g, of the substance taken.

Ester value (2.3.26). 75 to 95, determined by subtracting the Acid value from the Saponification value.

Ratio number. The Ester value divided by the Acid value is between 5 and 19.

Saponification value (2.3.37). 87 to 104, determined by the following method. Weigh accurately about 2.0 g, add 30 ml of a mixture of equal volumes of xylene and ethanol (95 per cent) and a few glass beads, heat until dissolved, add 25.0 ml of 0.5 M ethanolic potassium hydroxide and heat under a reflux condenser for 3 hours. Titrate the hot solution immediately with 0.5 M hydrochloric acid using 1 ml of
phenolphthalein solution as indicator, bringing the solution back to boil several times during the titration ($n_1$ ml). Repeat the procedure omitting the substance under examination ($n_2$ ml). Calculate the Saponification value from the expression $28.05(n_2 - n_1)/w$, where $w$ is the weight, in g, of the substance taken.

**Fats, fatty acids, Japan wax and resin.** Boil 5 g for 10 minutes with 80 ml of a 10 per cent w/v solution of sodium hydroxide, replace the water lost by evaporation, cool, filter the solution through a plug of glass wool and acidify with hydrochloric acid; no precipitate is produced.

**Ceresin, paraffin and other waxes.** To 3.0 g in a 100-ml round-bottomed flask add 30 ml of a 4 per cent w/v solution of potassium hydroxide in aldehyde-free ethanol (95 per cent) and boil gently under a reflux condenser for 2 hours. Remove the condenser and immediately insert a thermometer, place the flask in a water-bath at 80° and allow to cool with continuous swirling. The solution may be opalescent, but no precipitate is formed before the temperature reaches 65°.

**Glycerin and other polyhydric alcohols.** To 0.2 g add 10 ml of ethanolic potassium hydroxide solution, heat under a reflux condenser in a water-bath for 30 minutes, add 50 ml of 1 M sulphuric acid, cool and filter. Rinse the flask and filter with 1 M sulphuric acid, combine the filtrate and washings and dilute to 100 ml with 1 M sulphuric acid (solution A). Into two matched test-tubes introduce, respectively, 1 ml of solution A and 1 ml of a 0.001 per cent w/v solution of glycerin in 1 M sulphuric acid (solution B). Add 0.5 ml of a 1.07 per cent w/v solution of sodium periodate to each tube, mix, allow to stand for 5 minutes, add to each tube 1 ml of decolorised fuchsine solution and mix; any precipitate disappears. Place the tubes in a beaker containing water at 40° and observe for 10 to 15 minutes during cooling. Any bluish violet colour in the tube containing solution A is not more intense than that in the tube containing solution B (0.5 per cent w/v, calculated as glycerin).

**Storage.** Store in well-closed containers.

### Bentonite

Bentonite is a natural, colloidal, hydrated aluminium silicate that has been processed to remove grit and non-swelling components of the ore.

**Description.** A very fine, pale buff or cream-coloured to greyish-white powder, free or almost free from gritty particles.

**Identification**

Fuse 1 g with 2 g of anhydrous sodium carbonate, warm the residue with 10 ml of water, filter, wash the filter with 5 ml of water and reserve the combined filtrate and washings. Dissolve the residue in 10 ml of dilute hydrochloric acid; the solution gives the reactions of aluminium salts, (2.3.1). Add to the reserved filtrate and washings 3 ml of hydrochloric acid; a gelatinous precipitate is produced.

### Tests

**pH** (2.4.24). 9.0 to 10.5, determined in a 2.0 per cent w/v suspension in water.

**Heavy metals** (2.3.13). To 5.0 g add 7.5 ml of 2 M hydrochloric acid and 27.5 ml of water, boil for 5 minutes, centrifuge and filter the supernatant liquid. Wash the residue with water, filter, combine the filtrates and dilute to 50 ml with water. To 5 ml of the solution add 5 ml of water, 10 ml of hydrochloric acid and 25 ml of 4-methyl-2-pentanone, shake for 2 minutes, allow the layers to separate and evaporate the aqueous layer to dryness on a water-bath. Dissolve the residue in 1 ml of 5 M acetic acid, dilute to 25 ml and filter. The resulting solution complies with the limit test for heavy metals, Method D (50 ppm). Prepare the standard using lead standard solution (1 ppm Pb).

**Sedimentation volume.** In a mortar, mix 6.0 g with 0.3 g of light magnesium oxide, freshly calcined. Mix the powder progressively with 200 ml of water. Shake for 1 hour and place 100 ml of the suspension in a 100-ml graduated cylinder. After 24 hours the volume of the clear supernatant liquid is not greater than 2 ml.

**Swelling power.** Add 2.0 g in twenty portions at intervals of 2 minutes to 100 ml of a 1 per cent w/v solution of sodium lauryl sulphate in a 100-ml graduated cylinder about 3 cm in diameter. Allow each portion to settle before adding the next and let it stand for 2 hours. The apparent volume of the sediment at the bottom of the cylinder is not less than 24 ml.

**Coarse particles.** To 20 g add 1000 ml of water and mix for 15 minutes at not less than 5000 rpm. Transfer to a wet sieve of nominal aperture of 75 mm, previously dried at 100° to 105° and weighed, and wash with three quantities, each of 500 ml, of water, ensuring that any agglomerates are dispersed. Dry at 100° to 105° and weigh. The weight of the matter on the sieve is not more than 0.1 g (0.5 per cent).

**Microbial contamination** (2.2.9). 1 g is free from Escherichia coli.

**Loss on drying** (2.4.19). Not more than 15.0 per cent, determined on 1.0 g by drying in an oven at 105°.

### Benzalkonium Chloride

Benzalkonium Chloride is a mixture of alkylbenzyl-dimethylammonium chlorides, the alkyl groups having chain lengths of C₈ to C₁₈.

Benzalkonium Chloride contains not less than 95.0 per cent and not more than 104.0 per cent of alkylbenzyldimethyl-
ammonium chlorides, calculated as $\text{C}_{22}\text{H}_{40}\text{ClN}$ on the dried basis.

**Description.** A white or yellowish-white powder or gelatinous, yellowish-white fragments, hygroscopic, soapy to the touch.

**Identification**

A. Dilute 0.1 g with 10 ml of water. To 5 ml add 1.5 ml of *dilute nitric acid*; a white precipitate is produced which is soluble in ethanol (95 per cent). To the remainder add 1.5 ml of mercuric chloride solution; a white precipitate is produced which is soluble in ethanol (95 per cent).

B. Dissolve 0.25 g in 1 ml of sulphuric acid, add 0.1 g of potassium nitrate, heat on a water-bath for 5 minutes, cool, dilute with water to 10 ml, add 0.5 g of zinc powder, and heat on a water-bath for 5 minutes. To 2 ml of the clear supernatant liquid add 0.5 ml of sodium nitrite solution, cool in ice and add to 3 ml of 2-naphthol solution; an orange red colour is produced.

C. To 25 mg add 1 ml of 2 M nitric acid; a white precipitate is produced which dissolves on addition of 5 ml of ethanol (95 per cent). The resulting solution gives reaction A of chlorides (2.3.1).

**Tests**

**Acidity or alkalinity.** Dissolve 0.5 g in 50 ml of carbon dioxide-free water, add 0.1 ml of bromocresol purple solution and titrate with 0.1 M hydrochloric acid or with 0.1 M sodium hydroxide. Not more than 0.1 ml is required to change the colour of the solution.

**Ammonia compounds.** Boil 0.1 g with 3 ml of sodium hydroxide solution; no odour of ammonia is produced.

**Foreign amines.** Dissolve 0.1 g in 5 ml of water and add 3 ml of 1 M sodium hydroxide; no precipitate is formed. Heat to boiling; the odour of amines is not perceptible.

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Water** (2.3.43). Not more than 10 per cent, determined on 0.3 g.

**Assay.** Weigh accurately about 2.0 g, dissolve in sufficient water to produce 100.0 ml. Transfer 25.0 ml to a separating funnel, add 25 ml of chloroform, 10 ml of 0.1 M sodium hydroxide and 10.0 ml of a freshly prepared 5 per cent w/v solution of potassium iodide. Shake well, allow to separate and discard the chloroform layer. Shake the aqueous solution with three further quantities, each of 10 ml, of chloroform and discard the chloroform layer. Add 40 ml of hydrochloric acid, cool and titrate with 0.05 M potassium iodate until the solution becomes pale brown in colour. Add 2 ml of chloroform and continue the titration until the chloroform becomes colourless. Titrate a mixture of 20 ml of water, 10.0 ml of a freshly prepared 5 per cent w/v solution of potassium iodide and 40 ml of hydrochloric acid with 0.05 M potassium iodate in a similar manner; the difference between the titrations represents the amount of 0.05 M potassium iodate required. 1 ml of 0.05 M potassium iodate is equivalent to 0.0354 g of $\text{C}_{22}\text{H}_{40}\text{ClN}$.

**Storage.** Avoid contact with metals.

**Benzalkonium Chloride Solution**

Benzalkonium Chloride Solution is a solution of a mixture of alkylbenzyldimethylammonium chlorides, the alkyl groups having chain lengths of C\textsubscript{8} to C\textsubscript{18}. It may contain ethanol (95 per cent). In making Benzalkonium Chloride Solution, the ethanol (95 per cent) may be replaced by Industrial Methylated Spirit, diluted so as to be of equivalent strength.

Benzalkonium Chloride Solution contains not less than 49.0 per cent w/v and not more than 51.0 per cent w/v of alkylbenzyldimethylammonium chlorides, calculated as $\text{C}_{22}\text{H}_{40}\text{ClN}$. It may contain not more than 16.0 per cent v/v of ethanol, C\textsubscript{2}H\textsubscript{6}O.

**Description.** A clear, colourless or slightly yellow, syrupy liquid; odour, aromatic.

**Identification**

A. Dilute 0.2 ml with 10 ml of water. To 5 ml add 1.5 ml of *dilute nitric acid*; a white precipitate is produced which is soluble in ethanol (95 per cent). To the remainder add 1.5 ml of mercuric chloride solution; a white precipitate is produced which is soluble in ethanol (95 per cent).

B. Evaporate 0.5 ml to dryness on a water-bath, dissolve the residue in 1 ml of sulphuric acid, add 0.1 g of potassium nitrate, heat on a water-bath for 5 minutes, cool, dilute with water to 10 ml, add 0.5 g of zinc powder, and heat on a water-bath for 5 minutes. To 2 ml of the clear supernatant liquid add 0.5 ml of sodium nitrite solution, cool in ice and add to 3 ml of 2-naphthol solution; an orange red colour is produced.

C. To 0.05 ml add 1 ml of 2 M nitric acid; a white precipitate is produced which dissolves on addition of 5 ml of ethanol (95 per cent). The resulting solution gives reaction A of chlorides (2.3.1).

**Tests**

**Acidity or alkalinity.** Dissolve 1.0 g in 50 ml of carbon dioxide-free water, add 0.1 ml of bromocresol purple solution and titrate with 0.1 M hydrochloric acid or with 0.1 M sodium hydroxide. Not more than 0.1 ml is required to change the colour of the solution.

**Ammonia compounds.** Boil 0.2 ml with 3 ml of sodium hydroxide solution; no odour of ammonia is produced.
**Foreign amines.** To a volume containing 0.1 g of benzalkonium chloride add sufficient water to produce 5 ml and add 3 ml of 1 M sodium hydroxide; no precipitate is formed. Heat to boiling; the odour of amines is not perceptible.

**Ethanol (if present)** (2.3.45). Not more than 16.0 per cent v/v, determined by Method I or II, as applicable.

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Assay.** Weigh accurately about 4.0 g, dissolve in sufficient water to produce 100.0 ml. Transfer 25.0 ml to a separating funnel, add 25 ml of chloroform, 10 ml of 0.1 M sodium hydroxide and 10.0 ml of a freshly prepared 5 per cent w/v solution of potassium iodide. Shake well, allow to separate and discard the chloroform layer. Shake the aqueous solution with three further quantities, each of 10 ml, of chloroform and discard the chloroform layer. Add 40 ml of hydrochloric acid, cool and titrate with 0.05 M potassium iodate until the solution becomes pale brown in colour. Add 2 ml of chloroform and continue the titration until the chloroform becomes colourless. Titrate a mixture of 20 ml of water, 10.0 ml of a freshly prepared 5 per cent w/v solution of potassium iodide and 40 ml of hydrochloric acid with 0.05 M potassium iodate in a similar manner, the difference between the titrations represents the amount of 0.05 M potassium iodate required.

1 ml of 0.05 M potassium iodate is equivalent to 0.0354 g of C16H20N2, both calculated on the anhydrous basis. Determine the relative density (2.4.29), and calculate the amount of C16H20N2, weight in volume. Avoid contact with metals.

**Labelling.** The label states, where appropriate, the content of ethanol (95 per cent) or Industrial Methylated Spirit.

**Benzathine Penicillin**

Benzathine Benzylpenicillin; Benzathine Penicillin G

\[
\text{C}_{16}\text{H}_{20}\text{N}_{2}\text{S}_{2}(\text{C}_{16}\text{H}_{18}\text{N}_{2}\text{O}_{4}\text{S})_{2}\quad \text{Mol. Wt. 909.1}
\]

Benzathine Penicillin is \(N,N^\prime\)-dibenzylethlenediaminomonomium bis[[6R]-6-(2 phenylacetamido)penicillanate] containing a variable amount of water.

Benzathine Penicillin contains not less than 96.0 per cent and not more than 100.5 per cent of \(\text{C}_{16}\text{H}_{20}\text{N}_{2}(\text{C}_{16}\text{H}_{18}\text{N}_{2}\text{O}_{4}\text{S})_{2}\) and not less than 24.0 per cent and not more than 27.0 per cent of \(\text{C}_{16}\text{H}_{20}\text{N}_{2}\), both calculated on the anhydrous basis.

**Description.** A white, crystalline powder; almost odourless.

**Identification**

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with benzathine penicillin RS.

B. Shake 0.1 g with 1 ml of 1 M sodium hydroxide for 2 minutes, add 2 ml of ether, shake for 1 minute and allow to separate. Evaporate 1 ml of the ether layer to dryness, dissolve the residue in 2 ml of glacial acetic acid and add 1 ml of potassium dichromate solution; a golden yellow precipitate is formed.

C. Shake 0.1 g with 2 ml of 1 M sodium hydroxide for 2 minutes, extract the mixture with two quantities, each of 3 ml, of ether, evaporate the combined extracts and dissolve the residue in 1 ml of ethanol (50 per cent). Add 5 ml of picric acid solution, heat at 90° for 5 minutes and allow to cool slowly; the precipitate, after recrystallisation from ethanol (25 per cent) containing a small amount of picric acid, melts at about 214° (2.4.21).

D. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

**Tests**

**pH** (2.4.24). 5.0 to 7.5, determined in a saturated solution.

**Related substances.** Determine by liquid chromatography (2.4.14).

Prepare the solutions immediately before use. Avoid any overheating during the preparation of the solutions.

**Test solution.** Dissolve an accurately weighed quantity of about 70 mg of the substance under examination in 25 ml of methanol with the aid of ultrasound (for about 2 minutes). Dilute to 50.0 ml with a solution containing 6.8 g per litre of potassium dihydrogen phosphate and 1.02 g per litre of disodium hydrogen phosphate.

**Reference solution (a).** Dissolve an accurately weighed quantity of about 70 mg of benzathine penicillin RS in 25 ml of methanol with the aid of ultrasound (for about 2 minutes). Dilute to 50.0 ml with a solution containing 6.8 g per litre of potassium dihydrogen phosphate and 1.02 g per litre of disodium hydrogen phosphate.

**Reference solution (b).** Dilute 1 ml of reference solution (a) to 100 ml with mobile phase A.
Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: A. a mixture of 10 volumes of a 34 g per litre solution of potassium dihydrogen phosphate adjusted to pH 3.5 with phosphoric acid, 30 volumes of methanol and 60 volumes of water,
- B. a mixture of 10 volumes of a 34 g per litre solution of potassium dihydrogen phosphate adjusted to pH 3.5 with phosphoric acid, 30 volumes of water and 60 volumes of methanol,
- temperature 40°,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 220 nm,
- a 20 µl loop injector.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
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<tbody>
<tr>
<td>0 – 10</td>
<td>75 → 0</td>
<td>25 → 100</td>
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<td>10 – 20</td>
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<td>20 – 55</td>
<td>75 → 0</td>
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<td>55 – 70</td>
<td>75 → 0</td>
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</tbody>
</table>

Inject reference solution (a). Relative retention time with reference to benzylpenicillin: benzathine = 0.3 to 0.4; benzylpenicilloic acids benzathide = about 2.4. If necessary, adjust the concentration of methanol in the mobile phase.

Inject the test solution and reference solution (b). The area of any secondary peak obtained with the test solution corresponding to benzylpenicilloic acids benzathide is not more than twice the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (2 per cent). The area any other secondary peak obtained with the test solution is not more than the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Water** (2.3.43). 5.0 to 8.0 per cent, determined on 0.3 g.

**Assay.** Determine by liquid chromatography, (2.4.14) as given under the test for Related substances using the following mobile phase.

*Mobile phase.* a mixture of 10 volumes of phosphate buffer solution pH 3.5, 35 volumes of methanol, and 55 volumes of water.

Inject alternately the test solution and reference solution (a).

Calculate the percentage contents of C₁₆H₂₀N₂ and of C₁₆H₂₀N₂₂(C₁₆H₁₈N₂O₄S)₂. Calculate the content of C₁₆H₃₀N₂₂(C₁₆H₁₈N₂O₄S)₂, by multiplying the percentage content of benzylpenicillin by 1.36.

Benzathine Penicillin intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

**Bacterial endotoxins** (2.2.3). Not more than 0.13 Endotoxin Unit per ml of a solution prepared in the following manner. Suspend 20 mg of the substance under examination in 20 ml of 0.1 M sodium hydroxide, dilute 1 ml to 100 ml and use the supernatant liquid.

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage.** Store protected from moisture at a temperature not exceeding 30°. If the material is intended for use in the manufacture of parenteral preparations the container should be sterile and sealed so as to exclude micro-organisms.

**Labelling** The label states whether or not the contents are intended for use in the manufacture of parenteral preparations.

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**Benzathine Penicillin Injection**

**Benzathine Benzylpenicillin Injection; Benzathine Penicillin G Injection**

Benzathine Penicillin Injection is a sterile material consisting of Benzathine Penicillin with or without suspending agents, buffering agents and other excipients. It is filled in a sealed container.

The injection is constituted by suspending the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

**Storage.** The constituted suspension should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Benzathine Penicillin Injection contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of benzathine penicillin, C₁₆H₃₀N₂₂(C₁₆H₁₈N₂O₄S)₂.

**Description.** A white crystalline powder, almost odourless. The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

**Identification**

A. Shake 0.1 g with 1 ml of 1 M sodium hydroxide for 2 minutes, add 2 ml of ether, shake for 1 minute and allow to separate. Evaporate 1 ml of the ether layer to dryness, dissolve the
residue in 2 ml of glacial acetic acid and add 1 ml of potassium dichromate solution; a golden yellow precipitate is formed.

B. Shake 0.1 g with 2 ml of 1 M sodium hydroxide for 2 minutes, extract the mixture with two quantities, each of 3 ml, of ether; evaporate the combined extracts and dissolve the residue in 1 ml of ethanol (50 per cent). Add 5 ml of picric acid solution, heat at 90° for 5 minutes and allow to cool slowly; the precipitate, after recrystallisation from ethanol (25 per cent) containing a small quantity of picric acid, melts at about 214° (2.4.21).

C. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

**Tests**

**pH** (2.4.24). 5.0 to 7.5, determined in a suspension obtained by reconstituting as directed on the label.

**Consistency.** To a quantity containing 60,000 Units add 2 ml of water and shake thoroughly. The resulting suspension passes through a 23G hypodermic needle.

**Related substances.** Determine by liquid chromatography (2.4.14). Prepare the solutions immediately before use. Avoid any overheating during the preparation of the solutions.

**Test solution.** Dissolve an accurately weighed quantity containing about 70 mg of Benzathine Penicillin in 25 ml of methanol with the aid of ultrasound (for about 2 minutes). Dilute to 50.0 ml with a solution containing 6.8 g per litre of potassium dihydrogen phosphate and 1.02 g per litre of disodium hydrogen phosphate.

**Reference solution (a).** Dissolve an accurately weighed quantity of about 70 mg of benzathine penicillin RS in 25 ml of methanol with the aid of ultrasound (for about 2 minutes). Dilute to 50.0 ml with a solution containing 6.8 g per litre of potassium dihydrogen phosphate and 1.02 g per litre of disodium hydrogen phosphate.

**Reference solution (b).** Dilute 1 ml of reference solution (a) to 100 ml with mobile phase A.

**Chromatographic system**
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: A. a mixture of 10 volumes of a 34 g per litre solution of potassium dihydrogen phosphate adjusted to pH 3.5 with phosphoric acid, 30 volumes of methanol and 60 volumes of water,
- B. a mixture of 10 volumes of a 34 g per litre solution of potassium dihydrogen phosphate adjusted to pH 3.5 with phosphoric acid, 30 volumes of water and 60 volumes of methanol,
- temperature 40°,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 220 nm,
- a 20 µl loop injector.

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Inject reference solution (a). Relative retention time with reference to benzylpenicillin: benzathine = 0.3 to 0.4; benzylpenicilloic acids benzathide = about 2.4. If necessary, adjust the concentration of methanol in the mobile phase.

Inject the test solution and reference solution (b). The area of any secondary peak obtained with the test solution corresponding to benzylpenicilloic acids benzathide is not more than twice the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (2 per cent). The area any other secondary peak obtained with the test solution is not more than the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (1 per cent). Disregard any peak with an area 0.05 times the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Bacterial endotoxins** (2.2.3) Not more than 0.13 Endotoxin Unit per ml of a solution prepared by suspending 20 mg of the substance under examination in 20 ml of 0.1 M sodium hydroxide, diluting 1 ml to 100 ml and using the supernatant.

**Water** (2.3.43). 5.0 to 8.0 per cent, determined on 0.3 g.

**Assay.** Determine by liquid chromatography, (2.4.14) as given under the test for Related substances using the following mobile phase.

**Mobile phase.** a mixture of 10 volumes of phosphate buffer solution pH 3.5, 35 volumes of methanol, and 55 volumes of water.

Inject alternately the test solution and reference solution (a). Calculate the content of C₁₆H₂₀N₂₄(C₁₆H₁₈N₂O₄S)₂ by multiplying the percentage content of benzylpenicillin by 1.36.

**Labelling.** The label states (1) the directions for constituting the suspension; (2) the names of any added buffering agents or other pharmaceutical aids; (3) that the preparation is meant for intramuscular injection only.
Fortified Benzathine Penicillin Injection

Fortified Benzathine Benzylpenicillin Injection; Fortified Benzathine Penicillin G Injection

Benzathine Penicillin Injection is a sterile material consisting of Benzathine Penicillin and Procaine Penicillin with or without suspending agents, buffering agents and other excipients. It is filled in a sealed container.

The injection is constituted by suspending the contents of the sealed container in the requisite amount of sterile Water for Injections containing Benzylpenicillin Sodium immediately before use.

Storage. The constituted suspension should be used immediately after preparation but, in any case, within the period and under the conditions recommended by the manufacturer.

Fortified Benzathine Penicillin Injection contains not less than 90.0 per cent and not more than 125.0 per cent of the stated amount of benzathine penicillin, not less than 95.0 per cent and not more than 125.0 per cent of the stated amount of procaine penicillin, not less than 90.0 per cent and not more than 130.0 per cent of the stated amount of benzylpenicillin sodium, all in terms of Units of penicillin.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. Give the reaction for penicillins (2.3.1).
B. Give reaction B of penicillins and cephalosporins (2.3.1).
C. Shake 0.1 g with 1 ml of 1 M sodium hydroxide for 2 minutes, add 2 ml of ether, shake for 1 minute and allow to separate. Evaporate 1 ml of the ether layer to dryness, dissolve the residue in 2 ml of glacial acetic acid and add 1 ml of potassium dichromate solution; a golden yellow precipitate is formed.
D. Give the reactions of sodium salts (2.3.1).

Tests

Stability. Using an aseptic technique prepare the suspension as directed on the label in an individual unopened container and determine the concentration of benzylpenicillin sodium by the method described below using an accurately measured quantity of the suspension, withdrawn aseptically from the container. Store the remainder of the suspension in the closed container at 4°C for 7 days and then repeat the determination of benzylpenicillin sodium.

The concentration of benzylpenicillin sodium in the stored injection is not less than 80 per cent of the concentration found in the freshly prepared suspension.

Consistency. To a quantity containing 600,000 Units of Benzathine Penicillin, 300,000 Units each of Procaine Penicillin and Benzylpenicillin add 2 ml of water and shake thoroughly. The resulting suspension passes readily through a 22G hypodermic needle.

Bacterial endotoxins (2.2.3). Not more than 0.13 Endotoxin Unit per ml of a solution prepared by suspending 20 mg of the substance under examination in 20 ml of 0.1 M sodium hydroxide, diluting 1 ml to 100 ml and using the supernatant.

Water (2.3.43). Not more than 7.5 per cent, determined on 0.3 g.

Assay. For benzathine penicillin — Shake a quantity of the mixed contents of 10 containers containing 1 g of Benzathine Penicillin with 30 ml of a saturated solution of sodium chloride and 10 ml of 5 M sodium hydroxide and extract with four successive quantities, each of 50 ml, of ether. Wash the combined ether extracts with three successive quantities, each of 5 ml, of water, extracting each aqueous washing with the same 25 ml of ether. Combine the ether extracts, evaporate to a low bulk, add 2 ml of ethanol and evaporate to dryness. Dissolve the residue in 50 ml of glacial acetic acid and titrate with 0.1 M perchloric acid using 1 ml of 1-naphtholbenzein solution as indicator.

1 ml of 0.1 M perchloric acid is equivalent to 0.04545 g of C_{16}H_{20}N_{5}O_{7}S_{2}. Calculate the apparent content of Benzathine Penicillin.

Calculate the content of procaine penicillin, as determined by the method given below in the weight of the sample used in this assay, multiply this content by a factor of 1.544 and deduct the figure from the apparent content of benzathine penicillin; the result is the content of benzathine penicillin. (1 mg of benzathine penicillin is approximately equivalent to 1330 Units of penicillin).

For procaine penicillin — To a quantity of the mixed contents of 10 containers containing 0.25 g of Procaine Penicillin add 100 ml of water, shake well, dilute to 200.0 ml with water, mix and filter. Dilute 5.0 ml of the filtrate to 250.0 ml with buffer solution pH 7.0 and measure the absorbance of the resulting solution at the maximum at about 290 nm, using buffer solution pH 7.0 as the blank (2.4.7). Calculate the content of procaine penicillin taking 310 as the specific absorbance at 290 nm. (1 mg of procaine penicillin is equivalent to 1009 Units of penicillin).

For benzylpenicillin sodium — Shake a quantity of the mixed contents of 10 containers containing 0.15 g of Benzylpenicillin Sodium with water until dissolved and dilute to 500.0 ml with water. Dilute 25.0 ml of the resulting solution to 100.0 ml with phosphate buffer pH 6.8. Place two quantities, each of 2.0 ml, of the resulting solution in separate stoppered tubes. To one tube add 10.0 ml of imidazole-mercury reagent, mix, stopper...
the tube and immerse in a water-bath at 60° for 35 minutes, swirling occasionally. Remove from the water-bath and cool rapidly to 20° (solution A). Add 10.0 ml of imidazole solution to the second tube, mix, stopper the tube and allow to stand at 20° for 35 minutes, swirling occasionally (solution B). Without delay measure the absorbance of solutions A and B at about 325 nm (2.4.7), using as the blank a mixture of 2.0 ml of water and 10.0 ml of imidazole-mercury reagent for solution A and a mixture of 2.0 ml of water and 10.0 ml of imidazole solution for solution B. Calculate the content of total penicillins as C_{16}H_{17}N_{2}NaO_{4}S from the difference between the absorbances for solution B. Calculate the content of total penicillins as a mixture of 2.0 ml of

**Tests**

**Water** (2.3.43). Not more than 8.0 per cent, determined on the powdered tablets.

**Other tests.** Comply with the tests stated under Tablets.

**Related substances.** Determine by liquid chromatography (2.4.14).

Prepare the solutions immediately before use. Avoid any overheating during the preparation of the solutions.

**Test solution.** Weigh and powder 20 tablets. Dissolve an accurately weighed quantity containing about 70 mg of Benzathine Penicillin in 25 ml of methanol with the aid of ultrasound (for about 2 minutes) and allow to stand for 15 minutes. Dilute to 50.0 ml with a solution containing 6.8 g per litre of potassium dihydrogen phosphate and 1.02 g per litre of disodium hydrogen phosphate.

**Reference solution (a).** Dissolve an accurately weighed quantity of about 70 mg of benzathine penicillin RS in 25 ml of methanol with the aid of ultrasound (for about 2 minutes). Dilute to 50.0 ml with a solution containing 6.8 g per litre of potassium dihydrogen phosphate and 1.02 g per litre of disodium hydrogen phosphate.

**Reference solution (b).** Dilute 1 ml of reference solution (a) to 100 ml with mobile phase A.

**Benzathine Penicillin Tablets**

Benzathine Benzylpenicillin Tablets; Benzathine Penicillin G Tablets

Benzathine Penicillin Tablets contain Benzathine Penicillin equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated number of Units of penicillin.

**Identification**

A. Shake 0.1 g with 1 ml of 1 M sodium hydroxide for 2 minutes, add 2 ml of ether, shake for 1 minute and allow to separate. Evaporate 1 ml of the ether layer to dryness, dissolve the residue in 2 ml of glacial acetic acid and add 1 ml of potassium dichromate solution; a golden yellow precipitate is formed.

B. Shake 0.1 g with 2 ml of 1 M sodium hydroxide for 2 minutes, extract the mixture with two quantities, each of 3 ml, of ether; evaporate the combined extracts and dissolve the residue in 1 ml of ethanol (50 per cent). Add 5 ml of picric acid solution, heat at 90° for 5 minutes and allow to cool slowly; the precipitate, after recrystallisation from ethanol (25 per cent) containing a small quantity of picric acid, melts at about 214° (2.4.21).

C. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

**Chromatographic system**

- a stainless steel column 25 cm x 4 mm, packed with octadecylsilyl silica gel (5 µm),
- temperature 40°,
- mobile phase: A. a mixture of 10 volumes of a 34 g per litre solution of potassium dihydrogen phosphate adjusted to pH 3.5 with phosphoric acid, 30 volumes of methanol and 60 volumes of water,
- B. a mixture of 10 volumes of a 34 g per litre solution of potassium dihydrogen phosphate adjusted to pH 3.5 with phosphoric acid, 30 volumes of water and 60 volumes of methanol,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 220 nm,
- a 20 µl loop injector.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 10</td>
<td>75 → 0</td>
<td>25 → 100</td>
</tr>
<tr>
<td>10 – 20</td>
<td>75 → 0</td>
<td>25 → 100</td>
</tr>
<tr>
<td>20 – 55</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>55 – 70</td>
<td>75 → 0</td>
<td>25 → 100</td>
</tr>
</tbody>
</table>

Inject reference solution (a). Relative retention time with reference to benzylpenicillin: benzathine = 0.3 to 0.4;
benzylpenicilloic acids benzathide = about 2.4. If necessary, adjust the concentration of methanol in the mobile phase.

Inject the test solution and reference solution (b). The area of any secondary peak obtained with the test solution corresponding to benzylpenicilloic acids benzathide is not more than twice the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (2 per cent). The area any other secondary peak obtained with the test solution is not more than the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (1 per cent). Disregard any peak with an area 0.05 times the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Assay.** Determine by liquid chromatography, (2.4.14) as given under the test for Related substances using the following mobile phase.

**Mobile phase:** a mixture of 10 volumes of phosphate buffer solution pH 3.5, 35 volumes of methanol, and 55 volumes of water.

Inject alternately the test solution and reference solution (a). Calculate the percentage content of $C_{20}H_{31}NO$.HCl by multiplying the percentage content of benzylpenicillin by 1.36.

**Storage.** Store at a temperature not exceeding 30°.

### Benzhexol Hydrochloride

**Trihexyphenidyl Hydrochloride**

Benzhexol Hydrochloride is $(RS)$-1-cyclohexyl-1-phenyl-3-piperidinopropan-1-ol hydrochloride.

Benzhexol Hydrochloride contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{20}H_{31}NO$.HCl, calculated on the dried basis.

**Description.** A white or creamy-white, crystalline powder; odourless or almost odourless.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with benzhexol hydrochloride RS or with the reference spectrum of benzhexol hydrochloride.

B. Dissolve 0.5 g in 5 ml of warm methanol and make just alkaline to litmus paper with 5 M sodium hydroxide; a precipitate is produced, which, after recrystallisation from methanol melts at about 114° (2.4.21).

C. Gives the reactions of chlorides (2.3.1).

**Tests**

**pH** (2.4.24). 5.2 to 6.2, determined in a solution prepared by dissolving 1.0 g in 50 ml of carbon dioxide-free water with the aid of heat, cooling and diluting to 100.0 ml with the same solvent.

**Piperidylpropiophenone.** Dissolve 0.1 g in a mixture of 40 ml of water and 1 ml of 1 M hydrochloric acid with the aid of heat, cool and add sufficient water to produce 100.0 ml. The absorbance of the resulting solution at about 247 nm is not more than 0.5 (2.4.7).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.7 g and dissolve in 50 ml of anhydrous glacial acetic acid previously neutralised using 1-naphtholbenzein solution as indicator, warming and cooling, if necessary. Add 15 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid to the full colour change of the indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.03379 g of $C_{20}H_{31}NO$.HCl.

### Benzhexol Tablets

**Benzhexol Hydrochloride Tablets; Trihexyphenidyl Hydrochloride Tablets**

Benzhexol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of benzhexol hydrochloride, $C_{20}H_{31}NO$.HCl.

**Identification**

A. Shake a quantity of the powdered tablets with 20 ml of water and filter. The filtrate yields a yellow precipitate with trinitrophenol solution and a white precipitate with 5 M sodium hydroxide.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel $G$.

**Mobile phase.** A mixture of 90 volumes of chloroform and 10 volumes of methanol.
Test solution. Shake a quantity of the powdered tablets with sufficient chloroform to produce a solution containing 0.2 per cent w/v of Benzhexol Hydrochloride and filter.

Reference solution. A 0.2 per cent w/v solution of benzhexol hydrochloride RS in chloroform.

Apply to the plate 10 µl of each solution. After development remove the plate, allow it to dry in air and spray with dilute potassium iodobismuthate solution. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Uniformity of content. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

Test solution. Disperse well one tablet in 5.0 ml of water in an ultrasonic bath, add 10 ml of methanol, shake for 15 minutes, dilute to 25.0 ml with methanol, mix and filter through a filter with a maximum pore size of 0.2 mm.

Reference solution. A solution containing 0.008 per cent w/v of benzhexol hydrochloride RS and 0.004 per cent w/v of 3-piperidylpropiophenone hydrochloride RS in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilica gel (5 µm) (such as Resolve C18),
- mobile phase: 800 volumes of acetonitrile, 200 volumes of water and 0.2 volume of triethylamine, the pH of the mixture being adjusted to 4.0 with phosphoric acid,
- flow rate. 2 ml per minute,
- spectrophotometer set at 210 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the resolution factor between the two principal peaks in the chromatogram obtained with the reference solution is greater than 4.0.

Inject alternately the test solution and the reference solution.

Calculate the content of C_{20}H_{31}NO.HCl in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Determine by liquid chromatography (2.4.14), using the following solutions.

Test Solution. Disperse well a quantity of the powdered tablets containing about 5 mg of Benzhexol Hydrochloride in 5.0 ml of water in an ultrasonic bath, add 10 ml of methanol, shake for 15 minutes, dilute to 25.0 ml with methanol mix and filter through a filter with a maximum pore size of 0.2 µm.

Reference solution. A solution containing 0.02 per cent w/v of benzhexol hydrochloride RS and 0.01 per cent w/v of 3-piperidylpropiophenone hydrochloride RS in the mobile phase.

Carry out the chromatographic procedure described under Uniformity of content.

Calculate the content of C_{20}H_{31}NO.HCl in the tablets.

Benzocaine

C_{6}H_{11}NO_{2} Mol. Wt. 165.2

Benzocaine is ethyl 4-aminobenzoate.

Benzocaine contains not less than 99.0 per cent and not more than 101.0 per cent of C_{6}H_{11}NO_{2}, calculated on the dried basis.

Description. Colourless crystals or a white, crystalline powder; odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with benzocaine RS or with the reference spectrum of benzocaine.

B. Dissolve 10 mg in 1 ml of water with the aid of one drop of dilute hydrochloric acid and add 2 drops of a 10 per cent w/v solution of sodium nitrite and 2 drops of a solution of 10 mg of 2-naphthol in 5 ml of sodium hydroxide solution; a deep red colour is produced. On setting aside the solution for some time, a scarlet precipitate is produced.

C. Dissolve 0.2 g in 10 ml of water with the aid of dilute hydrochloric acid (solution A) and divide into 2 parts. To one part of solution A add iodine solution; a precipitate is obtained (distinction from orthocaine).

D. To the other part of solution A add potassium mercuri-iodide solution; no precipitate is obtained (distinction from procaine).

Tests

Appearance of solution. A 5.0 per cent w/v solution in ethanol (95 per cent) is clear (2.4.1), and colourless (2.4.1).

Acidity or alkalinity. Dissolve 0.5 g in 5 ml of ethanol (95 per cent), add 10 ml of water and one drop of phenolphthalein solution; no pink colour is produced. Add 0.5 ml of 0.01 M sodium hydroxide; the solution develops a pink colour.
**Benzoic Acid**

![COOH]

\[ C_7H_6O_2 \quad \text{Mol. Wt. 122.1} \]

Benzoic Acid contains not less than 99.5 per cent and not more than 100.5 per cent of \( C_7H_6O_2 \), calculated on the anhydrous basis.

**Description.** Colourless, light crystals, scales or needles; odour, slight and characteristic.

**Identification.**

A. Warm gently 0.2 g with 20 ml of water, add 1 ml of 1 M sodium hydroxide and filter. To the filtrate add ferric chloride test solution; a buff coloured precipitate is produced.

B. When examined in the range 220 nm to 360 nm, a 0.001 per cent w/v solution in methanol shows an absorption maximum only at about 225 nm; absorbance at about 225 nm, about 0.8 (2.4.7).

C. A 1 per cent w/v solution is acid to methyl red solution.

**Tests**

**Appearance of solution.** A 5.0 per cent w/v solution in ethanol (95 per cent) is clear (2.4.1), and colourless (2.4.1).

**Arsenic** (2.3.10). Mix 5.0 g with 3 g of anhydrous sodium carbonate, add 10 ml of bromine solution and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite and dissolve the cooled residue in 16 ml of brominated hydrochloric acid and 45 ml of water. Remove the excess of bromine with 2 ml of stannous chloride \( \text{AsT} \). The resulting solution complies with the limit test for arsenic (2 ppm).

**Chlorides.** Dissolve 0.2 g in 20 ml of dilute nitric acid and add few drops of silver nitrate solution; no turbidity is produced immediately.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over phosphorus pentoxide at a pressure of 1.5 to 2.5 kPa.

**Assay.** Weigh accurately about 0.4 g and dissolve in a mixture of 25 ml of hydrochloric acid and 50 ml of water. Cool to \( 10^\circ \). Determine by the nitrite titration (2.3.31).

1 ml of 0.1 M sodium nitrite is equivalent to 0.01652 g of \( C_7H_6O_2 \).

**Storage.** Store protected from light.

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**Compound Benzoic Acid Ointment**

Benzoic and Salicylic Acids Ointment; Whitfield’s Ointment

Compound Benzoic Acid Ointment is an ointment containing 6.0 per cent w/w of Benzoic Acid and 3.0 per cent w/w of Salicylic Acid in a suitable ointment base. Other strengths...
may also be prepared with Benzoic Acid and Salicylic Acid being in the ratio of about 2 to 1.

Compound Benzoic Acid Ointment contains not less than 5.7 per cent and not more than 6.3 per cent w/w of benzoic acid, C\textsubscript{7}H\textsubscript{6}O\textsubscript{2}, and not less than 2.85 per cent and not more than 3.15 per cent w/w of salicylic acid, C\textsubscript{7}H\textsubscript{6}O\textsubscript{3}.

**Identification**

Carry out the method for thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 80 volumes of toluene and 20 volumes of glacial acetic acid.

**Test solution.** Warm 1 g of the ointment with 10 ml of chloroform, cool and filter.

**Reference solution.** A solution containing 0.6 per cent w/v of benzoic acid and 0.3 per cent w/v of salicylic acid in chloroform.

Apply to the plate 2 µl of each solution. After development, dry the plate in a current of air and examine in ultraviolet light at 254 nm. The two principal spots in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution. Examine the plate in ultraviolet light at 365 nm. A blue fluorescent spot in the chromatogram obtained with the test solution corresponds in colour and position to the one in the chromatogram obtained with the reference solution. Spray the plate with ferric chloride test-solution. The chromatogram obtained with the test solution shows a purple spot corresponding in position to the blue fluorescent spot observed in ultraviolet light at 365 nm and corresponding in colour and position to the spot in the chromatogram obtained with the reference solution.

**Tests**

**Assay.** For benzoic acid — Weigh accurately about 2.5 g, dissolve with the aid of gentle heat, as completely as possible, in 50 ml of a mixture of equal volumes of ethanol (95 per cent) and ether, previously neutralised to phenolphthalein solution and titrate with 0.1 M sodium hydroxide using phenolphthalein solution as indicator.

1 ml of 0.1 M sodium hydroxide, after deducting 1 ml for each 0.01381 g of C\textsubscript{7}H\textsubscript{6}O\textsubscript{2} in the weight of the ointment taken (calculated from the result of the Assay for salicylic acid) is equivalent to 0.01221 g of C\textsubscript{7}H\textsubscript{6}O\textsubscript{3}.

For salicylic acid — Weigh accurately about 2.5 g, dissolve with the aid of gentle heat, as completely as possible, in 50 ml of ether, and extract with 5 quantities, each of 10 ml, of a saturated solution of sodium bicarbonate, washing each extract with the same 50 ml of ether. Combine the aqueous extracts, cautiously add hydrochloric acid until the solution is distinctly acid to litmus paper and extract with 4 quantities, each of 25 ml, of ether; combine the extracts and evaporate the ether at a temperature below 40°. Dissolve the residue in 5 ml of 0.5 M sodium hydroxide, add 50.0 ml of 0.1 M bromine and 5 ml of hydrochloric acid, shake repeatedly during 15 minutes and allow to stand for 15 minutes. Add 10 ml of potassium iodide solution and titrate with 0.1 M sodium thiosulphate using starch solution, added towards the end of the titration, as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of bromine required.

1 ml of 0.1 M bromine is equivalent to 0.002302 g of C\textsubscript{7}H\textsubscript{6}O\textsubscript{3}.

**Storage.** Store at a temperature not exceeding 30°

**Benzoin**

Benzoin is the balsamic resin obtained from Styrax benzoin Dryander or Styrax paralleloneurus Perkins, known in commerce as Sumatra Benzoin or from Styrax tonkinensis (Pierre) Craib ex Hartwich, or other species of the Section Anthostyrax of the genus Styrax, known in commerce as Siam Benzoin (Fam. Styraceae).

Benzoin contains not less than 25.0 per cent of total balsamic acids, calculated as cinnamic acid, C\textsubscript{7}H\textsubscript{6}O\textsubscript{2}, in Sumatra Benzoin and as benzoic acid, C\textsubscript{7}H\textsubscript{6}O\textsubscript{2}, in Siam Benzoin, calculated on the dried basis.

**Description.** Unground Sumatra Benzoin — Blocks or lumps of varying size, made up of tears compacted together, with a reddish-brown, reddish-grey or greyish-brown resinous mass, known in commerce as block benzoin. It also occurs in the form of tears with cream-coloured to yellowish surfaces; when fractured they exhibit milky-white surfaces; odour, balsamic, which accentuates on digestion with boiling water.

Unground Siam Benzoin — Pebble-like tears of variable size and shape, compressed, yellowish-brown to rusty-brown externally, milky white on fracture, hard and brittle at ordinary temperatures but softened by heat; odour, balsamic.

**Tests**

A. To a solution in ethanol (95 per cent) add water; the solution becomes milky, and the mixture is acid to litmus paper.

B. Heat 0.5 g in a dry test-tube; it melts and evolves white fumes, which form a white needle-shaped crystalline sublimate.

C. Heat 0.5 g in a test-tube with 5 ml of potassium permanganate solution; a strong odour of benzaldehyde is obtained with Sumatra Benzoin.

**Identification**

A. To a solution in ethanol (95 per cent) add water; the solution becomes milky, and the mixture is acid to litmus paper.

B. Heat 0.5 g in a dry test-tube; it melts and evolves white fumes, which form a white needle-shaped crystalline sublimate.

C. Heat 0.5 g in a test-tube with 5 ml of potassium permanganate solution; a strong odour of benzaldehyde is obtained with Sumatra Benzoin.
D. Determine by thin-layer chromatography (2.4.17), coating the plate with kieselguhr.

**Mobile phase.** A mixture of 93 volumes of toluene and 7 volumes of ethyl acetate.

**Test solution.** Dissolve 2.0 g of the substance under examination in 100 ml of ethanol (95 per cent).

**Reference solution (a).** A 0.05 per cent w/v solution of benzoic acid RS in chloroform.

**Reference solution (b).** A 0.05 per cent w/v solution of cinnamic acid RS in chloroform.

**Reference solution (c).** A 0.05 per cent w/v solution of coniferyl benzoate RS in chloroform.

**Reference solution (d).** A 0.05 per cent w/v solution of cinnamoyl cinnamate RS in chloroform.

**Reference solution (e).** A 0.05 per cent w/v solution of propyl cinnamate RS in chloroform.

**Reference solution (f).** A 0.05 per cent w/v solution of cinnamoyl benzoate RS in chloroform.

After development, dry the plate in air until the odour of the solvent is no longer detectable and spray with anisaldehyde-sulphuric acid reagent. Heat the plate at 110° for 5 minutes and examine in ultraviolet light at 254 nm. In the case of Sumatra Benzoin, the chromatogram obtained with the test solution exhibits four intense spots corresponding to spots in the chromatograms obtained with reference solutions (b), (c), (d) and (e). In the case of Siam Benzoin, it exhibits intense spots corresponding to spots in the chromatograms obtained with reference solutions (a), (c), (d) and (f).

**Tests**

**Dammar gum.** Determine by thin-layer chromatography (2.4.17), coating the plate with aluminium oxide G.

**Mobile phase.** A mixture of 60 volumes of ether and 40 volumes of light petroleum (80° to 100°).

**Test solution.** Dissolve by warming 0.2 g of the substance under examination in 10 ml of ethanol (90 per cent) and centrifuge.

Apply to the plate 5 µl of the test solution. Allow the mobile phase to rise 10 cm. Dry the plate in air, spray with anisaldehyde-sulphuric acid reagent and heat at 100° to 105° for 5 minutes. The chromatogram does not show any prominent spot with an Rf value between 0.4 and 1.0.

**Foreign organic matter (2.6.1).** Not more than 1.0 per cent.

**Ethanol-soluble extractive.** Not less than 75.0 per cent in Sumatra Benzoin and not less than 90.0 per cent in Siam Benzoin, determined by the following method. Weigh accurately about 2 g, in coarse powder, in a tared extraction thimble and insert the thimble in a Soxhlet or other suitable continuous extraction apparatus. Place 0.1 g of sodium hydroxide in the receiving flask of the apparatus, extract with ethanol (95 per cent) until extraction is complete (about 5 hours), dry the thimble to constant weight at 105° and calculate the ethanol-soluble extractive from the increase in weight of the thimble.

**Acid-insoluble ash (2.3.19).** Not more than 1.0 per cent in Sumatra Benzoin and not more than 0.5 per cent in Siam Benzoin, determined on 2.0 g.

**Loss on drying (2.4.19).** Not more than 10.0 per cent, determined on 2.0 g, in coarse powder, by drying over phosphorus pentoxide at a pressure not exceeding 2.7 kPa for 4 hours.

**Assay.** Weigh accurately about 1.25 g and boil with 25 ml of dilute ethanolic potassium hydroxide solution under a reflux condenser for 1 hour. Remove the ethanol and digest the residue with 50 ml of hot water until diffused. Cool the liquid, add 150 ml of water and 1.5 g of magnesium sulphate dissolved in 50 ml of water. Mix thoroughly and set aside for 10 minutes. Filter, wash the residue on the filter with 20 ml of water, acidify the combined filtrate and washings with hydrochloric acid and extract with successive quantities of 50, 40, 30, 30 and 30 ml of ether. Combine the ether extracts and discard the aqueous portion. Extract with successive quantities of 20, 20, 10, 10 and 10 ml of sodium bicarbonate solution, washing each aqueous extract with the same 20 ml of ether. Discard the ether layers, acidify the combined aqueous extracts with hydrochloric acid and extract with successive quantities of 30, 20, 20 and 10 ml of chloroform, filtering each chloroform extract through a plug of cotton wool on which a layer of anhydrous sodium sulphate is placed. Evaporate the chloroform on a water-bath until about 10 ml remains and remove the remainder in a current of air stopping immediately when the last trace of solvent is removed. Dissolve the residue by warming with 10 ml of ethanol (95 per cent), previously neutralised to phenol red solution, cool and titrate with 0.1 M sodium hydroxide using phenol red solution as indicator.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.01482 g of total balsamic acids, calculated as cinnamic acid, C_9H_8O_2, in Sumatra Benzoin and 0.01221 g of total balsamic acids, calculated as benzoic acid, C_7H_6O_2, in Siam Benzoin.

**Storage.** Store protected from light at a temperature not exceeding 30°.

**Labelling.** The label states whether the material is Sumatra Benzoin or Siam Benzoin.
Compound Benzoin Tincture

Friars’ Balsam

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Benzoin, in moderately coarse powder</td>
<td>100 g</td>
</tr>
<tr>
<td>Prepared Storax</td>
<td>75 g</td>
</tr>
<tr>
<td>Tolu Balsam</td>
<td>25 g</td>
</tr>
<tr>
<td>Aloes, in moderately coarse powder</td>
<td>20 g</td>
</tr>
<tr>
<td>Ethanol (90 per cent)</td>
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</tbody>
</table>

Macerate the Benzoin, Prepared Storax, Tolu Balsam and Aloes with 800 ml of Ethanol (90 per cent) in a closed vessel for not less than 2 days with occasional shaking. Filter and pass sufficient Ethanol (90 per cent) through the filter to produce the required volume.

Compound Benzoin Tincture contains not less than 4.5 per cent w/v of total balsamic acids, calculated as cinnamic acid, C9H8O2.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with kieselguhr.

Mobile phase. A mixture of 93 volumes of toluene and 7 volumes of ethyl acetate.

Test solution. Dilute 1 ml of the tincture with 4 ml of ethanol (95 per cent).

Reference solution (a). A 0.05 per cent w/v solution of benzoic acid RS in chloroform.

Reference solution (b). A 0.05 per cent w/v solution of cinnamic acid RS in chloroform.

Reference solution (c). A 0.05 per cent w/v solution of coniferyl benzoate RS in chloroform.

Reference solution (d). A 0.05 per cent w/v solution of cinnamoyl cinnamate RS in chloroform.

Reference solution (e). A 0.05 per cent w/v solution of propyl cinnamate RS in chloroform.

Reference solution (f). A 0.05 per cent w/v solution of cinnamoyl benzoate RS in chloroform.

Apply to the plate 20 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable and spray with anisaldehyde-sulphuric acid reagent. Heat the plate at 110° for 5 minutes and examine in ultraviolet light at 254 nm. The chromatogram obtained with the test solution exhibits an intense spot corresponding to the spot in the chromatogram obtained with the reference solution (Prepared Storax).

B. Determine by thin-layer chromatography (2.4.17), coating the plate with kieselguhr.

Mobile phase. A mixture of 93 volumes of toluene and 7 volumes of ethyl acetate.

Test solution. Dilute 1 ml of the tincture with 4 ml of ethanol (95 per cent).

Reference solution (a). A 0.05 per cent w/v solution of benzoyl benzoate RS in chloroform.

Reference solution (b). A 0.05 per cent w/v solution of benzoyl cinnamate RS in chloroform.

Reference solution (c). A 0.05 per cent w/v solution of eugenol RS in chloroform.

Reference solution (d). A 0.05 per cent w/v solution of vanillin RS in chloroform.

Apply to the plate 20 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable and spray with anisaldehyde-sulphuric acid reagent. Heat the plate at 110° for 5 minutes and examine in ultraviolet light at 254 nm. The chromatogram obtained with the test solution exhibits spots corresponding to spots in the chromatograms obtained with reference solutions (a), (b), (c) and (d) (Tolu Balsam).

C. Determine by thin-layer chromatography (2.4.17), coating the plate with kieselguhr.

Mobile phase. A mixture of 93 volumes of toluene and 7 volumes of ethyl acetate.

Test solution. Dilute 1 ml of the tincture with 4 ml of ethanol (95 per cent).

Reference solution (a). A 0.05 per cent w/v solution of styrene RS in chloroform.

Apply to the plate 20 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable and spray with anisaldehyde-sulphuric acid reagent. Heat the plate at 110° for 5 minutes and examine in ultraviolet light at 254 nm. The chromatogram obtained with the test solution exhibits an intense spot corresponding to the spot in the chromatogram obtained with the reference solution (Prepared Storax).

D. Carry out the method for thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 100 volumes of ethyl acetate, 13.5 volumes of methanol and 10 volumes of water.

Test solution. Dilute 1 ml of the tincture with 4 ml of ethanol (95 per cent).
Reference solution. A 0.5 per cent w/v solution of barbaloin RS in methanol.

Apply to the plate 50 µl of each solution as bands 20 mm long and not more than 3 mm wide. Allow the mobile phase to rise 10 cm. Dry the plate in air until the odour of the solvent is no longer detectable, spray with a 10 per cent w/v solution of potassium hydroxide in methanol and examine in ultraviolet light at 365 nm. The chromatogram obtained with the test solution exhibits a yellow fluorescent band corresponding to the band obtained in the chromatogram obtained with the reference solution and a light blue fluorescent band with a lower Rf value due to aloesine. Heat the plate at 110° for 5 minutes; a violet fluorescent band just below the band corresponding to barbaloin may also be seen in the chromatogram obtained with the test solution (Aloes).

Tests

Weight per ml (2.4.29). 0.870 g to 0.885 g.

Ethanol content. 70.0 to 77.0 per cent v/v, determined by Method II (2.3.45).

Total solids. Not less than 13.5 per cent w/v, determined on 1 ml by drying in an oven at 105° for 4 hours.

Assay. Evaporate 10 ml to a thick consistency on a water-bath. Boil the residue with 25 ml of ethanolic potassium hydroxide solution under a reflux condenser for 1 hour. Remove the ethanol and digest the residue with 50 ml of hot water until diffused. Cool the liquid, add 150 ml of water and 1.5 g of magnesium sulphate dissolved in 50 ml of water. Mix thoroughly and set aside for 10 minutes. Filter, wash the residue on the filter with 20 ml of water, acidify the combined filtrate and washings with hydrochloric acid and extract with successive quantities of 50, 40, 30, 30 and 30 ml of ether. Combine the ether extracts and discard the aqueous portion. Extract with successive quantities of 20, 20, 10, 10 and 10 ml of sodium bicarbonate solution, washing each aqueous extract with the same 20 ml of ether. Discard the ether layers, acidify the combined aqueous extracts with hydrochloric acid and extract with successive quantities of 30, 20, 20 and 10 ml of chloroform, filtering each chloroform extract through a plug of cotton wool on which a layer of anhydrous sodium sulphate is placed. Evaporate the chloroform on a water-bath until about 10 ml remains and remove the remainder in a current of air stopping immediately when the last trace of solvent is removed. Dissolve the residue by warming with 10 ml of ethanol (95 per cent), previously neutralised to phenol red solution, cool and titrate with 0.1 M sodium hydroxide using phenol red solution as indicator.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.01482 g of total balsamic acids, calculated as cinnamic acid, C₉H₈O₂.

Storage. Store protected from light in tightly-closed containers and avoid exposure to direct sunlight and to excessive heat.

Labelling. The label states that it is flammable.

Benzyl Alcohol

C₇H₈O Mol. Wt. 108.1

Benzyl Alcohol contains not less than 97.0 per cent of C₇H₈O.

Description. A colourless liquid; almost odourless; taste, sharp and burning.

Identification

Add three drops to a strong potassium permanganate solution, acidified with sulphuric acid; benzaldehyde, recognizable by its odour, is produced.

Tests

Wt. per ml (2.4.29). 1.64 g to 1.05 g.

Distillation range (2.4.8). None distils below 200° and not less than 94 per cent distils between 202° and 208°.

Refractive index (2.4.27). 1.536 to 1.542.

Acid Value (2.3.23). Not more than 0.5.

Chlorinated compounds. Mix 2.0 g with 50 ml of amyl alcohol in a dry flask, add in small quantities 3 g of sodium, connect the flask to a reflux air condenser, warm gently until the evolution of hydrogen ceases, and boil gently for one hour. Cool the liquid to a little below 100° add 50 ml of water; 5.0 ml of 0.1M silver nitrate, and 20 ml of nitric acid, and titrate the excess of silver nitrate with 0.1 M ammonium thiocyanate, using ferric ammonium sulphate solution as indicator. Repeat the operation without the sample; the difference between the titrations does not exceed 0.3 ml.

Benzaldehyde. Mix in a stoppered cylinder 10 ml with 10 ml of aldehyde-free alcohol and 20 ml of hydroxylamine hydrochloride solution. Allow to stand for five minutes and titrate with 0.1 M sodium hydroxide to the same green colour as that shown by 20 ml of hydroxylamine hydrochloride solution contained in a similar cylinder, both solutions being viewed down the axes of the cylinders; not more than 1.4 ml of 0.1 M sodium hydroxide is required.

Assay. To 1.5 g add 25 ml of a mixture of 1 volume of acetic anhydride and 7 volumes of pyridine and heat on a water-bath for thirty minutes. Cool, add 25 ml of water, and titrate with 1 M sodium hydroxide, using phenolphthalein solution as indicator. Repeat the operation without the substance under examination; the difference between the titrations represents the amount of alkali required by the benzyl alcohol.

1 ml of 1 M sodium hydroxide is equivalent to 0.1081 g of C₇H₈O.
Storage. Store protected from moisture in a container with minimum space above the level of the liquid.

**Benzyl Benzoate**

\[
\text{C}_{14}\text{H}_{12}\text{O}_2 \quad \text{Mol. Wt. 212.6}
\]

Benzyl Benzoate is the benzyl ester of benzoic acid.

Benzyl Benzoate contains not less than 99.0 per cent and not more than 100.5 per cent w/w of \(\text{C}_{14}\text{H}_{12}\text{O}_2\).

**Description.** Colourless crystals or a clear, colourless, oily liquid; odour, faintly aromatic.

**Identification**

A. Boil 2 g with 25 ml of ethanolic potassium hydroxide solution for 2 hours in a flask fitted with a reflux condenser. Remove the ethanol on a water-bath, add 50 ml of water to the liquid remaining in the flask and distill until the liquid distilling is no longer turbid. Preserve the distillate for test B. To the liquid remaining in the flask add dilute hydrochloric acid till it is neutral and divide the mixture into two parts. To one part add ferric chloride test solution; a buff-coloured precipitate is produced. To the other part add hydrochloric acid; a white crystalline precipitate of benzoic acid is produced.

B. To the distillate obtained in test A, add 2.5 g of potassium permanganate and 2 ml of sodium hydroxide solution, boil for 15 minutes in a flask fitted with a reflux condenser, cool and filter. To the filtrate add dilute hydrochloric acid till it is neutral and divide the mixture into two parts. To one part add ferric chloride test solution; a buff-coloured precipitate is produced. To the other part add hydrochloric acid; a white crystalline precipitate of benzoic acid is produced.

**Tests**

**Congealing temperature** (2.4.10). Not below 17.0°.

**Relative density** (2.4.29). 1.113 g to 1.118 g.

**Refractive index** (2.4.27). 1.567 to 1.569.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Assay.** Boil a convenient quantity of ethanol (95 per cent) thoroughly to expel carbon dioxide and neutralise to phenolphthalein solution. Weigh accurately about 2 g of the substance under examination, dissolve in 5 ml of the neutralised ethanol contained in a hard-glass flask and neutralise the free acid in the solution with 0.5 \(M\) ethanolic potassium hydroxide using 0.2 ml of phenolphthalein solution as indicator. Add 40 ml of 0.5 \(M\) ethanolic potassium hydroxide and boil under a reflux condenser on a water-bath for 1 hour. Add 20 ml of water and titrate the excess of alkali with 0.5 \(M\) hydrochloric acid using a further 0.2 ml of phenolphthalein solution as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the alkali required to saponify the benzyl benzoate.

1 ml of 0.5 \(M\) ethanolic potassium hydroxide is equivalent to 0.1061 g of \(\text{C}_{14}\text{H}_{12}\text{O}_2\).

**Storage.** Store protected from light and in well-filled containers.

**Benzyl Benzoate Application**

Benzyl Benzoate Application contains 25 per cent w/w of Benzyl Benzoate in a suitable oil-in-water emulsified basis.

Benzyl Benzoate Application contains not less than 22.5 per cent and not more than 27.5 per cent w/w of benzyl benzoate, \(\text{C}_{14}\text{H}_{12}\text{O}_2\).

**Assay.** Weigh accurately about 8.0 g and dissolve in 10 ml of ethanol (95 per cent) previously neutralised with 0.1 \(M\) sodium hydroxide contained in a hard-glass flask and neutralise the free acid in the solution with 0.5 \(M\) ethanolic potassium hydroxide using 0.2 ml of phenolphthalein solution as indicator. Add 40 ml of 0.5 \(M\) ethanolic potassium hydroxide and boil under a reflux condenser on a water-bath for 1 hour. Add 20 ml of water and titrate the excess of alkali with 0.5 \(M\) hydrochloric acid using a further 0.2 ml of phenolphthalein solution as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the alkali required to saponify the benzyl benzoate.

1 ml of 0.5 \(M\) ethanolic potassium hydroxide is equivalent to 0.1061 g of \(\text{C}_{14}\text{H}_{12}\text{O}_2\).

**Labelling.** The label states that the contents should be shaken before use.

**Benzylpenicillin Potassium**

Penicillin G Potassium

\[
\text{C}_{16}\text{H}_{17}\text{KN}_{2}\text{O}_{4}\text{S} \quad \text{Mol. Wt. 372.5}
\]
Benzylpenicillin Potassium is potassium (6R)-6-(2-phenylacetamido)penicillanate, produced by the growth of certain strains of Penicillium notatum or related organisms, or obtained by any other means.

Benzylpenicillin Potassium contains not less than 96.0 per cent and not more than 100.5 per cent of penicillins, calculated as C_{16}H_{17}KN_{2}O_{4}S on the dried basis.

**Description.** A white or almost white, crystalline powder.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with benzylpenicillin potassium RS.

B. Gives reaction A of potassium salts (2.3.1).

**Tests**

**pH** (2.4.24). 5.5 to 7.5, determined in a 10.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +270° to +300°, determined in a 2.0 per cent w/v solution in carbon dioxide-free water.

**Light absorption** (2.4.7). Dissolve 94 mg in sufficient water to produce 50.0 ml. Measure the absorbance of the solution at about 325 nm, at about 280 nm and at the maximum at about 264 nm, diluting the solution, if necessary, for the measurement at the maximum at about 264 nm. Absorbances at about 325 nm and 280 nm, not more than 0.10 and that at the maximum at about 264 nm, calculated on the basis of the undiluted solution (0.188 per cent w/v), 0.80 to 0.88.

**Related substances.** Determine by liquid chromatography (2.4.14) as given under Assay. Inject reference solution (d) and elute isocratically using the chosen mobile phase. Inject test solution (b) and start the elution isocratically. Immediately after elution of the benzylpenicillin peak start the following linear gradient:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
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<tr>
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<td>30 → 100</td>
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<td>0</td>
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</tr>
<tr>
<td>35 – 50</td>
<td>70</td>
<td>30</td>
<td>re-equilibration</td>
</tr>
</tbody>
</table>

Inject water and use the same elution pattern to obtain a blank. In the chromatogram obtained with test solution (b) the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (d) (1.0 per cent).

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°C.

**Assay.** Determine by liquid chromatography (2.4.14). Prepare the solutions immediately before use.

**Test solution (a).** Dissolve 50.0 mg of the substance under examination in water and dilute to 50.0 ml with the same solvent.

**Test solution (b).** Dissolve 80.0 mg of the substance under examination in water and dilute to 20.0 ml with the same solvent.

**Reference solution (a).** Dissolve 50.0 mg of benzylpenicillin potassium RS in water and dilute to 50.0 ml with the same solvent.

**Reference solution (b).** Dissolve 10 mg of benzylpenicillin potassium RS and 10 mg of phenylacetic acid RS in water and dilute to 50.0 ml with the same solvent.

**Reference solution (c).** Dilute 1.0 ml of reference solution (a) to 20.0 ml with water. Dilute 1.0 ml of the solution to 50.0 ml with the same solvent.

**Reference solution (d).** Dilute 4.0 ml of reference solution (a) to 100.0 ml with water.

**Chromatographic system**

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilil silica gel (5 µm),
- mobile phase: A. a mixture of 10 volumes of a 68 g per litre solution of potassium dihydrogen phosphate adjusted to pH 3.5 with a 500 g per litre solution of dilute phosphoric acid, 30 volumes of methanol and 60 volumes of water,
- B. a mixture of 10 volumes of a 68 g per litre solution of potassium dihydrogen phosphate adjusted to pH 3.5 with a 500 g per litre solution of dilute phosphoric acid, 40 volumes of water and 50 volumes of methanol,
- flow rate. 1 ml per minute,
- spectrophotometer set at 225 nm,
- a 20 µl loop injector.

Equilibrate the column with a mobile phase ratio A:B of 70:30. Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is at least 6.0 (if necessary, adjust the ratio A:B of the mobile phase) and the capacity factor for the second peak (benzylpenicillin) is 4.0 to 6.0.

Inject reference solution (c). Adjust the system to obtain a peak with a signal-to-noise ratio of at least 3.

Inject alternately test solution (a) and reference solution (a). Calculate the percentage content of benzylpenicillin potassium by multiplying the percentage content of benzylpenicillin sodium by 1.045.

**Benzylpenicillin Potassium intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.**
Bacterial endotoxins (2.2.3). Not more than 0.16 Endotoxin Unit per mg.

Benzylpenicillin Potassium intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture at a temperature not exceeding 30°. If it is intended for use in the manufacture of parenteral preparations, the container should be sterile and sealed so as to exclude micro-organisms.

Labelling. The label states whether or not the contents are intended for use in the manufacture of parenteral preparations.

Benzylpenicillin Sodium

Penicillin G Sodium

C_{16}H_{17}N_{2}NaO_{4}S  Mol. Wt. 356.4

Benzylpenicillin Sodium is sodium (6R)-6-(2-phenylacetamido) penicillanate, produced by the growth of certain strains of Penicillium notatum or related organisms, or obtained by any other means.

Benzylpenicillin Sodium contains not less than 96.0 per cent and not more than 100.5 per cent of penicillins, calculated as C_{16}H_{17}N_{2}NaO_{4}S on the dried basis.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with benzylpenicillin sodium RS.

B. Gives reaction A of sodium salts (2.3.1).

Tests

pH (2.4.24). 5.5 to 7.5, determined in a 10.0 per cent w/v solution.

Specific optical rotation (2.4.22). +285° to +310°, determined in a 2.0 per cent w/v solution in carbon dioxide-free water.

Light absorption (2.4.7). Dissolve 90 mg in sufficient water to produce 50.0 ml. Measure the absorbance of the solution at about 325 nm, at about 280 nm and at the maximum at about 264 nm, diluting the solution, if necessary, for the measurement at about 264 nm. Absorbances at about 325 nm and 280 nm, not more than 0.10 and that at the maximum at about 264 nm, calculated on the basis of the undiluted solution (0.18 per cent w/v), 0.80 to 0.88.

Related substances. Determine by liquid chromatography (2.4.14) as given under Assay. Inject reference solution (d) and elute isocratically using the chosen mobile phase. Inject test solution (b) and start the elution isocratically. Immediately after elution of the benzylpenicillin peak start the following linear gradient:

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</tr>
<tr>
<td>35 – 50</td>
<td>70</td>
<td>30</td>
<td>re-equilibration</td>
</tr>
</tbody>
</table>

Inject water and use the same elution pattern to obtain a blank. In the chromatogram obtained with test solution (b) the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (d) (1.0 per cent).

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography, (2.4.14).

Prepare the solutions immediately before use.

Test solution (a). Dissolve 50.0 mg of the substance under examination in water and dilute to 50.0 ml with the same solvent.

Test solution (b). Dissolve 80.0 mg of the substance under examination in water and dilute to 20.0 ml with the same solvent.

Reference solution (a). Dissolve 50.0 mg of benzylpenicillin sodium RS in water and dilute to 50.0 ml with the same solvent.

Reference solution (b). Dissolve 10 mg of benzylpenicillin sodium RS and 10 mg of phenylacetic acid RS in water and dilute to 50.0 ml with the same solvent.

Reference solution (c). Dilute 1.0 ml of reference solution (a) to 20.0 ml with water. Dilute 1.0 ml of the solution to 50.0 ml with the same solvent.

Reference solution (d). Dilute 4.0 ml of reference solution (a) to 100.0 ml with water.

Chromatographic system

- a stainless steel a column 25 cm x 4.6 mm, packed with octadecysilil silica gel (5 µm),
- mobile phase: A. a mixture of 10 volumes of a 68 g per litre solution of potassium dihydrogen phosphate adjusted to pH 3.5 with a 500 g per litre solution of dilute phosphoric acid, 30 volumes of methanol and 60 volumes of water,

- B. a mixture of 10 volumes of a 68 g per litre solution of potassium dihydrogen phosphate adjusted to pH 3.5 with a 500 g per litre solution of dilute phosphoric acid, 40 volumes of water and 50 volumes of methanol,
— flow rate. 1 ml per minute,
— spectrophotometer set at 225 nm,
— a 20 µl loop injector.

Equilibrate the column with a mobile phase ratio A:B of 70:30.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is at least 6.0 (if necessary, adjust the ratio A:B of the mobile phase) and the capacity factor for the second peak (benzylpenicillin) is 4.0 to 6.0.

Inject reference solution (c). Adjust the system to obtain a peak with a signal-to-noise ratio of at least 3.

Inject alternately test solution (a) and reference solution (a).

Calculate the percentage content of C_{16}H_{17}N_{2}O_{4}S.

Benzylpenicillin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.16 Endotoxin Unit per mg.

Benzylpenicillin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture at a temperature not exceeding 30°. If it is intended for use in the manufacture of parenteral preparations, the container should be sterile and sealed so as to exclude micro-organisms.

Labelling. The label states whether or not the contents are intended for use in the manufacture of parenteral preparations.

**Benzylpenicillin Injection**

Penicillin G Injection

Benzylpenicillin Injection is a sterile material consisting of Benzylpenicillin Potassium or Benzylpenicillin Sodium with or without buffering agents and other excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Benzylpenicillin Injection contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of penicillins, calculated as C_{16}H_{17}N_{2}O_{4}S.

**Description.** A white or almost white crystalline powder.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with benzylpenicillin potassium RS or benzylpenicillin sodium RS.

B. Gives reaction A of potassium or sodium salts (2.3.1).

**Tests**

**pH** (2.4.24). 5.5 to 7.5, determined in a 10.0 per cent w/v solution.

**Related substances.** Determine by liquid chromatography (2.4.14) as given under Assay. Inject reference solution (d) and elute isocratically using the chosen mobile phase. Inject test solution (b) and start the elution isocratically. Immediately after elution of the benzylpenicillin peak start the following linear gradient:

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<td>70</td>
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</tr>
</tbody>
</table>

Inject water and use the same elution pattern to obtain a blank. In the chromatogram obtained with test solution (b) the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (d) (1.0 per cent).

**Bacterial endotoxins** (2.2.3). Not more than 0.16 Endotoxin Unit per mg.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°C.

**Assay.** Determine by liquid chromatography, (2.4.14).

Prepare the solutions immediately before use.

Determine the weight of the contents of 10 containers.

Test solution (a). Dissolve 50.0 mg of the mixed contents of the 10 containers in water and dilute to 50.0 ml with the same solvent.
Teat solution (b). Dissolve 80.0 mg of the substance under examination in water and dilute to 20.0 ml with the same solvent.

Reference solution (a). Dissolve 50.0 mg of benzylpenicillin sodium RS in water and dilute to 50.0 ml with the same solvent.

Reference solution (b). Dissolve 10 mg of benzylpenicillin sodium RS and 10 mg of phenylacetic acid RS in water and dilute to 50.0 ml with the same solvent.

Reference solution (c). Dissolve 10 mg of benzylpenicillin sodium RS and 10 mg of phenylacetic acid RS in water and dilute to 50.0 ml with the same solvent.

Reference solution (d). Dilute 4.0 ml of reference solution (a) to 100.0 ml with water.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilica gel (5 µm),
- mobile phase: A. a mixture of 10 volumes of a 68 g per litre solution of potassium dihydrogen phosphate adjusted to pH 3.5 with a 500 g per litre solution of dilute phosphoric acid, 30 volumes of methanol and 60 volumes of water,
  B. a mixture of 10 volumes of a 68 g per litre solution of potassium dihydrogen phosphate adjusted to pH 3.5 with a 500 g per litre solution of dilute phosphoric acid, 40 volumes of water and 50 volumes of methanol,
- flow rate. 1 ml per minute,
- spectrophotometer set at 225 nm,
- a 20 µl loop injector.

Equilibrate the column with a mobile phase ratio A:B of 70:30. Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is at least 6.0 (if necessary, adjust the ratio A:B of the mobile phase) and the capacity factor for the second peak (benzylpenicillin) is 4.0 to 6.0.

Inject reference solution (c). Adjust the system to obtain a peak with a signal-to-noise ratio of at least 3. Inject alternately test solution (a) and reference solution (a). Calculate the content of benzylpenicillin sodium in the injection. 1 mg of C₁₆H₁₇N₂NaO₄S is equivalent to 0.9383 mg of C₁₆H₁₉N₂O₄S.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states (1) whether the contents are Benzylpenicillin Potassium or Benzylpenicillin Sodium; (2) the name of any added buffering agents.

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Betahistine Hydrochloride

\[
\text{\begin{align*}
\text{C}_8\text{H}_{12}\text{N}_2, 2\text{HCl} \\
\text{Mol. Wt. 209.12}
\end{align*}}
\]

Betahistine Dihydrochloride is N-methyl-2-(2-pyridyl) ethylamine dihydrochloride.

Betahistine Hydrochloride contains not less than 98.5 per cent and not more than 102.0 per cent of C₈H₁₄Cl₂N₂·2HCl, calculated on the dried basis.

Description. A white to off-white, crystalline powder; sometimes clumped, odourless or almost odourless, very hygroscopic.

Identification
A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with betahistine hydrochloride RS.
B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.
C. Gives the reaction A of chlorides (2.3.12).

Tests

Appearance of solution. A 10 per cent w/v solution in water is clear (2.4.1) and not more intensely coloured than reference solution B8 (2.4.1).

pH (2.4.24). 2.0 to 3.0, determined in a 10 per cent w/v solution in water.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 40 mg of the substance under examination in 100 ml of the mobile phase.

Reference solution (a). A 0.04 per cent w/v solution of betahistine hydrochloride RS in the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 3.0 mm packed with octadecysilane chemically bonded to porous silica (5 µm),
- mobile phase: dissolve 0.45 g ammonium acetate and 0.4 ml glacial acetic acid in 650 ml of water, add 350 ml of acetonitrile and add 2.88 g of sodium laurylsulphate and mix,
flow rate, 0.5 ml per minute,
spectrophotometer set at 254 nm,
a 10 µl loop injector.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 2000 theoretical plates.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

**Heavy metals** (2.3.13). 1 g complies with the limit test for heavy metals, Method C (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 2.0 per cent, determined on 1 g by drying in an oven at 105°.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 40 mg of the substance under examination in 100.0 ml of the mobile phase.

**Reference solution.** A 0.04 per cent w/v solution of betahistine hydrochloride RS in the mobile phase.

Chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of C₁₈H₂₂Cl₂N₄·2HCl.

**Storage.** Store protected from light.

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**Betaistine Tablets**

Betaistine Hydrochloride Tablets

Betaistine Tablets contain Betaistine Dihydrochloride.

Betaistine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of betaistine dihydrochloride, C₁₈H₂₂Cl₂N₄·2HCl.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6).

Compare the spectrum with that obtained with betahistine hydrochloride RS.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

**Tests**

**Dissolution** (2.5.2).

**Apparatus.** No 2

**Medium.** Phosphate citrate buffer pH 6.8.

**Speed and time.** 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 256 nm (2.4.7). Calculate the content of C₁₈H₂₂Cl₂N₄·2HCl in the medium from the absorbance obtained from a solution of known concentration of betaistine hydrochloride RS in the same medium.

D. Not less than 80 per cent of the stated amount of C₁₈H₂₂Cl₂N₄·2HCl.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablet containing 32 mg of Betaistine Dihydrochloride, disperse in 50 ml of mobile phase and dilute to 100 ml with mobile phase and filter.

**Reference solution (a).** A 0.032 per cent w/v solution of betahistine hydrochloride RS in mobile phase.

**Reference solution (b).** Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadeциlsilil silica (5 µm),
- column temperature 50°,
- mobile phase: dissolve 2.76 g of sodium dihydrogen phosphate monohydrate and 1.60 g of sodium dodecylsulphate in 600 ml of water, add 1.2 g of hexylamine and 400 ml of acetonitrile, and mix, adjusted pH to 3.5 with orthophosphoric acid,
- flow rate, 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 100 µl loop injector.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 2000 theoretical plates.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than 1.5 times the area of the peak in the
chromatogram obtained with the reference solution (b) (1.5 per cent).

**Uniformity of content.** Comply with the tests stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay.

**Test solution.** Crush one tablet in 100 ml volumetric flask. Add about 50 ml of mobile phase and swirl for 10 minutes, make up to volume with mobile phase and filter.

Calculate the content of C$_8$H$_{14}$Cl$_2$N$_2$.2HCl.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablet containing 32 mg of Betahistine Dihydrochloride, disperse in 50 ml of mobile phase and dilute to 100.0 ml with mobile phase and filter.

**Reference solution.** A 0.032 per cent w/v solution of betahistine hydrochloride RS in mobile phase.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilyl silica (5 µm),
- column temperature 50º,
- mobile phase: dissolve 2.76 g of sodium dihydrogen phosphate monohydrate and 1.6 g of sodium dodecylsulphate in 600 ml of water. Add 0.4 g of hexylamine and 400 ml of acetonitrile, adjust the pH to 3.5 with orthophosphoric acid,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 2000 theoretical plates. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of C$_8$H$_{14}$Cl$_2$N$_2$.2HCl.

**Storage.** Store protected from light.

**Betamethasone**

Betamethasone is 9α-fluoro-11β,17α,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione.

Betamethasone contains not less than 96.0 per cent and not more than 104.0 per cent of C$_{22}$H$_{29}$FO$_5$, calculated on the dried basis.

**Description.** A white to creamy-white powder; odourless.

**Identification**

*Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with betamethasone RS or with the reference spectrum of betamethasone.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with a suitable silica gel containing a fluorescent indicator with an optimal intensity at 254 nm (such as Merck silica gel 60 F254).

**Mobile phase.** A mixture of 85 volumes of ether, 10 volumes of toluene and 5 volumes of 1-butanol saturated with water.

**Test solution.** Dissolve 25 mg of the substance under examination in a mixture of 90 volumes of chloroform and 10 volumes of methanol.

**Reference solution (a).** A 0.25 per cent w/v solution of betamethasone RS in a mixture of 90 volumes of chloroform and 10 volumes of methanol.

**Reference solution (b).** A 0.125 per cent w/v solution of each of the substance under examination and betamethasone RS in the same solvent mixture.

**Reference solution (c).** A 0.125 per cent w/v solution of each of the substance under examination and dexamethasone RS in the same solvent mixture.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and spray with ethanolic sulphuric acid (20 per cent). Heat at 120º for 10 minutes or until spots are produced, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution is similar in colour in daylight, in fluorescence in ultraviolet light at 365 nm, position and size to the principal spot in the chromatogram obtained with reference solution (a) and the chromatogram obtained with reference solution (b) shows only one spot. The test is not valid unless the chromatogram obtained with reference solution (c) shows two principal spots that are close to one another but separated.

C. Heat 0.5 ml of chromic-sulphuric acid in a test-tube (5 cm x about 6 mm) in a naked flame until white fumes are evolved; the solution wets the sides of the tube readily and there is no greasiness. Add 2 or 3 mg of the substance under examination
and again heat in a naked flame until white fumes appear; the solution does not wet the sides of the tube and does not pour easily from the tube.

D. Place 2 ml of a 0.01 per cent w/v solution in ethanol in a stoppered tube, add 10 ml of phenylhydrazine solution, mix, warm in a water-bath at 60° for 20 minutes and cool immediately; absorbance of the resulting solution at about 450 nm, not more than 0.25, (2.4.7).

Tests
Specific optical rotation (2.4.22). +114.0° to +122.0°, determined in a 0.5 per cent w/v solution in dioxan.

Light absorption (2.4.7). Absorbance of a 0.001 per cent w/v solution in ethanol (95 per cent) at the maximum at about 240 nm, 0.37 to 0.40.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25.0 mg of the substance under examination in a mixture of equal volumes of acetonitrile and methanol and dilute to 10.0 ml with the same solvent.

Reference solution (a). Dissolve 2 mg of betamethasone RS and 2 mg of methylprednisolone RS in mobile phase A and dilute to 100.0 ml with the same mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm ξ 4.6 mm, packed with octadecylsilyl silica gel (5 µm), temperature 45°,
- mobile phase: A. a mixture of 250 ml of acetonitrile and 700 ml of water, allowed to equilibrate, sufficient water added to produce 1000 ml and mixed, B. acetonitrile,
- flow rate. 2.5 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–15</td>
<td>100 → 0</td>
<td>0</td>
<td>isocratic</td>
</tr>
<tr>
<td>15–40</td>
<td>0 → 100</td>
<td>100</td>
<td>linear gradient</td>
</tr>
<tr>
<td>41–46</td>
<td>100 → 0</td>
<td>0</td>
<td>equilibration</td>
</tr>
</tbody>
</table>

Equilibrate the column with mobile phase B for at least 30 minutes and then with mobile phase A for 5 minutes. For subsequent chromatograms, use the conditions described from 40 minutes to 46 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is not less than 50 per cent of the full scale of the recorder.

Inject reference solution (a). When the chromatograms are recorded in the conditions described above, the retention times are: methylprednisolone, about 11.5 minutes and betamethasone, about 12.5 minutes. The test is not valid unless the resolution between the peaks corresponding to methylprednisolone and betamethasone is at least 1.5; if necessary, adjust the concentration of acetonitrile in mobile phase A.

Inject separately a mixture of equal volumes of acetonitrile and methanol as blank, the test solution and reference solution (b). In the chromatogram obtained with the test solution: the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and not more than one such peak has an area greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all the peaks, other than the principal peak, is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent). Disregard any peak due to the blank and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. Dissolve 0.1 g in alcohol and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of the solution to 100.0 ml with alcohol. Measure the absorbance of the resulting solution at the maximum at about 238.5 nm (2.4.7).

Calculate the percentage content of C22H29FO5 taking 395 as the specific absorbance at 238.5 nm.

Storage. Store protected from light.

Betamethasone Tablets

Betamethasone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of betamethasone, C22H29FO5.

Identification

Powder a few tablets and extract with chloroform. Evaporate the extract to dryness. The residue complies with the following tests.
A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with betamethasone RS or with the reference spectrum of betamethasone.

B. Place 2 ml of a 0.01 per cent w/v solution in ethanol in a stoppered tube, add 10 ml of phenylhydrazine solution, mix, warm in a water-bath at 60° for 20 minutes and cool immediately; absorbance of the resulting solution at about 450 nm, not more than 0.25 (2.4.7).

C. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Solvent mixture.** A mixture of 90 volumes of acetone and 10 volumes of formamide.

**Mobile phase.** Chloroform.

**Test solution.** Dissolve 25 mg of the residue in 10 ml of the solvent mixture.

**Reference solution (a).** Dissolve 25 mg of betamethasone RS in 10 ml of the solvent mixture.

**Reference solution (b).** Mix equal volumes of the test solution and reference solution (a).

**Reference solution (c).** Mix equal volumes of the test solution and a 0.25 per cent w/v solution of dexamethasone RS in the solvent mixture.

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with ethanolic sulphuric acid (20 per cent v/v). Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot. The chromatogram obtained with reference solution (c) shows two closely running spots.

**Tests**

**Related substances.** Transfer a quantity of the powdered tablets containing about 2 mg of Betamethasone to a glass-stoppered 50-ml centrifuge tube. Pipette 20 ml of ethanol (95 per cent) into the tube, shake for 2 minutes and allow to stand for 20 minutes with occasional shaking. Centrifuge the mixture for 5 minutes. Pipette 10 ml of the clear supernatant liquid into a glass-stoppered tube and evaporate the ethanol on a water-bath with the aid of a current of air to about 0.5 ml, then evaporate without heat to dryness. Pipette 1 ml of a mixture of 9 volumes of chloroform and 1 volume of methanol, insert the stopper and mix. Centrifuge, if necessary, to remove any insoluble material. Use this solution as the test solution.

Determine by thin-layer chromatography (2.4.17), coating the plate with a suitable silica gel containing a fluorescent indicator with an optimal intensity at 254 nm (such as Merck silica gel 60 F254).

**Mobile phase.** A mixture of 77 volumes of dichloromethane, 15 volumes of ether, 8 volumes of methanol and 1.2 volumes of water.

**Reference solution (a).** A 0.02 per cent w/v solution of the substance under examination in a mixture of 90 volumes of chloroform and 10 volumes of methanol.

**Reference solution (b).** A 0.01 per cent w/v solution of the substance under examination in the same solvent mixture.

**Reference solution (c).** A 0.1 per cent w/v solution of each of the substance under examination and prednisone RS in the same solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of solvents is no longer detectable and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

**Dissolution (2.5.2).**

**Apparatus.** No 1

**Medium.** 900 ml of water and 1 ml of 0.05 per cent w/v solution of testosterone RS (internal standard) in methanol.

**Speed and time.** 50 rpm and 45 minutes.

Use one tablet in the vessel for each test.

Withdraw a suitable volume of the medium and filter. Determine by liquid chromatography (2.4.14)

**Test solution.** The filtrate obtained as given above.

**Reference solution.** Dilute a mixture of 1.0 ml each of a 0.05 per cent w/v solution of betamethasone RS in methanol and 1 ml of a 0.05 per cent w/v solution of testosterone RS in methanol to 900 ml with water.
Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 60 volumes of methanol and 40 volumes of water,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

D: Not less than 75 per cent of the stated amount of C22H29FO5.

Uniformity of content. Comply with the test stated under Tablets. Determine by liquid chromatography (2.4.14).

Test solution. Finely crush one tablet, add 20.0 ml of a 0.002 per cent w/v solution of hydrocortisone (internal standard) in methanol (50 per cent), shake for 10 minutes and filter through a glass-fibre filter paper.

Reference solution. A solution containing 0.0025 per cent w/v of betamethasone RS and 0.002 per cent w/v of hydrocortisone.

NOTE — Protect the solutions from light.

Chromatographic system
- a stainless steel column 20 cm x 5 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 53 volumes of water and 47 volumes of methanol.
- flow rate. 1.4 ml per minute,
- spectrophotometer set at 238 nm,
- a 20 µl loop injector.

Calculate the content of C22H29FO5 in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 2.5 mg of Betamethasone, add 20.0 ml of methanol (50 per cent), shake for 10 minutes and filter through a glass-fibre paper.

Reference solution (a). A solution containing 0.0125 per cent w/v of betamethasone RS and 0.01 per cent w/v of hydrocortisone RS (internal standard).

Reference solution (b). Prepare in the same manner as the test solution but use 20.0 ml of a 0.01 per cent w/v solution of hydrocortisone in methanol (50 per cent) in place of 20.0 ml of methanol (50 per cent).

NOTE — Protect the solutions from light.

Carry out the chromatographic procedure described under Uniformity of content. Calculate the content of C22H29FO5 in the tablets.

Storage. Store protected from light.

Betamethasone Sodium Phosphate

Betamethasone Sodium Phosphate is 9α-fluoro-11β,17α,21-trihydroxy-16β-methyl-pregna-1,4-diene-3,20-dione disodium phosphate.

Betamethasone Sodium Phosphate contains not less than 96.0 per cent and not more than 103.0 per cent of C22H29FNa2O8P, calculated on the anhydrous basis.

Description. A white or almost white powder; odourless; very hygroscopic.

Identification
A. To 2 ml of a 0.013 per cent w/v solution in ethanol (95 per cent) in a stoppered tube add 10 ml of phenylhydrazine-sulphuric acid solution, mix, warm in a water-bath at 60° for 20 minutes and cool immediately. Absorbance of the resulting solution at the maximum at about 450 nm, not more than 0.13 (2.4.7).

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A freshly prepared mixture of 30 volumes of isopropyl alcohol, 10 volumes of acetic acid and 10 volumes of water.

Test solution. Dissolve 0.25 g of the substance under examination in 100 ml of water.

Reference solution (a). A 0.25 per cent w/v solution of betamethasone sodium phosphate RS.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Reference solution (c). A mixture of equal volumes of the test solution and a 0.25 per cent w/v solution of prednisolone sodium phosphate RS.

Apply to the plate 2 µl of each solution. After development, dry the plate in air until the odour of solvents is no longer detectable, spray with ethanolic sulphuric acid (20 per cent), heat at 120° for 10 minutes, allow to cool, and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to C22H29FNa2O8P Mol. Wt. 516.4
that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot and the chromatogram obtained with reference solution (c) shows two closely running spots.

C. Heat 0.5 ml of chromic-sulphuric acid in a test-tube (5 cm × about 6 mm) in a naked flame until white fumes are evolved; the solution wets the sides of the tube readily and there is no greasiness. Add 2 or 3 mg of the substance under examination and again heat in a naked flame until white fumes appear; the solution does not wet the sides of the tube and does not pour easily from the tube.

D. Dissolve 2 mg in 2 ml of sulphuric acid and allow to stand for 5 minutes; no red colour or yellowish-green fluorescence is produced (distinction from prednisolone sodium phosphate and hydrocortisone sodium phosphate).

E. Heat gently 40 mg with 2 ml of sulphuric acid until white fumes are evolved, add nitric acid dropwise until oxidation is complete and cool. Add 2 ml of water, heat until white fumes are again evolved, cool, add 10 ml of water and neutralise to litmus paper with dilute ammonia solution. The solution gives the reactions of sodium salts and of phosphates (2.3.1).

**Tests**

**Appearance of solution.** A 2.0 per cent w/v solution is clear (2.4.1), and colourless (2.4.1).

**pH** (2.4.24). 7.5 to 9.0, determined in a 0.5 per cent w/v solution.

**Specific optical rotation** (2.4.22). +98.0° to +104°, determined in a 1.0 per cent w/v solution.

**Light absorption** (2.4.7). Ratio of the absorbance of the solution prepared as directed under Assay at the maximum at about 241 nm to that at about 263 nm, 1.70 to 1.90.

**Inorganic phosphate.** Not more than 0.5 per cent, calculated as PO₄, determined by the following method. Weigh accurately about 25 mg, dissolve in 10 ml of water; add 4 ml of dilute sulphuric acid, 1 ml of ammonium molybdate solution and 2 ml of methylaminophenol with sulphite solution and allow to stand for 15 minutes. Add sufficient water to produce 25.0 ml, allow to stand for further 15 minutes and measure the absorbance of the resulting solution at the maximum at about 730 nm (2.4.7). Calculate the content of phosphate from a calibration curve prepared by treating suitable aliquots of a 0.00143 per cent w/v solution of potassium dihydrogen phosphate in a similar manner.

**Free betamethasone and other derivatives.** Determine by thin-layer chromatography, (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** Methanol.

**Test solution.** Dissolve 1.0 g of the substance under examination in 10 ml of methanol.

**Reference solution (a).** A 1.0 per cent w/v solution of betamethasone sodium phosphate RS in methanol.

**Reference solution (b).** A 0.02 per cent w/v solution of betamethasone RS in methanol.

Apply to the plate 2 µl of each solution. After development, dry the plate in air for 5 minutes and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution other than that corresponding to betamethasone sodium phosphate RS is not more intense than the spot in the chromatogram obtained with reference solution (b).

**Water** (2.3.43). Not more than 8.0 per cent, determined on 0.5 g.

**Assay.** Weigh accurately about 0.2 g and dissolve in sufficient water to produce 200.0 ml. Dilute 5.0 ml to 250.0 ml with water and measure the absorbance of the resulting solution at the maximum at about 241 nm (2.4.7). Calculate the content of C₂₂H₂₈FNa₂O₈P, taking 297 as the specific absorbance at 241 nm.

**Storage.** Store protected from light and moisture.

**Betamethasone Eye Drops**

Betamethasone Eye Drops are a sterile solution of Betamethasone Sodium Phosphate in Purified Water.

Betamethasone Eye Drops contain not less than 90.0 per cent and not more than 110.0 per cent of betamethasone sodium phosphate, C₂₂H₂₈FNa₂O₈P.

**Identification**

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 60 volumes of butanol, 20 volumes of acetic anhydride and 20 volumes of water.

**Test solution.** Dilute the eye drops suitably with water to get a solution containing 0.1 per cent w/v of Betamethasone Sodium Phosphate.

**Reference solution (a).** A 0.1 per cent w/v solution of betamethasone sodium phosphate RS in water.

**Reference solution (b).** A mixture of equal volumes of the test solution and reference solution (a).

**Reference solution (c).** A mixture of equal volumes of reference solution (a) and 0.1 per cent w/v of prednisolone sodium phosphate RS in water.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air, heat at 110° for 10 minutes and examine in ultraviolet light at 254 nm. The
chromatograms obtained with the test solution, reference solution (a) and reference solution (b) show single principal spots with similar Rf values. The chromatogram obtained with reference solution (c) shows two principal spots with almost identical Rf values.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

C. To a volume containing 0.2 mg of Betamethasone Sodium Phosphate, add slowly 1 ml of sulphuric acid and allow to stand for 2 minutes. A brownish yellow colour but no red colour or yellowish green fluorescence is produced.

Tests

**pH** (2.4.24). 7.0 to 8.5.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dilute the eye drops if necessary to obtain a solution containing 0.1 per cent w/v of Betamethasone Sodium Phosphate.

**Reference solution (a).** Dilute 1 volume of the test solution to 50 volumes with water.

**Reference solution (b).** A solution containing 0.006 per cent w/v each of betamethasone sodium phosphate RS and betamethasone RS.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilyl silica gel (10 µm) (such as Spherisorb ODS 1),
- column temperature, 60º,
- mobile phase: a mixture of 60 volumes of citro-phosphate buffer pH 5.0 and 40 volumes of methanol,
- flow rate. 2 ml per minute,
- spectrophotometer set at 241 nm,
- a 20 µl loop injector.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to betamethasone sodium phosphate and betamethasone is at least 3.5.

Inject the test solution and reference solution (a) and record the chromatogram for three times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any peak corresponding to betamethasone is not more than 1.3 times the area of the principal peak in the chromatogram obtained with reference solution (a). The area of any other secondary peak is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a). The sum of the areas of all the secondary peaks is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a).

**Other tests.** Comply with the tests stated under Eye Drops.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution (a).** Mix a quantity of the eye drops containing 5 mg of Betamethasone Sodium Phosphate with 10 ml of methanol and dilute to 25.0 ml with water.

**Test solution (b).** Mix a quantity of the eye drops containing 5 mg of Betamethasone Sodium Phosphate with 10 ml of a 0.06 per cent w/v solution of hydrocortisone (internal standard) in methanol and dilute to 25.0 ml with water.

**Reference solution.** Mix 5.0 ml of a 0.1 per cent w/v solution of betamethasone sodium phosphate RS in water (solution A) and 10 ml of the internal standard solution and dilute to 25.0 ml with water.

Chromatographic system
- a stainless steel column 20 cm x 5 mm, packed with octadecylsilyl silica gel (10 µm) (such as Spherisorb ODS 1),
- mobile phase: a mixture of 55 volumes of citro-phosphate buffer pH 5.0 and 45 volumes of methanol,
- flow rate. 2 ml per minute.
- spectrophotometer set at 241 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject test solutions (a), (b) and reference solution.

Calculate the content of C22H28FNa2O8P in the eye drops.

**Storage.** Store protected from light.

**Betamethasone Injection**

Betamethasone Sodium Phosphate Injection

Betamethasone Injection is a sterile solution of Betamethasone Sodium Phosphate in Water for Injections.

Betamethasone Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of betamethasone, C22H29FO5.

**Description.** A clear, colourless solution.

**Identification**

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A freshly prepared mixture of 30 volumes of 1-butanol, 10 volumes of acetic anhydride and 10 volumes of water.
**Test solution.** Dilute the injection, if necessary, with water so that it contains the equivalent of 2 mg of betamethasone per ml.

**Reference solution (a).** A 0.25 per cent w/v solution of betamethasone sodium phosphate RS in water.

**Reference solution (b).** A mixture of equal volumes of the test solution and reference solution (a).

**Reference solution (c).** A mixture of equal volumes of the test solution and a 0.25 per cent w/v solution of prednisolone sodium phosphate RS in water.

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of solvents is no longer detectable, spray with ethanolic sulphuric acid (20 per cent), heat at 120° for 10 minutes, allow to cool, and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot and the chromatogram obtained with reference solution (c) shows two closely running spots. Secondary spots due to excipients may also be seen in the chromatograms obtained with the test solution and reference solutions (b) and (c).

B. To a volume containing 4 mg of betamethasone, add 1 ml of water and sufficient ethanol to produce 40 ml. To 2 ml of this solution in a stoppered tube add 10 ml of phenylhydrazine solution, mix, warm in a water-bath at 60° for 20 minutes and cool immediately; absorbance of the resulting solution at the maximum at about 450 nm, not more than 0.1 (2.4.7).

**Tests**

**pH (2.4.24).** 7.5 to 9.0

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** Measure accurately a volume containing about 20 mg of betamethasone and add sufficient water to produce 50.0 ml. To 5.0 ml add 20 ml of water and 2 ml of 0.1 M hydrochloric acid and shake with two quantities, each of 25 ml, of ether. Wash the ethereal solutions separately with 2, 1 and 1 ml of water, add the washings to the acid solution and discard the ether solutions. To the combined acid solution and the washings add 2 ml of 0.1 M sodium hydroxide and sufficient water to produce 200.0 ml. Measure the absorbance of the resulting solution at the maximum at about 241 nm (2.4.7), using as the blank a solution prepared in a similar manner but omitting the substance under examination. Calculate the content of C₂₂H₂₉FO₅, taking 391 as the specific absorbance at 241 nm.

**Storage.** Store protected from light at a temperature not exceeding 30°.

**Labelling.** The label states the strength in terms of the equivalent amount of betamethasone in a suitable dose-volume.

**Betamethasone Sodium Phosphate Tablets**

Betamethasone Sodium Phosphate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of betamethasone, C₂₂H₂₉FO₅.

**Identification**

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A freshly prepared mixture of 30 volumes of 1-butanol, 10 volumes of acetic anhydride and 10 volumes of water.

**Test solution.** Dissolve a quantity of the powdered tablets containing 2 mg of betamethasone in 25 ml of water, add 2.5 g of sodium chloride and 1 ml of hydrochloric acid, extract with 25 ml of chloroform and discard the chloroform layer. Extract with 2.5 ml of tributyl phosphate and discard the aqueous layer.

**Reference solution (a).** Prepare in the same manner as the test solution but using 2.5 mg of betamethasone sodium phosphate RS instead of the substance under examination.

**Reference solution (b).** A mixture of equal volumes of the test solution and reference solution (a).

**Reference solution (c).** A mixture of equal volumes of the test solution and a solution prepared in the same manner as the test solution but using 2.5 mg of prednisolone sodium phosphate RS instead of the substance under examination.

Apply to the plate 2 µl of each solution. After development, dry the plate in air until the odour of solvents is no longer detectable, spray with ethanolic sulphuric acid (20 per cent), heat at 120° for 10 minutes, allow to cool, and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot and the chromatogram obtained with reference solution (c) shows two closely running spots. Secondary spots due to excipients may also be seen in the chromatograms obtained with the test solution and reference solutions (b) and (c).

B. Mix a quantity of the powdered tablets containing 0.4 mg of betamethasone with 1 ml of sulphuric acid and allow to stand for 5 minutes; a pale yellow colour is produced.

**Tests**

**Disintegration (2.5.1).** Maximum time, 5 minutes.
Uniformity of content. Comply with the test stated under Tablets. Determine by liquid chromatography (2.4.14).

Test solution. Powder one tablet and dissolve as completely as possible in 5 ml of water and add 5 ml of a 0.006 per cent w/v solution of hydrocortisone (internal standard) in methanol.

Reference solution. A mixture of equal volumes of a 0.0065 per cent w/v solution of betamethasone sodium phosphate RS in water and a 0.006 per cent w/v solution of hydrocortisone in methanol.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm) (such as Spherisorb ODS),
- mobile phase: a mixture of 55 volumes of citrophosphate buffer pH 5.0 and 45 volumes of methanol,
- flow rate. 2 ml per minute,
- spectrophotometer set at 241 nm,
- a 20 µl loop injector.

Calculate the content of C_{27}H_{37}FO_{6} in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14)

Test solution. Weigh and powder 20 tablets. To a quantity of the powder containing 1.25 mg of betamethasone add 25.0 ml of a 0.006 per cent w/v solution of hydrocortisone (internal standard) in methanol and dilute to 50.0 ml with water.

Reference solution. A mixture of equal volumes of a 0.0065 per cent w/v solution of betamethasone sodium phosphate RS in water and a 0.006 per cent w/v solution of hydrocortisone in methanol.

Carry out the chromatographic procedure described under Uniformity of content. Calculate the content of C_{27}H_{37}FO_{6} in the tablets.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of betamethasone.

Betamethasone Valerate

Betamethasone Valerate contains not less than 96.0 per cent and not more than 102.0 per cent of C_{27}H_{37}FO_{6}, calculated on the dried basis.

Description. A white to creamy-white powder.

Identification
A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with betamethasone valerate RS or with the reference spectrum of betamethasone valerate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Solvent mixture. A mixture of 90 volumes of acetone and 10 volumes of formamide.

Mobile phase. A mixture of 30 volumes of toluene and 10 volumes of chloroform.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

Reference solution (a). Dissolve 25 mg of betamethasone RS in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 1 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120 °C for 15 minutes and spray the hot plate with ethanolic sulphuric acid (20 per cent v/v). Heat at 120 °C for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. In the Assay, the retention time of the principal peak in the chromatogram obtained with the test solution corresponds to that of the peak due to betamethasone valerate RS in the chromatogram obtained with the reference solution.

D. Heat 50 mg with 2 ml of 0.5 M ethanolic potassium hydroxide in a water-bath for 5 minutes. Cool, add 2 ml of sulphuric acid (50 per cent v/v) and boil gently for 1 minute; the odour of ethyl valerate is perceptible.
Tests

Specific optical rotation (2.4.22). +75.0° to +82.0°, determined in a 1.0 per cent w/v solution in dioxan.

Light absorption (2.4.7). Absorbance of a 0.002 per cent w/v solution in ethanol at the maximum at about 240 nm, 0.63 to 0.67.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately 4 mg of the substance under examination add 10 ml of the mobile phase and shake well to dissolve.

Chromatographic system
- a stainless steel column 15 cm × 4.6 mm packed with octadecylsilil silica gel (3 to 10 µm),
- mobile phase: a mixture of 55 volumes of acetonitrile, 45 volumes of water and 0.1 volume of glacial acetic acid,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 10 µl loop injector.

Inject the test solution. The resolution between betamethasone valerate and any impurity is not less than 1.5 and the column efficiency is not less than 9000 theoretical plates.

Inject the test solution. Measure all the peak responses. Calculate the content of each impurity as a percentage of the sum of all the peak responses (1.0 per cent). Not more than 2.0 per cent of total impurities is found.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately about 60 mg of the substance under examination add 10 ml of glacial acetic acid in methanol and dilute to 100.0 ml with the same solvent. To 5.0 ml of this solution add 10.0 ml of reference solution (b) and mix.

Reference solution (a). Weigh accurately a suitable quantity of betamethasone valerate RS and dissolve in a 0.1 per cent v/v solution of glacial acetic acid in methanol to obtain a solution containing a known concentration of about 0.6 mg per ml. To 5.0 ml of this solution add 10.0 ml of reference solution (b) and mix.

Reference solution (b). A 0.04 per cent w/v solution of betamethasone dipropionate RS in a 0.1 per cent v/v solution of glacial acetic acid in methanol.

Chromatographic system
- a stainless steel column 30 cm × 4.0 mm, packed with octadecylsilil silica gel (3 to 10 µm),
- mobile phase: a mixture of 30 volumes of acetonitrile and 20 volumes of water,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 254 nm,
- a 10 µl loop injector.

Inject reference solution (a). The relative retention times are about 1.7 for beclomethasone dipropionate and 1.0 for betamethasone valerate, The resolution between betamethasone valerate and beclomethasone dipropionate is not less than 4.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the percentage content of C27H37FO6.

Storage. Store protected from light.

Betamethasone Valerate Ointment

Betamethasone Valerate Ointment contains Betamethasone Valerate in a suitable ointment base.

Betamethasone Valerate Ointment contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of betamethasone, C22H29FO5.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 20 volumes of chloroform, 2 volumes of acetone and 1 volume of ethanol.

Test solution. Heat a quantity of the ointment containing 1 mg of betamethasone with 10 ml of methanol on a water-bath until it boils, shake vigorously, cool in ice for 30 minutes, filter, evaporate the filtrate to dryness in a current of nitrogen with gentle heating and dissolve the residue in 0.5 ml of chloroform.

Reference solution. A 0.24 per cent w/v solution of betamethasone valerate RS in chloroform.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, heat at 105° for 5 minutes and spray while hot with alkaline tetrazolium blue solution. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to betamethasone valerate RS in the chromatogram obtained with the reference solution.

Tests

Microbial contamination (2.2.9). 1.0 g is free from Staphylococcus aureus and Pseudomonas aeruginosa.
Other tests. Complies with the tests stated under Ointments.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Heat a quantity of the accurately weighed ointment containing 2.5 mg of betamethasone with 10.0 ml of 0.04 per cent w/v solution of beclomethasone dipropionate RS (internal standard) in methanol containing 0.1 per cent v/v of glacial acetic acid and 5.0 ml of methanol containing 0.1 per cent v/v of glacial acetic acid on a water-bath until it boils, shake vigorously, cool in ice for 30 minutes, centrifuge and decant the supernatant solution into a stoppered flask.

Reference solution. Mix 5 ml of a 0.06 per cent w/v solution of betamethasone valerate RS in methanol containing 0.1 per cent v/v of glacial acetic acid and 10.0 ml of a 0.04 per cent w/v solution of beclomethasone dipropionate RS in methanol containing 0.1 per cent v/v of glacial acetic acid.

Chromatographic system
- a stainless steel column 30 cm × 4.0 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 60 volumes of acetonitrile and 40 volumes of water,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 240 nm,
- a 20 µl loop injector.

Inject the reference solution. The relative retention times should be about 1.7 for beclomethasone dipropionate and 1.0 for betamethasone valerate.

Inject alternately the test solution and the reference solution.

Calculate the content of C_{22}H_{29}FO_{5} in the ointment.

Storage. Store protected from light. Avoid exposure to excessive heat.

Labelling. The label states the strength in terms of the equivalent amount of betamethasone.

Biperiden Hydrochloride

Biperiden Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of C_{21}H_{29}NO.HCl, calculated on the dried basis.

Description. A white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with biperiden hydrochloride RS.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 100 volumes of toluene, 5 volumes of diethylamine and 5 volumes of methanol.

Test solution. Dissolve 0.5 g of the substance under examination in 100 ml of methanol.

Reference solution (a). A 0.5 per cent w/v solution of biperiden hydrochloride RS in methanol.

Reference solution (b). Dissolve 5 mg of (SR)-1-[(1RS, 2RS, 4RS)-bicyclo[2.2.1]hept-5-en-2-yl]-1-phenyl-3-(piperidin-1-yl)-propan-1-ol (endo form) in reference solution (a) and dilute to 2 ml with the same solution.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). Spray with dilute iodobismuthate solution and examine in daylight. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. To about 20 mg add 5 ml of phosphoric acid; a green colour develops.

D. Gives reaction A of chlorides (2.3.1).

Tests

Appearance of solution. A 0.2 per cent solution in carbon dioxide-free water is not more opalescent than opalescence standard OS2 (2.4.1), and is colourless (2.4.1).

pH (2.4.24). 5.0 to 6.5, determined in a 0.2 per cent w/v solution.

Related substances. Determine by gas chromatography (2.4.13).

Test solution. Dissolve 1.0 g of the substance under examination in 100 ml of methanol.

Biperiden Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of C_{21}H_{29}NO.HCl, calculated on the dried basis.

Description. A white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with biperiden hydrochloride RS.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 100 volumes of toluene, 5 volumes of diethylamine and 5 volumes of methanol.

Test solution. Dissolve 0.5 g of the substance under examination in 100 ml of methanol.

Reference solution (a). A 0.5 per cent w/v solution of biperiden hydrochloride RS in methanol.

Reference solution (b). Dissolve 5 mg of (SR)-1-[(1RS, 2RS, 4RS)-bicyclo[2.2.1]hept-5-en-2-yl]-1-phenyl-3-(piperidin-1-yl)-propan-1-ol (endo form) in reference solution (a) and dilute to 2 ml with the same solution.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). Spray with dilute iodobismuthate solution and examine in daylight. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. To about 20 mg add 5 ml of phosphoric acid; a green colour develops.

D. Gives reaction A of chlorides (2.3.1).

Tests

Appearance of solution. A 0.2 per cent solution in carbon dioxide-free water is not more opalescent than opalescence standard OS2 (2.4.1), and is colourless (2.4.1).

pH (2.4.24). 5.0 to 6.5, determined in a 0.2 per cent w/v solution.

Related substances. Determine by gas chromatography (2.4.13).

Test solution. Dissolve 1.0 g of the substance under examination in 100 ml of methanol.
**Reference solution (a).** Dilute 1.0 ml of the test solution to 100 ml with methanol and mix. Dilute 10 ml of the resulting solution to 100 ml with methanol.

**Reference solution (b).** To 1.0 ml of the test solution add 10 ml of methanol and 10 mg of (SR)-1-[(1RS, 2RS, 4RS)-bicyclo[2.2.1]hept-5-en-2-yl]-1-phenyl-3-(piperidin-1-yl) propan-1-ol (endo form) and sufficient methanol to produce 100 ml.

**Chromatographic system**
- a fused-silica capillary column, 50 m × 0.25 mm coated with poly (vinyl-phenylmethyl siloxane with thickness of 0.25 µm,
- flame ionisation detector,
- temperature:
  - column. 200° for 5 minutes, then raised at the rate of 2° per minute to 270°,
  - inlet port at 250° and detector at 300°,
- flow rate. 0.4 ml per minute of nitrogen (the carrier gas) and a split ratio of 1:250.

Inject 2 µl of each solution. When using a recorder, adjust the sensitivity of the system so that the heights of the two principal peaks in the chromatogram obtained with reference solution (b) are not less than 50 per cent of the full scale of the recorder. The test is not valid unless, in the chromatogram obtained with reference solution (a), the resolution between the first peak due to biperiden and the second peak due to (SR)-1-[(1RS, 2RS, 4RS)-bicyclo[2.2.1]hept-5-en-2-yl]-1-phenyl-3-(piperidin-1-yl)propan-1-ol (endo form) is at least 2.5; the principal peak in the chromatogram obtained with reference solution (a) has a signal-to-noise ratio of at least 6. For peaks with relative retention times outside the above-mentioned range, the area of any peak, other than the principal peak, is not greater than 0.5 per cent of the area of the principal peak and the sum of the areas of such peaks is not greater than 0.5 per cent of the area of the principal peak. Disregard any peak with an area less than 0.05 per cent of the area of the principal peak in the chromatogram obtained with the test solution.

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method D (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

**Assay.** Weigh accurately about 0.5 g, dissolve in 80 ml of anhydrous glacial acetic acid, warming slightly, if necessary to effect solution and cool. Add 10 ml of mercuric acetate solution and titrate with 0.1 M perchloric acid, using 0.1 ml of crystal violet solution as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.03479 g of C_{21}H_{29}NO, HCl.

**Storage.** Store protected from light.

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**Biperiden Tablets**

Biperiden Hydrochloride Tablets

Biperiden Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of biperiden hydrochloride, C_{21}H_{29}NO, HCl.

**Identification**

Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 100 volumes of methanol and 1.5 volumes of strong ammonia solution.

**Test solution.** Shake a quantity of the powdered tablets containing about 10 mg of Biperiden Hydrochloride with 5 ml of water and disperse the powder with the aid of ultra sound for a few minutes. Add 5 ml of methanol and mix again for 15 minutes. Filter the solution into a separator, add 2 ml of 1 M sodium hydroxide and 10 ml of chloroform and shake for 3 minutes. Filter the chloroform layer into a stoppered flask and use the filtrate.

**Reference solution.** Prepare in a similar manner using 10 mg of biperiden hydrochloride RS in place of the substance under examination.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and expose it to iodine vapours till spots appear. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

**Tests**

**Dissolution** (2.5.2).

Apparatus. No 1
Medium. 500 ml of 0.1 M hydrochloric acid.
Speed and time. 50 rpm and 45 minutes.

Use one tablet in the vessel for each test.

Withdraw 75 ml of the solution and filter through a membrane filter disc with an average pore diameter not greater than
1.0 μm, rejecting the first few ml of the filtrate. Transfer 50.0 ml of the clear filtrate into a suitable container, adjust the pH to 5.3 with 0.1 M sodium hydroxide. Transfer this solution to a 100-ml volumetric flask and dilute with water to volume and mix.

Prepare a reference solution by weighing accurately about 80 mg of biperiden hydrochloride RS in sufficient methanol to produce 100.0 ml. Dilute 5.0 ml of this solution to 500.0 ml with 0.1 M hydrochloric acid and mix. Transfer 25.0 ml of the resulting solution into a suitable container and adjust the pH to 5.3 with 0.1 M sodium hydroxide and dilute to 100.0 ml with water (2 μg per ml).

Prepare a blank solution by treating 50 ml of water in place of the clear filtrate in the same manner as described for the test solution beginning at the words “adjust the pH to 5.3……

Transfer 20.0 ml of the solutions into individual separators, each containing 10.0 ml of phosphate-buffered bromocresol purple solution. Add 40.0 ml of chloroform to each and shake for 10 minutes. After the layers have separated, filter each chloroform extract through a filter paper into separate, glass-stoppered flasks, discarding the first 10 ml of each filtrate.

Measure the absorbances of the solutions at the maximum at about 408 nm against the blank solution (2.4.7). Calculate the content of C21H29NO,HCl in the medium from the absorbance obtained from the reference solution.

D: Not less than 75 per cent of the stated amount of C21H29NO,HCl.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 2 mg of Biperiden Hydrochloride and transfer to a 50-ml volumetric flask, add 12.5 ml of water and heat on a steam-bath for 15 minutes. Cool, dilute with methanol to volume and mix. Transfer 5.0 ml of the resulting solution to a separator, add 10.0 ml of phosphate-buffered bromocresol purple solution, extract with two quantities, each of 20 ml, of chloroform and allow to separate. Filter the chloroform extracts into a 50-ml volumetric flask through filter paper and make to volume. Measure the absorbance of the resulting solution at the maximum at about 408 nm (2.4.7). Calculate the content of C21H29NO,HCl in the medium from the absorbance obtained from the reference solution.

Bisacodyl is bis(4-acetoxyphenyl)-2-pyridylmethane.

Bisacodyl contains not less than 98.0 per cent and not more than 101.0 per cent of C22H19NO4, calculated on the dried basis.

Description. A white or almost white, crystalline powder; odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with bisacodyl RS or with the reference spectrum of bisacodyl.

B. When examined in the range 230 nm to 360 nm, a 0.001 per cent w/v solution in 0.1 M potassium hydroxide in methanol shows an absorption maximum only at about 248 nm; absorbance at about 248 nm, about 0.65 (2.4.7).

Tests

Acidity or alkalinity. Shake 1.0 g with 20 ml of carbon dioxide-free water, boil, cool and filter. Add 0.2 ml of 0.01 M sodium hydroxide and 0.1 ml of methyl red solution. The resulting solution is yellow and not more than 0.4 ml of 0.01 M hydrochloric acid is required to change the colour of the solution to red.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 0.5 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.3 g and dissolve in 50 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.03614 g of C22H19NO4.

Storage. Store protected from light.
Bisacodyl Suppositories
Bisacodyl Suppositories contain Bisacodyl in a suitable suppository basis.
Bisacodyl Suppositories contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of bisacodyl, C22H19NO4.

Identification
A. Dissolve a quantity of the suppositories containing 0.15 g of Bisacodyl as completely as possible in 150 ml of light petroleum (40° to 60°), filter, wash the residue with light petroleum (40° to 60°) until free from fatty material and dry at about 100°. Wash with a very small quantity of warm chloroform and dissolve the residue in 10 ml of a 1 per cent w/v solution of sulphuric acid (solution A). To 2 ml of the solution add one drop of potassium mercuri-iodide solution; a white precipitate is produced.
B. To 2 ml of solution A add sulphuric acid; a reddish-violet colour is produced.
C. Boil 2 ml of solution A with a few drops of nitric acid; a yellow colour is produced. Cool and add 5 M sodium hydroxide; the colour becomes yellowish-brown.

Tests
Uniformity of content. Comply with the test stated under Tablets.
Powder one tablet, shake with 70 ml of chloroform for 30 minutes and dilute with sufficient chloroform to produce 100.0 ml. Mix well, filter and discard the first few ml of the filtrate. Measure the absorbance of the filtrate at the maximum at about 264 nm (2.4.7). Calculate the content of C22H19NO4 taking 148 as the specific absorbance at 264 nm.

Bismuth Subcarbonate
Bismuth Carbonate
Bismuth Subcarbonate contains not less than 80.0 per cent and not more than 82.5 per cent of Bi, calculated on the dried basis.

Description. A white or almost white powder; odourless.

Identification
A. Gives the reactions of bismuth salts (2.3.1).
B. Gives reaction A of carbonates (2.3.1).

Tests
Appearance of solution. Shake 5.0 g with 10 ml of water, add 20 ml of nitric acid. Heat to dissolve, cool and dilute to 100 ml with water (solution A). Solution A is not more opalescent than opalescence standard OS2 (2.4.1), and is colourless (2.4.1).
**Alkalis and alkaline-earth metals.** Not more than 1.0 per cent, determined by the following method. To 1.0 g add 10 ml of water and 10 ml of 5 M acetic acid, boil for 2 minutes, cool, filter and wash the residue with 20 ml of water. To the combined filtrate and washings add 2 ml of 2 M hydrochloric acid and 20 ml of water. Boil, pass hydrogen sulphide through the boiling solution until no further precipitate is produced, filter and wash the residue with water. Evaporate the combined filtrate and washings to dryness on a water-bath and add 0.5 ml of sulphuric acid, ignite gently and allow to cool.

**Arsenic** (2.3.10). To 0.5 g in a distillation flask add 5 ml of water and 7 ml of sulphuric acid, cool and add 5 g of hydrazine reducing mixture and 10 ml of hydrochloric acid. Connect the flask to an air-condenser, heat gradually to boiling during 15 to 30 minutes and continue heating at such a rate that the distillation proceeds steadily and until the volume in the flask is reduced by half, or until 5 minutes after the condenser has become full of steam. Discontinue distillation before fumes of sulphur trioxide are evolved. Collect the distillate in a tube containing 15 ml of water cooled in ice. Wash the condenser with water and dilute the combined distillate and washings to 25 ml with water. The resulting solution complies with the limit test for arsenic (5 ppm). Use 2.5 ml of arsenic standard solution (1 ppm As) diluted to 25 ml with water to prepare the standard.

**Copper.** To 5 ml of solution A add 2 ml of 10 M ammonia, dilute to 50 ml with water and filter. To 10 ml of the filtrate add 1 ml of a 0.1 per cent w/v solution of sodium diethylthiocarbamate. Any colour produced is not more intense than that produced by treating at the same time and in the same manner a solution containing 0.25 ml of copper standard solution (10 ppm Cu) diluted to 10 ml with water (50 ppm).

**Lead.** To 10 ml of solution A add 10 ml of 1 M sulphuric acid; the solution does not become cloudy.

**Silver.** To 2.0 g add 1 ml of water and 4 ml of nitric acid. Heat gently to dissolve and dilute to 11 ml with water. Cool, add 2 ml of 1 M hydrochloric acid and allow to stand for 5 minutes protected from light. Any opalescence produced is not more intense than that obtained by treating at the same time and in the same manner a solution containing 0.25 ml of silver standard solution (5 ppm Ag), 2 ml of 1 M hydrochloric acid and 1 ml of nitric acid (25 ppm).

**Chlorides** (2.3.12). To 10 ml of solution A add 4 ml of nitric acid and 20 ml of water; the resulting solution complies with the limit test for chlorides (500 ppm).

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.5 g, dissolve in 3 ml of nitric acid and dilute to 250 ml with water. Add strong ammonia solution until cloudiness is first observed, add 0.5 ml of nitric acid and heat to 70°, maintaining the solution at this temperature until the solution becomes completely clear. Add about 50 mg of xylenol orange mixture and titrate with 0.1 M disodium edetate until the colour changes from pinkish-violet to lemon yellow.

1 ml of 0.1 M disodium edetate is equivalent to 0.02090 g of Bi.

**Storage.** Store protected from light.

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**Bleomycin Sulphate**

The sulphate salt of bleomycin, a mixture of basic cytotoxic glycopeptides produced by the growth of *Streptomyces verticillus* or produced by other means. Its main components are bleomycin A₂ and bleomycin B₂. Bleomycin A₂ sulphate is 

\[
\text{C}_{55}\text{H}_{84}\text{N}_{17}\text{O}_{21}\text{S}_{3}\text{H}_{2}\text{SO}_{4}
\]

Mol. Wt. 1516.6

(Bleomycin A₂ Sulphate)

\[
\text{C}_{55}\text{H}_{84}\text{N}_{17}\text{O}_{21}\text{S}_{3}\text{H}_{2}\text{SO}_{4}
\]

Mol. Wt. 1523.6

(Bleomycin B₂ Sulphate)

Bleomycin Sulphate is the sulphate salt of bleomycin, a mixture of basic cytotoxic glycopeptides produced by the growth of *Streptomyces verticillus* or produced by other means. Its main components are bleomycin A₂ and bleomycin B₂. Bleomycin A₂ sulphate is \(N^1\)-[3-(dimethylsulphonio)propyl]bleomycinamide hydrogen sulphate and Bleomycin B₂ is \(N^1\)-(guanidinobutyl)bleomycinamide sulphate.

Bleomycin Sulphate contains not less than 1.5 and not more than 2.0 Units of bleomycin per mg and the content of bleomycins is: bleomycin A₂, between 55 per cent and 70 per cent; bleomycin B₂, between 25 per cent and 32 per cent; sum of bleomycin A₂ and bleomycin B₂, not less than 85 per cent; demethylbleomycin A₂, not more than 5.5 per cent; other related substances, not more than 9.5 per cent.

**Description.** A white or cream-coloured, amorphous powder.

**CAUTION — Bleomycin Sulphate must be handled with care, avoiding contact with the skin and inhalation of airborne particles.**
Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with bleomycin sulphate RS.

B. Gives the reactions of sulphates (2.3.1).

Tests

pH (2.4.24). 4.5 to 6.0, determined in a solution containing 10 Units per ml.

Copper. Not more than 0.02 per cent determined by Method A or by Method B.

A. Weigh accurately about 50 mg, transfer to a 60-ml separator and dissolve in 10.0 ml of 0.1 M hydrochloric acid. Add 10 ml of a 0.01 per cent w/v solution of zinc bis (diphenyl dithiocarbamate) in carbon tetrachloride and shake vigorously for 1 minute. Allow the layers to separate, filter the lower layer through 1 g of anhydrous sodium sulphate. Treat similarly 10.0 ml of copper standard solution (10 ppm Cu) and measure the absorbances (2.4.7) of the two solutions at the maximum at about 435 nm, using carbon tetrachloride as the blank.

B. Determine by atomic absorption spectrophotometry (2.4.2) measuring at 324.7 nm using an air-acetylene flame and a solution prepared in the following manner: Dissolve about 50 mg of the substance under examination in water and dilute to 10.0 ml with the same solvent. Use copper solution AAS suitably diluted with water, for preparing the reference solutions.

Content of bleomycins. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve the substance under examination in freshly boiled and cooled water so as to give a solution containing about 2.5 Units per ml. This solution should be stored at 2° to 8° until just before use.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilyl silica gel (5 µm) (such as Nucleosil C18),
- mobile phase: Transfer 0.96 g of sodium 1-pentanesulphonate to a 1000-ml volumetric flask, add 5.0 ml of glacial acetic acid and 900 volumes of water. Mix and adjust the pH to 4.3 with strong ammonia solution (1.86 g of disodium edetate may be included if needed for satisfactory chromatography). Adjust the volume with water, mix well, filter and degas before use. Use a linear gradient of 10 per cent to 40 per cent methanol, which also is filtered and degassed before use, mixed with this solution,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- a 10 µl loop injector.

After the final conditions are reached (about 60 minutes) allow the chromatography to proceed with the final gradient mixture for an additional 20 minutes or until demethylbleomycin A₂ is eluted.

Inject the test solution and proceed with gradient elution, pumping the mobile phase mixture under the conditions mentioned above for about 80 minutes or until the demethylbleomycin A₂ is eluted. The usual order of elution is bleomycinic acid, bleomycin A₁ (first principal peak), bleomycin A₅, bleomycin B₂ (second principal peak), bleomycin B₄, and demethylbleomycin A₂ (retention time relative to bleomycin A₅, between 1.5 and 2.5).

Measure the peak responses of all peaks. Calculate the percentage contents of each bleomycin component by comparing the ratios of the individual areas of the peaks with that of the total area of all bleomycins.

Loss on drying (2.4.19). Not more than 6.0 per cent, determined on 0.5 g by drying in an oven over phosphorous pentoxide at 60° at a pressure not exceeding 0.25 kPa for 3 hours.

Assay. Determine by the microbiological assay of antibiotics, Method A or B (2.2.10), and express the result in Units per mg.

Bleomycin Sulphate intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 10.0 Endotoxin Units per unit of bleomycin.

Bleomycin Sulphate intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. If the material is sterile, it should be stored in sterile, tamper-evident containers and sealed so as to exclude microorganisms.

Labelling. The label states (1) the strength with respect to Bleomycin Sulphate as the number of bleomycin Units per mg; (2) whether or not the contents are intended for use in the manufacture of parenteral preparations.

Bleomycin Injection

Bleomycin sulphate injection

Beomycin injection is a sterile freeze dried material consisting of Bleomycin sulphate with or without excipients. It is filled in a sealed container.
The injection is constituted by dissolving the contents of the sealed container in the requisite amount of the liquid stated on the label before use.

The constituted solution complies with the tests for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Bleomycin injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of bleomycin and the content of bleomycins is: bleomycin A₂, between 55 per cent and 70 per cent; bleomycin B₂, between 25 and 32 per cent; sum of bleomycin A₂ and bleomycin B₂, not less than 85 per cent; demethylbleomycin A₂, not more than 5.5 per cent; other related substances, not more than 9.5 per cent.

The contents of the sealed container comply with the tests stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. Determine by infra-red absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with bleomycin sulphate RS.

B. Gives the reactions of sulphates (2.3.1).

Tests

pH (2.4.24). 4.5 to 6.0, determined in a solution containing 10 Units per ml.

Copper. Not more than 0.02 per cent, determined by Method A or Method B

A. Weigh accurately a quantity containing about 50 mg of bleomycin, transfer to a 60-ml separator and dissolve in 10.0 ml of 0.1 M hydrochloric acid. Add 10 ml of a 0.01 per cent w/v solution of zinc bis (diphenyl dithiocarbamate) in carbon tetrachloride and shake vigorously for 1 minute. Allow the layers to separate, filter the lower layer through 1 g of anhydrous sodium sulphate. Treat similarly 10.0 ml of copper standard solution (10 ppm Cu) and measure the absorbances (2.4.7) of the two solutions at the maximum at about 435 nm, using carbon tetrachloride as the blank.

B. Determine by atomic absorption spectrophotometry (2.4.2) measuring at 324.7 nm using an air-acetylene flame and a solution prepared in the following manner: Weigh accurately a quantity containing about 75 mg of bleomycin, dissolve in water and dilute to 10.0 ml with the same solvent. Use copper solution AAS suitably diluted with water, for preparing the reference solutions.

Content of bleomycins. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a suitable quantity dissolve in freshly boiled and cooled water and dilute to obtain a solution containing about 2.5 Units per ml. This solution should be stored at 2° to 8° until just before use.

Chromatographic system

– a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilyl silica gel (5 µm) (such as Nucleosil 7C18),
– mobile phase: Transfer 0.96 g of sodium 1-pentanesulphonate to a 1000-ml volumetric flask, add 5.0 ml of glacial acetic acid and 900 volumes of water. Mix and adjust the pH to 4.3 with strong ammonia solution (1.86 g of disodium edetate may be included if needed for satisfactory chromatography). Adjust the volume with water, mix well, filter and degas before use. Use a linear gradient of 10 per cent to 40 per cent methanol, which also is filtered and degassed before use, mixed with this solution,
– flow rate. 1.5 ml per minute,
– spectrophotometer set at 254 nm,
– a 10 µl loop injector.

After the final conditions are reached (about 60 minutes) allow the chromatography to proceed with the final gradient mixture for an additional 20 minutes or until demethylbleomycin A₂ is eluted.

Inject the test solution and proceed with gradient elution, pumping the mobile phase mixture under the conditions mentioned above for about 80 minutes or until the demethylbleomycin A₂ is eluted. The usual order of elution is bleomycinic acid, bleomycin A₂ (first principal peak), bleomycin A₃, bleomycin B₂ (second principal peak), bleomycin B₄ and demethylbleomycin A₂ (retention time relative to bleomycin A₂, between 1.5 and 2.5).

Measure the peak responses of all the peaks. Calculate the percentage contents of each bleomycin component by comparing the ratios of the individual areas of the peaks with that of the total area of all bleomycins.

Bacterial endotoxins (2.2.3). Not more than 10.0 Endotoxin Units per unit of bleomycin.

Loss on drying (2.4.19). Not more than 6.0 per cent, determined by drying the combined contents of two containers in an oven at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. Determine the weight of the contents of 10 containers. Mix the contents of the containers and determine by the microbiological assay of antibiotics, Method A or B (2.2.10) and express the results in Units per vial.

Storage. The sealed container should be protected from light.
Labelling. The label states the total number of units contained in the sealed container.

Boric Acid

H₃BO₃  Mol. Wt. 61.8

Boric Acid contains not less than 99.5 per cent and not more than 100.5 per cent of H₃BO₃, calculated on the dried basis.

Description. A white, crystalline powder or colourless shiny plates unctuous to the touch or white crystals; odourless.

Identification

A. Dissolve 0.1 g by gently warming with 5 ml of methanol to which a few drops of sulphuric acid have been added. Ignite the solution; the flame has a green border.
B. Dissolve 3.0 g in 90 ml of boiling distilled water, cool; the solution is faintly acid.

Tests

Appearance of solution. A 3.5 per cent w/v solution in boiling water is clear (2.4.1), and colourless (2.4.1).

pH (2.4.24). 3.8 to 4.8, determined in the solution obtained in Identification test B.

Solubility in ethanol. Dissolve 1.0 g in 10 ml of boiling ethanol (95 per cent); the solution is not more opalescent than opalescence standard OS2 (2.4.1), and colourless (2.4.1).

Arsenic (2.3.10). Dissolve 1.0 g in 50 ml of water containing 2 g of citric acid and add 0.1 ml of stannous chloride solution AsT and 10 ml of hydrochloric acid. The resulting solution complies with the limit test for arsenic (10 ppm).

Heavy metals (2.3.13). A solution produced by dissolving 1.0 g in 2 ml of dilute acetic acid and diluting with sufficient water to produce 25 ml complies with the limit test for heavy metals, Method A (20 ppm).

Sulphates (2.3.17). Dissolve 0.33 g in 10 ml of boiling water and dilute to 15 ml with water. The solution complies with the limit test for sulphates (450 ppm).

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over silica gel for 5 hours.

Assay. Weigh accurately about 2.0 g, dissolve in a mixture of 50 ml of water and 100 ml of glycerin, previously neutralised to phenolphthalein solution. Titrate with 1 M sodium hydroxide using phenolphthalein solution as indicator.

1 ml of 1 M sodium hydroxide is equivalent to 0.06183 g of H₃BO₃.

Labelling. The label states that it is not meant for internal use.

Bromhexine Hydrochloride

C₈H₁₄Br₂N₂.HCl  Mol. Wt. 412.6

Bromhexine Hydrochloride is 2-amino-3,5-dibromobenzyl(cyclohexyl)methylamine hydrochloride.

Bromhexine Hydrochloride contains not less than 98.5 per cent and not more than 101.5 per cent of C₈H₁₄Br₂N₂.HCl, calculated on the dried basis.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Identification

Test A may be omitted if tests B, C, D and E are carried out.
Tests B, C and D may be omitted if tests A and E are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with bromhexine hydrochloride RS or with the reference spectrum of bromhexine hydrochloride.
B. In the test for Related Substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).
C. Dissolve about 25 mg in a mixture of 1 ml of 1 M sulphuric acid and 50 ml of water, add 2 ml of dichloromethane and 5 ml of a freshly prepared 2 per cent w/v solution of chloramine T and shake; a brownish yellow colour is produced in the lower layer.
D. A solution prepared by dissolving about 1 mg in 3 ml of 0.1 M hydrochloric acid gives the reaction for primary aromatic amines (2.3.1).
E. Dissolve about 20 mg in 1 ml of methanol and add 1 ml of water. The solution gives reaction A of chlorides (2.3.1).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 66 volumes of 1-butanol, 17 volumes of glacial acetic acid and 17 volumes of water.

Test solution (a). Dissolve 2.0 g of the substance under examination in 100 ml of methanol.
**Test solution (b).** Dilute 10 ml of test solution (a) to 100 ml with methanol.

**Reference solution (a).** Dilute 2.5 ml of test solution (b) to 100 ml with methanol.

**Reference solution (b).** Dilute 15 ml of reference solution (a) to 20 ml with methanol and mix.

**Reference solution (c).** A 0.2 per cent w/v solution of bromhexine hydrochloride RS in methanol.

Apply to the plate in small quantities a total of 20 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). The test is not valid unless there is a clearly visible spot in the chromatogram obtained with reference solution (b).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°C.

**Assay.** Weigh accurately about 0.3 g, dissolve in 70 ml of ethanol (95 per cent), add 1 ml of 0.1 M hydrochloric acid and titrate with 0.1 M sodium hydroxide, determining the endpoint potentiometrically (2.4.25). Record the volume added between the two inflections.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.04126 g of C₁₄H₂₀Br₂N₂·HCl.

**Storage.** Store protected from light.

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**Bromhexine Tablets**

Bromhexine Hydrochloride Tablets

Bromhexine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of bromhexine hydrochloride, C₁₄H₂₀Br₂N₂·HCl.

**Identification**

A. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows an absorption maximum only at about 317 nm.

B. Suspend a quantity of the powdered tablets containing 0.1 g of Bromhexine Hydrochloride in 5 ml of dilute ammonia solution and extract with two quantities, each of 20 ml, of chloroform. Wash the combined extracts with 5 ml of water, filter through anhydrous sodium sulphate and evaporate the filtrate to dryness using a rotary evaporator. If necessary, scratch the inside of the flask with a glass rod to induce crystallisation. Mix the residue with 1 g of sodium carbonate, heat at a dull red heat for 10 minutes, allow to cool, extract with water and filter. The filtrate, after acidification with 2 M nitric acid, yields reaction A of bromides (2.3.1).

C. Shake a quantity of the powdered tablets containing 20 mg of Bromhexine Hydrochloride with 10 ml methanol and filter. The filtrate gives reaction A of chlorides (2.3.1).

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 90 volumes of heptane and 10 volumes of ethanol.

**Test solution.** Shake a quantity of the powdered tablets containing 0.1 g of Bromhexine Hydrochloride with 10 ml of methanol for 5 minutes, centrifuge and mix 9 volumes of the supernatant liquid with 1 volume of strong ammonia solution.

**Reference solution.** Dilute 1 volume of the test solution to 40 volumes with methanol.

Apply to the plate 20 µl of each solution. After development, dry the plate in a current of warm air, place in a tank containing a freshly prepared 10 per cent w/v solution of sodium nitrite in 5 M hydrochloric acid and allow to stand for 1 minute. Remove the plate and immediately spray with a 0.5 per cent w/v solution of N-(1-naphthyl)ethylenediamine dihydrochloride in methanol. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 8 mg of Bromhexine Hydrochloride, shake with 50 ml of 0.1 M methanolic hydrochloric acid for 30 minutes, add sufficient 0.1 M methanolic hydrochloric acid to produce 100.0 ml and filter. Measure the absorbance of the filtrate at the maximum at about 317 nm (2.4.7). Calculate the content of C₁₄H₂₀Br₂N₂·HCl taking 87 as the specific absorbance at 317 nm.

**Storage.** Store protected from light.
Bromocriptine Mesylate

C_{32}H_{40}BrN_{5}O_{5},CH_{4}O_{3}S  
Mol. Wt. 750.7

Bromocriptine Mesylate is (5'S)-2-bromo-12'-hydroxy-2'-(1-methylethyl)-5'-2-(2-methylpropyl)ergotaman-3',6',18-trione methanesulphonate

Bromocriptine Mesylate contains not less than 98.0 per cent and not more than 101.0 per cent of C_{32}H_{40}BrN_{5}O_{5},CH_{4}O_{3}S, calculated on the dried basis.

Description. A white or slightly coloured, fine crystalline powder; very sensitive to light.

NOTE — Carry out the tests as rapidly as possible without exposure to daylight and with minimum exposure to artificial light.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B, C, D and E may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry in a mineral oil dispersion (2.4.6). Compare the spectrum with that obtained with bromocriptine mesylate RS or with the reference spectrum of bromocriptine mesylate.

B. Dissolve 5 mg in 5 ml of methanol and dilute to 100 ml with 0.01 M hydrochloric acid. The resulting solution, when examined in the range 230 nm to 360 nm (2.4.7) shows an absorption maximum at about 305 nm and a minimum at about 270 nm; absorbance at about 305 nm, 0.60 to 0.68.

C. In the test for Related substances, the principal band in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (d).

D. To about 0.1 g add 5 ml of 2 M hydrochloric acid, shake for 5 minutes, filter and add 1 ml of a 6 per cent w/v solution of barium chloride to the filtrate; it remains clear. Mix another 0.1 g with 0.5 g of anhydrous sodium carbonate and ignite until a white residue is obtained. After cooling, dissolve the residue in 5 ml of water (solution A); solution A gives the reactions of sulphates (2.3.1).

E. Solution A gives reaction A of bromides (2.3.1).

Tests

Appearance of solution. A 1.0 per cent w/v solution in methanol is clear (2.4.1), and not more intensely coloured than reference solution BS5, YS5 or BYS5 (2.4.1).

pH (2.4.24). 3.1 to 3.8, determined in a 1.0 per cent w/v solution in a mixture of 2 volumes of methanol and 8 volumes of water.

Specific optical rotation (2.4.22). +95° to +105°, determined in a 1.0 per cent w/v solution in a mixture of equal volumes of methanol and dichloromethane.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 100 volumes of ether, 88 volumes of dichloromethane, 3 volumes of 2-propanol, 1.5 volumes of water and 0.1 volume of strong ammonia solution.

Test solution (a). Prepare freshly a solution containing 1 g of the substance under examination in 100 ml of a mixture of 4 volumes of dichloromethane, 3 volumes of ethanol (95 per cent) and 3 volumes of methanol.

Test solution (b). Freshly dilute 10 ml of test solution (a) to 100 ml with the same solvent mixture.

Reference solution (a). Dilute 2 ml of test solution (b) to 50 ml with the same solvent mixture.

Reference solution (b). Dilute 10 ml of reference solution (a) to 20 ml with the same solvent mixture.

Reference solution (c). Dilute 10 ml of reference solution (b) to 20 ml with the same solvent mixture.

Reference solution (d). A 0.1 per cent w/v solution of bromocriptine mesylate RS in the same solvent mixture.

Apply to the plate, as 1-cm bands, 10 µl of each solution. Apply test solution (a) to the plate as the last solution and develop the chromatogram immediately in an unsaturated tank. After development, allow the plate to dry in a current of cold air, spray with ethanolic ammonium molybdate solution and heat at 100° until bands are visible (about 10 minutes). Any secondary band in the chromatogram obtained with test solution (a) is not more intense than the principal band in the chromatogram obtained with reference solution (a) and not more than one such band is more intense than the principal band in the chromatogram obtained with reference solution (b) and not more than one other such band is more intense...
than the principal band in the chromatogram obtained with solution (c).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 3.0 per cent, determined on 0.5 g by drying in an oven over phosphorus pentoxide at 80° at a pressure of 1.5 to 2.5 kPa for 5 hours.

**Assay.** Weigh accurately about 0.5 g, dissolve in 80 ml of a mixture of 10 volumes of anhydrous glacial acetic acid and 70 volumes of acetic anhydride. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.07507 g of C$_{32}$H$_{40}$BrN$_5$O$_5$,CH$_4$O$_3$S.

**Storage.** Store protected from light in a deep freezer (temperature not exceeding –15°).

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**Bromocriptine Capsules**

Bromocriptine Mesylate Capsules

Bromocriptine Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of bromocriptine, C$_{32}$H$_{40}$BrN$_5$O$_5$.

**NOTE —** Carry out the tests as rapidly as possible without exposure to daylight and with minimum exposure to artificial light.

**Identification**

A. Shake a quantity of the contents of the capsules containing 10 mg of bromocriptine with 50 ml of methanol for 30 minutes, centrifuge and dilute 5 ml of the supernatant liquid to 20 ml with methanol. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 305 nm and a minimum at about 270 nm.

B. In the test for Related substances, the principal band in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (d).

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 100 volumes of ether, 88 volumes of dichloromethane, 3 volumes of 2-propanol, 1.5 volumes of water and 0.1 volume of strong ammonia solution.

Prepare the following solutions freshly:

- Test solution (a). Shake a quantity of the contents of the capsules containing 20 mg of bromocriptine with 10 ml of methanol for 20 minutes and centrifuge.
- Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with methanol.
- Reference solution (a). Dilute 3 ml of test solution (a) to 100 ml with methanol.
- Reference solution (b). Dilute 1 ml of test solution (a) to 100 ml with methanol.
- Reference solution (c). Dilute 1 ml of test solution (a) to 200 ml with methanol.
- Reference solution (d). A 0.023 per cent w/v solution of bromocriptine mesylate RS in methanol.

Apply to the plate, as 1-cm bands, 50 µl of each solution. Apply test solution (a) to the plate as the last solution and develop the chromatogram immediately in an unsaturated tank. After development, allow the plate to dry in a current of cold air, spray with ethanolic ammonium molybdate solution and heat at 100° until bands are visible (about 10 minutes). In the chromatogram obtained with test solution (a) any secondary band is not more intense than the band in the chromatogram obtained with reference solution (a). Not more than one such band is more intense than the band in the chromatogram obtained with reference solution (b) and not more than two further such bands are more intense than the band in the chromatogram obtained with reference solution (c). Disregard any band within 20 mm of the line of application.

**Uniformity of content.** Comply with the test stated under Capsules.

Empty the contents of one capsule, crush, if necessary, add 10.0 ml of methanol, shake vigorously and centrifuge. If necessary, dilute the solution appropriately and carry out the procedure described under the Assay beginning at the words “measure the absorbance.....”.

**Other tests.** Comply with the tests stated under Capsules.

**Assay.** Weigh accurately a quantity of the mixed contents of 20 capsules containing about 25 mg of bromocriptine and shake vigorously with 30 ml of methanol. Dilute to 100.0 ml with methanol and filter. Dilute further with methanol to yield a final concentration of about 50 mcg per ml and measure the absorbance of the resulting solution at the maximum at about 305 nm (2.4.7). Calculate the content of C$_{32}$H$_{40}$BrN$_5$O$_5$ from the absorbance obtained by repeating the operation using bromocriptine mesylate RS equivalent to 25 mg of bromocriptine instead of the substance under examination.

**Storage.** Store protected from light.

**Labelling.** The label states the strength in terms of the equivalent amount of bromocriptine.
Bromocriptine Tablets

Bromocriptine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of bromocriptine, C_{32}H_{40}BrN_5O_5.

**NOTE** — Carry out the tests as rapidly as possible without exposure to daylight and with minimum exposure to artificial light.

**Identification**

A. Shake a quantity of powdered tablets containing about 20 mg of bromocriptine with 20 ml of methanol, filter, evaporate the filtrate to dryness on a water-bath and dry at 105° for 1 hour. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with bromocriptine mesylate RS or with the reference spectrum of bromocriptine mesylate.

B. Shake a quantity of the powdered tablets containing about 10 mg of bromocriptine with 50 ml of methanol for 30 minutes, centrifuge and dilute 5 ml of the supernatant liquid to 20 ml with methanol. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 305 nm and a minimum at about 270 nm.

C. In the test for Related substances, the principal band in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (d).

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 100 volumes of ether, 88 volumes of dichloromethane, 3 volumes of 2-propanol, 1.5 volumes of water and 0.1 volume of strong ammonia solution.

Prepare the following solutions freshly:

**Test solution (a).** Shake a quantity of the powdered tablets containing 10 mg of bromocriptine with 25 ml of a mixture of equal volumes of chloroform and methanol for 30 minutes, filter through a sintered glass filter (porosity No. 4) and wash the residue with two quantities, each of 5 ml, of the same solvent mixture. Evaporate the filtrate and washings to dryness at 25° at a pressure of 2 kPa, dissolve the residue in 2 ml of the same solvent mixture and centrifuge.

**Test solution (b).** Dilute 1 ml of test solution (a) to 10 ml with the same solvent mixture.

**Reference solution (a).** Dilute 3 ml of test solution (a) to 100 ml with the same solvent mixture.

**Reference solution (b).** Dilute 1 ml of test solution (b) to 10 ml with the same solvent mixture.

**Reference solution (c).** Dilute 1 ml of test solution (b) to 20 ml with the same solvent mixture.

**Reference solution (d).** A 0.055 per cent w/v solution of bromocriptine mesylate RS in the same solvent mixture.

Apply to the plate, as 1-cm bands, 20 µl of each solution. Apply test solution (a) to the plate as the last solution and develop the chromatogram immediately in an unsaturated tank. After development, allow the plate to dry in a current of cold air, spray with ethanolic ammonium molybdate solution and heat at 100° until bands are visible (about 10 minutes). Any secondary band in the chromatogram obtained with test solution (a) is not more intense than the band in the chromatogram obtained with reference solution (a). Not more than one such band is more intense than the band in the chromatogram obtained with reference solution (b) and not more than a further two such bands are more intense than the band in the chromatogram obtained with solution (c). Disregard any band within 20 mm of the line of application.

**Uniformity of content.** Comply with the test stated under Tablets.

Finely crush one tablet, add 10.0 ml of methanol, shake vigorously and centrifuge. If necessary, dilute the solution appropriately and carry out the procedure described under the Assay beginning at the words “Measure the absorbance......”.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 2.5 mg of bromocriptine and shake vigorously with 30 ml of methanol. Dilute to 50.0 ml with methanol and filter. Measure the absorbance of the resulting solution at the maximum at about 305 nm (2.4.7). Calculate the content of C_{32}H_{40}BrN_5O_5 from the absorbance obtained by repeating the operation with bromocriptine mesylate RS equivalent to 25 mg of bromocriptine in 50 ml methanol and diluting 5.0 ml to 50.0 ml with methanol.

**Storage.** Store protected from light.

**Labelling.** The label states the strength in terms of the equivalent amount of bromocriptine.

**Bronopol**

![Bronopol Structure](image)

C_{2}H_{6}BrNO_{4}

Mol. Wt. 200.0

Bronopol is 2-bromo-2-nitropropane-1,3-diol.
Bronopol contains not less than 99.0 per cent and not more than 101.0 per cent of C₃H₆BrNO₄, calculated on the anhydrous basis.

**Description.** White or almost white crystals or crystalline powder.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with bronopol RS.

B. Dissolve 0.1 g in 10 ml of water, add 10 ml of 7.5 M sodium hydroxide and carefully with constant stirring and cooling, 0.5 g of nickel-aluminium alloy. Allow the reaction to subside, filter and carefully neutralise with nitric acid. The resulting solution gives reaction A of bromides (2.3.1).

**Tests**

**pH** (2.4.24). 5.0 to 7.0, determined on 1.0 per cent w/v solution.

**Related substances.** Determine by liquid chromatography (2.4.14)

**Test solution.** Dissolve 0.2 g of the substance under examination in 100 ml of mobile phase.

**Reference solution (a).** Dilute 5 ml of the test solution to 50 ml with the mobile phase. Further, dilute 1 ml of the solution to 100 ml with the mobile phase.

**Reference solution (b).** A solution containing 0.001 per cent w/v each of 2-methyl-2-nitropropan-1,3-diol and tris(hydroxymethyl)nitromethane in the mobile phase.

**Reference solution (c).** A solution containing 0.0002 per cent w/v each of 2-methyl-2-nitropropane-1,3-diol, 2-nitroethanol, sodium bromide and tris(hydroxymethyl)nitromethane in the mobile phase.

**Chromatographic system**

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- temperature 35°,
- mobile phase: a mixture of 189 volumes of water, 10 volumes of acetonitrile and 1 volume of a 10 per cent v/v solution of orthophosphoric acid, adjusting the pH to 3.0 using 2 M sodium hydroxide,
- flow rate. 1 ml per minute,
- spectrophotometer set at 214 nm,
- a 20 µl loop injector.

Inject reference solution (c). The test is not valid unless the resolution between the peaks corresponding to sodium bromide and tris (hydroxymethyl)nitromethane is at least 1.0 and the resolution between the peaks corresponding to tris(hydroxymethyl)nitromethane and 2-nitroethanol is at least 1.5.

In the chromatogram obtained with the test solution the area of any peaks corresponding to 2-methyl-2-nitropropane-1,3-diol and tris (hydroxymethyl) nitromethane are not more than the area of the corresponding peaks in the chromatogram obtained with reference solution (b) (0.5 per cent each) and the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 0.5 per cent, determined on 5.0 g.

**Assay.** In a flask fitted with a reflux condenser dissolve 0.4 g in 15 ml of water and add 15 ml of 7.5 M sodium hydroxide. Slowly, with caution, add 2 g of nickel-aluminium alloy through the reflux condenser, agitating the flask whilst cooling under running water. Allow the mixture to stand for 10 minutes and boil for 1 hour. Cool and filter under reduced pressure, washing the condenser, flask and residue with 150 ml of water. Combine the filtrate and washings, add 25 ml of nitric acid and 40 ml of 0.1 M silver nitrate, shake vigorously and titrate with 0.1 M ammonium thiocyanate using ammonium iron(III) sulphate solution as indicator. Carry out a blank titration.

1 ml of 0.1 M silver nitrate is equivalent to 0.020 g of C₃H₆BrNO₄.

**Storage.** Store protected from light.

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**Budesonide**

Inject the test solution, reference solution (a) and reference solution (b). Continue the chromatography for 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any peaks corresponding to 2-methyl-2-nitropropane-1,3-diol and tris (hydroxymethyl) nitromethane are not more than the area of the corresponding peaks in the chromatogram obtained with reference solution (b) (0.5 per cent each) and the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b).

Budesonide contains not less than 98.0 per cent and not more than 102.0 per cent of a mixture of epimers A and B, C₂₅H₃₄O₆, calculated on the dried basis.
**Description.** A white or almost white, crystalline powder.

**Identification**

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with budesonide RS or with the reference spectrum of budesonide.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

*Mobile phase.* Add a mixture of 1.2 volumes of water and 8 volumes of methanol to a mixture of 15 volumes of ether and 77 volumes of dichloromethane.

*Solvent mixture.* 1 volume of methanol and 9 volumes of methylene chloride.

*Test solution.* Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

*Reference solution (a).* A 0.25 per cent w/v solution of budesonide RS in the solvent mixture.

*Reference solution (b).* A solution containing 0.25 per cent w/v of triamcinolone acetonide RS and 0.25 per cent w/v of budesonide RS in the solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. Dissolve about 2 mg in 2 ml of sulphuric acid. A yellow colour appears in 5 minutes and the colour changes to brown or reddish-brown in 30 minutes. Add cautiously the solution to 10 ml of water and mix. The colour fades and a clear solution remains.

D. Dissolve about 1 mg in 2 ml of a solution containing 2 g of phosphomolybdic acid in a mixture of 10 ml of dilute sodium hydroxide solution, 15 ml of water and 25 ml of glacial acetic acid. Heat for 5 minutes on a water-bath. Cool in iced water for 10 minutes and add 3 ml of dilute sodium hydroxide solution. The solution turns blue.

**Tests**

**Related substances.** Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 50 mg of the substance under examination in 30 ml of acetonitrile. Add about 60 ml of phosphate buffer pH 3.2 and, if necessary, disperse with the aid of ultrasound to dissolve. Dilute with phosphate buffer pH 3.2 to 100 ml and allow to stand for at least 15 minutes before use and filter.

*Reference solution (a).* Dissolve 50 mg of budesonide RS in 30 ml of acetonitrile. Add about 60 ml of phosphate buffer pH 3.2 and disperse, if necessary, with the aid of ultrasound to dissolve. Dilute to 100 ml with phosphate buffer pH 3.2 and allow to stand for at least 15 minutes before use and filter.

*Reference solution (b).* Dilute reference solution (a) with the mobile phase to get a 0.00025 per cent w/v solution of budesonide.

Use the chromatographic system described in the Assay.

Inject reference solution (a). The test is not valid unless the resolution between epimer B peak and epimer A peak is not less than 1.5, the tailing factor for epimer B peak is not more than 1.5 and the relative standard deviation of sum of epimer A and epimer B peaks for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution: the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of the areas of all the peaks, other than the principal peak, is not greater than thrice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent).

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1 g by drying in an oven at 105º.

**Assay.** Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 50 mg of the substance under examination in 30 ml of acetonitrile and dilute to 100.0 ml with phosphate buffer solution pH 3.2 and filter.

*Reference solution.* Dissolve 50 mg of budesonide RS in 30 ml of acetonitrile and dilute to 100.0 ml with phosphate buffer solution pH 3.2.

**Chromatographic system**

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilyl silica (5 µm),
- mobile phase: a mixture of 34 volumes of acetonitrile and 66 volumes of a buffer solution prepared by adding 100 ml of 0.25 per cent w/v solution of orthophosphoric acid to 900 ml of 0.4 per cent w/v solution of sodium dihydrogen phosphate and adjusting the pH to 3.2, if necessary,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 240 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the resolution between epimer B peak and epimer A peak is not
less than 1.5, the tailing factor for epimer B peak is not more than 1.5, the column efficiency determined for epimer B peak is not less than 4000 theoretical plates and the relative standard deviation for the sum of epimer A and B peaks for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution. Calculate the content of \( \text{C}_{25}\text{H}_{34}\text{O}_6 \).

Storage. Store protected from light.

### Bupivacaine Hydrochloride

\[
\text{H}_3\text{C} \quad \text{N} \quad \text{H} \quad \text{N} \quad \text{H}_3\text{C} \quad \text{O} \quad \text{N} \quad \text{H} \quad \text{CH}_3 \quad \text{HCl, H}_2\text{O}
\]

\( \text{C}_{18}\text{H}_{28}\text{N}_2\text{O}, \text{HCl, H}_2\text{O} \)  
 Mol. Wt. 342.9

Bupivacaine Hydrochloride is 1-butyl-\( \text{N} \)-(2,6-dimethylphenyl)-2-piperidinecarboxamide hydrochloride monohydrate.

Bupivacaine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of \( \text{C}_{18}\text{H}_{28}\text{N}_2\text{O}, \text{HCl} \), calculated on the dried basis.

**Description.** A white, crystalline powder or colourless crystals; almost odourless.

**Identification.**

*Test A may be omitted if tests B, C, D and E are carried out.*

*Tests B, C and D may be omitted if tests A and E are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bupivacaine hydrochloride RS* or with the reference spectrum of bupivacaine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.05 per cent w/v solution in 0.01 M hydrochloric acid shows two absorption maxima at about 263 nm and 271 nm; absorbance at about 263 nm, about 0.70 and at about 271 nm, about 0.57.

C. Dissolve 0.1 g in 10 ml of water, add 2 ml of 2 M sodium hydroxide and shake with two quantities, each of 15 ml, of ether. Dry the combined ether extracts over anhydrous sodium sulphate, filter, evaporate the ether, recrystallise the residue from ethanol (90 per cent) and dry the residue at a pressure of 1.5 to 2.5 kPa. The melting range (2.4.21) of the residue is between 105° and 108° (2.4.21).

D. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

E. A 10 per cent w/v solution gives reaction A of chlorides (2.3.1).

### Tests

**Acidity or alkalinity.** To 10 ml of a 2.0 per cent w/v solution in carbon dioxide-free water add 0.2 ml of 0.01 M sodium hydroxide; the pH is not less than 4.7. Add 0.4 ml of 0.01 M hydrochloric acid; the pH is not more than 4.7 (2.4.24).

**Appearance of solution.** A 2.0 per cent w/v solution in carbon dioxide-free water is clear (2.4.1), and colourless (2.4.1).

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 100 volumes of methanol and 0.1 volume of strong ammonia solution.

**Test solution (a).** Dissolve 5.0 g of the substance under examination in 100 ml of methanol.

**Test solution (b).** Dilute 10 ml of test solution (a) to 100 ml with methanol.

**Reference solution (a).** Dilute 5 ml of test solution (b) to 100 ml with methanol.

**Reference solution (b).** A 0.5 per cent w/v solution of bupivacaine hydrochloride RS in methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with dilute potassium iodobismuthate solution. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

2,6-Dimethylaniline. To 2.0 ml of a 5.0 per cent w/v solution in methanol (solution A) add 1 ml of a freshly prepared 1 per cent w/v solution of 4-dimethylaminobenzaldehyde in methanol and 2 ml of glacial acetic acid and allow to stand for 10 minutes. Any yellow colour produced is not more intense than that obtained with a solution prepared at the same time and in the same manner using 2 ml of a 0.0005 per cent w/v solution of 2,6-dimethylaniline in methanol in place of solution A (100 ppm).

**Heavy metals** (2.3.13). A 10.0 per cent w/v solution in a mixture of 85 volumes of methanol and 15 volumes of water complies with the limit test for heavy metals Method D (10 ppm). Prepare the standard using lead standard solution (1 ppm Pb) obtained by diluting lead standard solution (20 ppm Pb) with a mixture of 85 volumes of methanol and 15 volumes of water.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.
**Bupivacaine Injection**

Bupivacaine Hydrochloride Injection

Bupivacaine Injection is a sterile solution of Bupivacaine Hydrochloride in Water for Injection.

Bupivacaine Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of anhydrous bupivacaine hydrochloride, C₁₈H₂₈N₂O.HCl.

**Identification**

A. To a volume containing 25 mg of anhydrous bupivacaine hydrochloride add 2 ml of strong ammonia solution, shake and filter. Wash the precipitate with water and dry at 60° at a pressure of 2 kPa for 16 hours. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with bupivacaine hydrochloride RS treated in the same manner or with the reference spectrum of bupivacaine hydrochloride, C₁₈H₂₈N₂O.HCl.

B. To a volume containing 50 mg of anhydrous bupivacaine hydrochloride add 15 ml of picric acid solution; the precipitate, after rapid washing with a small quantity of water followed by successive quantities, each of 2 ml, of methanol and ether melts at about 194° (2.4.21).

C. To a volume containing 50 mg of anhydrous bupivacaine hydrochloride add 2 ml of a 10 per cent w/v solution of disodium hydrogen phosphate and sufficient iodine solution to produce a distinct brown colour. Remove the excess iodine by adding 0.1 M sodium thiosulphate; no pink colour is produced.

**Tests**

**pH** (2.4.24). 4.0 to 6.5.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Assay.** To an accurately measured volume containing about 0.5 g of anhydrous bupivacaine hydrochloride add 5 ml of water and 2 ml of 1 M sodium hydroxide and extract with three quantities, each of 15 ml, of chloroform. Combine the chloroform extracts, add 2 ml of 1 M perchloric acid and evaporate the combined chloroform extracts on a water-bath. Add two successive quantities, each of 5 ml, of acetone and evaporate. Dissolve the residue in 50 ml of anhydrous glacial acetic acid. Add 15 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid, using crystal violet solution as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.03249 g of C₁₈H₂₈N₂O.HCl.

**Storage.** Store in single dose or multiple dose containers, preferably of Type 1 glass.
**Labelling.** The label states the strength in terms of the equivalent amount of anhydrous bupivacaine hydrochloride in a suitable dose-volume.

**Buprenorphine Hydrochloride**

![Chemical Structure of Buprenorphine Hydrochloride]

\[C_{29}H_{41}NO_4\cdot HCl\]  \[\text{Mol. Wt. 504.1}\]

Buprenorphine Hydrochloride is (6\(\text{R}\),7\(\text{R}\),14\(\text{S}\))-17-
cyclopropylmethyl-7,8-dihydro-7-[(1\(\text{S}\))-1-hydroxy-1,2,2-
trimethylpropyl]-6-\(\text{O}\)-methyl-6,14-ethano-17-normorphine
hydrochloride.

Buprenorphine Hydrochloride contains not less than 97.0 per
cent and not more than 102.0 per cent of \(C_{29}H_{41}NO_4\cdot HCl\),
calculated on the dried basis.

**Description.** A white to off-white, crystalline powder.

**Identification**

**A.** Determine by infrared absorption spectrophotometry (2.4.6).

Compare the spectrum with that obtained with **buprenorphine hydrochloride RS**.

**B.** When examined in the range 230 nm to 360 nm (2.4.7), a 0.01 per cent w/v solution in 0.01 M hydrochloric acid shows an absorption maximum at about 286 nm; absorbance at about 286 nm, about 0.33.

**C.** Dissolve about 5 mg in 5 ml of hot water, add 2 ml of dilute hydrochloric acid and 2 ml of a 2 per cent w/v solution of sodium nitrite and allow to stand for 10 minutes; a yellow colour is produced.

**D.** Dissolve 10 mg in 10 ml of hot water; add 2 ml of dilute nitric acid, shake and add 1 ml of silver nitrate solution; a white precipitate is produced.

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 85 volumes of toluene, 15 volumes of methanol and 0.5 volume of strong ammonia solution.

**Prepare the following solutions freshly.**

**Test solution.** Dissolve 0.25 g of the substance under examination in 50 ml of methanol.

**Reference solution.** Dilute 1 ml of the test solution to 100 ml with methanol. Mix well and dilute 10 ml of this solution to 20 ml with methanol.

Apply to the plate 5 \(\mu\)l of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm or expose to iodine vapours. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Heavy metals** (2.3.13). Moisten the residue obtained in the test for Sulphated ash with a few drops of hydrochloric acid and evaporate almost to dryness on a water-bath. Dissolve the residue in 10 ml of water by warming, cool, transfer to a test-tube with the aid of 10 ml of water and add 2 ml of dilute acetic acid. The solution complies with the limit test for heavy metals, Method A (20 ppm). Prepare the standard using 5 ml of lead standard solution (2 ppm Pb).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 0.5 g by drying in an oven at 105° for 4 hours.

**Assay.** Weigh accurately about 0.5 g, dissolve in 60 ml of anhydrous glacial acetic acid, add 10 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid, using 0.1 ml of crystal violet solution as indicator to a green end-point. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.05041 g of \(C_{29}H_{41}NO_4\cdot HCl\).

### Buprenorphine Injection

Buprenorphine Hydrochloride Injection

Buprenorphine Injection is a sterile solution of Buprenorphine Hydrochloride in Water for Injection.

Buprenorphine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of buprenorphine, \(C_{29}H_{41}NO_4\).

**Identification**

**A.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel HF254.

**Mobile phase.** A mixture of 85 volumes of toluene, 15 volumes of methanol and 0.5 volume of strong ammonia solution.
Test solution. Transfer a volume of the injection containing 1.5 mg of Buprenorphine Hydrochloride to a 125-ml separator, add 0.5 ml of dilute ammonia solution, shake and extract with three quantities, each of 10 ml, of chloroform, washing each chloroform extract with the same 10 ml of water and discard the water: Evaporate the combined chloroform extracts to dryness on a water-bath and dissolve the residue in 1.5 ml of chloroform.

Reference solution. Dissolve 1.5 mg of buprenorphine hydrochloride RS in 5 ml of 0.01 M hydrochloric acid, transfer the solution to a 125-ml separator and repeat the above procedure beginning at the words “add 0.5 ml of dilute ammonia solution.....”.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of air and examine in ultraviolet light at 254 nm or expose to iodine vapours. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. To a volume containing about 5 mg of Buprenorphine Hydrochloride in a 125-ml separator, add 1 ml of dilute ammonia solution and shake with three quantities, each of 10 ml, of chloroform. Wash each chloroform extract with the same 10 ml of water and discard the washings. Evaporate the combined chloroform extracts to dryness on a water-bath and dissolve the residue in 50 ml of 0.1 M hydrochloric acid. When examined in the range 230 to 360 nm (2.4.7) the resulting solution shows an absorption maximum only at about 286 nm.

Tests

pH (2.4.24). 3.5 to 6.5.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Measure accurately a volume containing 1.5 mg of buprenorphine and transfer to a 25-ml volumetric flask. Add 1 ml of 1 M hydrochloric acid, 2 ml of a 2 per cent w/v solution of sodium nitrite and shake well. Stopper the flask and allow to stand for 15 minutes. Dilute the solution to volume with dilute ammonia solution and measure the absorbance of the resulting solution at the maximum at about 460 nm (2.4.7), using as the blank a solution prepared in the same manner by treating 5 ml of water instead of the preparation under examination.

Calculate the content of C₂₉H₄₁NO₄ from the absorbance obtained by repeating the procedure with 5 ml of a solution containing buprenorphine hydrochloride RS equivalent to 0.03 per cent w/v of buprenorphine.

Labelling. The label states the strength in terms of the equivalent amount of buprenorphine in a suitable dose-volume.

Buprenorphine Tablets

Buprenorphine Hydrochloride Tablets

Buprenorphine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of buprenorphine, C₂₉H₄₁NO₄.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel HF254.

Mobile phase. A mixture of 85 volumes of toluene, 15 volumes of methanol and 0.5 volume of strong ammonia solution.

Test solution. Extract a quantity of the powdered tablets containing 1 mg of Buprenorphine Hydrochloride with three quantities, each of 10 ml, of methanol, filtering each extract through a sintered-glass filter (porosity No. 4). Evaporate the filtrate to dryness and dissolve the residue in 1 ml of methanol.

Reference solution. Dissolve 1 mg of buprenorphine hydrochloride RS in 1 ml of methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of air and examine in ultraviolet light at 254 nm or expose to iodine vapours. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Shake vigorously a quantity of the powdered tablets containing 2 mg of Buprenorphine Hydrochloride with 20 ml of hot water, filter and cool. The filtrate, when examined in the range 230 nm to 360 nm (2.4.7), shows an absorption maximum at about 286 nm; absorbance at about 286 nm, about 0.33.

Tests

Disintegration. The requirement of Disintegration does not apply.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 200 mcg of buprenorphine and transfer to a 125-ml separator. Add 10 ml of hot water, shake, add 1 ml of a 10 per cent w/v solution of sodium bicarbonate and shake well. Add 3 ml of a 10 per cent v/v solution of acetic acid, shake, add 3 ml of a 0.2 per cent w/v solution of metanil yellow and again shake well. Shake with 100 ml of chloroform for about 5 minutes and allow the two layers to separate over a period of 45 minutes. Collect the chloroform layer into another 250-ml separator and extract the chloroform layer with 50.0 ml of 1 M hydrochloric acid. Discard the chloroform layer, centrifuge the red acid layer and measure the absorbance at the maximum at about 530 nm (2.4.7), using 1 M hydrochloric acid as the blank. Calculate the
content of C₆H₁₄NO₄ from the absorbance obtained by repeating the procedure with 10.0 ml of a solution containing buprenorphine hydrochloride RS equivalent to 0.002 per cent w/v solution of buprenorphine beginning at the words “transfer to a 125-ml separator...”.

**Labelling.** The label states the strength in terms of the equivalent amount of buprenorphine.

**Busulphan**

C₆H₁₄O₆S₂  Mol. Wt. 246.3

Busulphan is 1,4-butanediol dimethanesulphonate.

Busulphan contains not less than 99.0 per cent and not more than 100.5 per cent of C₆H₁₄O₆S₂, calculated on the dried basis.

**Description.** A white or almost white, crystalline powder.

**Identification**

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with busulphan RS or with the reference spectrum of busulphan.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of equal volumes of acetone and toluene.

**Test solution.** Dissolve 1 g of the substance under examination in 100 ml of acetone.

**Reference solution.** A 1 per cent w/v solution of busulphan RS in acetone.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of hot air, spray with anisaldehyde solution and heat at 120°. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Heat 0.1 g with 5 ml of 1 M sodium hydroxide until a clear solution is obtained and allow to cool. To 2 ml of the solution add 0.1 ml of a 3 per cent w/v solution of potassium permanganate; the purple colour changes to violet, then to blue and finally to green. Filter and add 1 ml of ammoniacal silver nitrate solution; a precipitate is produced.

D. Fuse 0.1 g with 0.1 g of potassium nitrate and 0.25 g of potassium hydroxide, cool and dissolve the residue in 5 ml of water. Acidify with dilute hydrochloric acid and add a few drops of barium chloride solution; a white precipitate is produced.

**Tests**

**Appearance of solution.** Dissolve 0.25 g in 20.0 ml of acetonitrile, dilute to 25 ml with water and examine immediately. The solution is clear (2.4.1), and not more intensely coloured than reference solution BS6 (2.4.1).

**Acidity.** Dissolve 0.2 g in 50 ml of warm ethanol previously neutralised to methyl red solution and titrate with 0.1 M sodium hydroxide using methyl red solution as indicator; not more than 0.05 ml of 0.1 M sodium hydroxide is required.

**Sulphated ash (2.3.18).** Not more than 0.1 per cent.

**Loss on drying (2.4.19).** Not more than 2.0 per cent, determined on 1.0 g by drying in an oven over phosphorus pentoxide at 60° at a pressure of 1.5 to 2.5 kPa.

**Assay.** Weigh accurately about 0.25 g and shake with 50 ml of water. Boil under a reflux condenser for 30 minutes and, if necessary, restore the initial volume with water. Allow to cool and titrate with 0.1 M sodium hydroxide, using 0.3 ml of dilute phenolphthalein solution as indicator, until a pink colour is produced.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.01232 g of C₆H₁₄O₆S₂.

**Storage.** Store protected from light.

**Busulphan Tablets**

Busulphan Tablets contain not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of busulphan, C₆H₁₄O₆S₂. The tablets are coated.

**Identification**

A. Warm a quantity of the powdered tablets containing 10 mg of Busulphan with 10 ml of acetone, filter and evaporate the filtrate to dryness. Dry the residue at 60° at a pressure not exceeding 0.7 kPa for 1 hour. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with busulphan RS or with the reference spectrum of busulphan.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.
Tests

Disintegration (2.5.1). Maximum time, 15 minutes.

Uniformity of content. Comply with the test stated under Tablets.

Determine by gas chromatography (2.4.13).

Test solution. Add 1 ml of water to one tablet in a 50-ml volumetric flask and place in an ultrasonic bath until completely dispersed. Add 30 ml of acetone, shake for 15 minutes and dilute to 50.0 ml with acetone. Centrifuge and dilute a quantity of the supernatant liquid with acetone to produce a solution containing 0.0001 per cent w/v of Busulphan. To 5.0 ml of the resulting solution add 5 ml of a 30 per cent w/v solution of sodium iodide in acetone, stopper the flask lightly and heat in a water-bath at 50° for 90 minutes. Cool, add 10 ml of a 0.0001 per cent w/v solution of 1,5-diiodopentane (internal standard) in acetone, mix, add 10 ml of water and 20.0 ml of hexane, shake vigorously for 1 minute and allow to separate. Use the hexane layer.

Reference solution (a). Add 5 ml of a 30 per cent w/v solution of sodium iodide in acetone to 5.0 ml of a 0.0001 per cent w/v solution of busulphan RS in acetone, stopper the flask lightly and heat in a water-bath at 50° for 90 minutes. Cool, add 10 ml of the internal standard solution, mix, add 10 ml of water and 20.0 ml of hexane, shake vigorously for 1 minute and allow to separate. Use the hexane layer.

Reference solution (b). Prepare in the same manner as reference solution (a) but using 10 ml of acetone in place of internal standard solution.

Chromatographic system

- a glass column 1.5 m x 4 mm, packed with acid-washed, diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl),
- temperature: column. 140°, inlet port and detector at 240°,
- electron capture detector,
- flow rate. 30 ml per minute of the carrier gas.

Calculate the content of C₆H₁₄O₆S₂ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Determine by gas chromatography (2.4.13) as given under the test for Uniformity of content using the following test solution.

Test solution. Weigh accurately a quantity of the powdered tablets containing about 2.5 mg of Busulphan, add 5 ml of water and place in an ultrasonic bath until completely dispersed. Add 150 ml of acetone, shake for 15 minutes and dilute to 250.0 ml with acetone. Centrifuge and dilute 10.0 ml of the supernatant liquid to 100.0 ml with acetone. To 5.0 ml of the resulting solution add 5 ml of a 30 per cent solution of sodium iodide in acetone, stopper the flask lightly and heat in a water-bath at 50° for 90 minutes. Cool, add 10 ml of the internal standard solution, mix, add 10 ml of water and 20.0 ml of hexane, shake vigorously for 1 minute and allow to separate. Use the hexane layer.

Calculate the content of C₆H₁₄O₆S₂ in the tablets.

Storage. Store protected from light.

Butylated Hydroxyanisole

BHA

\[
\text{OH} \quad \text{C(CH₃)₃} \\
\text{OCH₃}
\]

C₁₁H₁₆O₂ \quad \text{Mol. Wt. 180.3}

Butylated Hydroxyanisole is 2-(1,1-dimethylethyl)-4-methoxyphenol containing not more than 10 per cent of 3-(1,1-dimethylethyl)-4-methoxyphenol.

Description. A white or almost white, crystalline powder or yellowish-white, waxy solid; odour, aromatic.

Identification

A. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

B. Dissolve about 0.1 g in 10 ml of ethanol (95 per cent), add 2 ml of a 2.0 per cent w/v solution of sodium tetraborate and a few crystals of 2,6-dichloroquinone-4-chlorimide; a blue colour is produced (distinction from butylated hydroxytoluene).

C. Dissolve a few crystals in 10 ml of ethanol (95 per cent), add 0.5 ml of a 0.2 per cent w/v solution of potassium ferricyanide and 0.5 ml of a 0.5 per cent w/v solution of ferric ammonium sulphate in 0.5 M sulphuric acid; a green to blue colour is produced.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. Dichloromethane.

Test solution (a). Dissolve 2.5 g of the substance under examination in 100 ml of dichloromethane.
Test solution (b). Dilute 1.0 ml of test solution (a) to 10 ml with dichloromethane.

Reference solution (a). A 0.25 per cent w/v solution of butylhydroxyanisole RS in dichloromethane.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 20 ml with dichloromethane.

Reference solution (c). Dissolve 50 mg of hydroquinone in 5 ml of ethanol (95 per cent) and dilute to 100 ml with dichloromethane. Dilute 1 ml of this solution to 10 ml with dichloromethane.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air and spray with a freshly prepared mixture of 10 volumes of potassium ferricyanide solution, 25 volumes of ferric chloride test solution and 65 volumes of water. In the chromatogram obtained with test solution (a), any violet-blue spot with an Rf value of about 0.35 (due to 3-(1,1-dimethylethyl)-4-methoxyphenol) is not more intense than the principal spot in the chromatogram obtained with reference solution (a) (10 per cent); any spot corresponding to hydroquinone is not more intense than the principal spot in the chromatogram obtained with reference solution (c) (0.2 per cent); any spot, besides the principal spot and any spots corresponding to 3-(1,1-dimethylethyl)-4-methoxyphenol and hydroquinone, is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Sulphated ash (2.3.18). Not more than 0.05 per cent.

Storage. Store protected from light.

Butylated Hydroxytoluene

BHT

\[
\begin{align*}
&\text{C}_{15}\text{H}_{24}\text{O} \\
&\text{Mol. Wt. 220.4}
\end{align*}
\]

Butylated Hydroxytoluene is 2,6-bis(1,1-dimethylethyl)-4-methoxyphenol.

Description. A white to yellowish white, crystalline powder.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B and C may be omitted if tests A, D and E are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with butylated hydroxytoluene RS.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution in ethanol shows an absorption maximum only at about 278 nm; absorbance at about 278 nm, between 0.40 and 0.45.

C. Dissolve about 10 mg in 2 ml of ethanol (95 per cent), add 1 ml of a 0.1 per cent w/v solution of testosterone propionate in ethanol (95 per cent) and 2 ml of 2 M sodium hydroxide, heat in a water-bath at 80° for 10 minutes and allow to cool; a blue colour is produced.

D. Dissolve about 0.1 g in 10 ml of ethanol (95 per cent), add 2 ml of a 2.0 per cent w/v solution of sodium tetraborate and a few crystals of 2,6-dichloroquinone-4-chlorimide; not more than a faint blue colour is produced (distinction from butylated hydroxyanisole).

E. Dissolve a few crystals in 10 ml of ethanol (95 per cent), add 0.5 ml of a 0.2 per cent w/v solution of potassium ferricyanide and 0.5 ml of a 0.2 per cent per cent w/v solution of ferric ammonium sulphate in 0.5 M sulphuric acid; a green to blue colour is produced.

Tests

Appearance of solution. A 10.0 per cent w/v solution in methanol is clear (2.4.1), and not more intensely coloured than reference solution YS5 or BYS5 (2.4.1).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. Dichloromethane.

Test solution. Dissolve 2 g of the substance under examination in 100 ml of methanol.

Reference solution. Dilute 1 ml of the test solution to 200 ml with methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with a freshly prepared mixture of 70 volumes of water, 20 volumes of a 10.5 per cent w/v solution of ferric chloride and 10 volumes of potassium ferricyanide solution. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.
Caffeine
Calamine
Aqueous Calamine Cream
Calamine Lotion
Calamine Ointment
Calciferol Capsules
Calciferol Injection
Calciferol Oral Solution
Calciferol Tablets
Calcium Carbonate
Calcium Chloride
Calcium Folinate
Calcium Folinate Injection
Calcium Gluconate
Calcium Gluconate Injection
Calcium Gluconate Tablets
Calcium Lactate
Calcium Lactate Tablets
Calcium Levulinate
Calcium Levulinate Injection
Calcium Pantothenate
Dibasic Calcium Phosphate
Tribasic Calcium Phosphate
Calcium Stearate
Capreomycin Sulphate
Capreomycin Injection
Captopril
Captopril Tablets
Caramel
Carbamazepine
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Chlorobutanol
Chlorocresol
Chloroform
Chloroquine Phosphate
Chloroquine Phosphate Injection
Chloroquine Phosphate Suspension
Chloroquine Phosphate Tablets
Chloroquine Sulphate
Chloroquine Sulphate Injection
Chloroquine Sulphate Tablets
Chloroquine Syrup
Chloroxylenol
Chloroxylenol Solution
Chlorpheniramine Maleate
Chlorpheniramine Injection
Chlorpheniramine Tablets
Chlorpromazine Hydrochloride
Chlorpromazine Injection
Chlorpromazine Tablets
Chlorpropamide
Chlorpropamide Tablets
Chlorthalidone
Chlorthalidone Tablets
Cholecalciferol
Chorionic Gonadotrophin
Chorionic Gonadotrophin Injection
Ciclesonide
Ciclesonide Inhalation
Cimetidine
Cimetidine Tablets
Cinnarizine
Cinnarizine Tablets
Ciprofloxacin
Ciprofloxacin Injection
Ciprofloxacin Hydrochloride
Ciprofloxacin Eye Drops
Ciprofloxacin Tablets
Cisplatin
Cisplatin Injection
Citric Acid
Citric Acid Monohydrate
Clarithromycin
Clarithromycin Tablets
Clobazam
Clobazam Capsules
Clofazimine
Clofazimine Capsules
Clomifene Citrate
Clomifene Tablets
Clomipramine Hydrochloride
Clomipramine Capsules
Clonazepam
Clonazepam Injection
Clonidine Hydrochloride
Clonidine Injection
Clonidine Tablets
Clotrimazole
Clotrimazole Cream
Clotrimazole Pessaries
Clove Oil
Cloxacillin Sodium
Cloxacillin Capsules
Cloxacillin Injection
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Caffeine

![Caffeine structure](Image)

\( C_{8}H_{10}N_{4}O_{2} \)  Mol. Wt. 194.2 (anhydrous)
\( C_{8}H_{10}N_{4}O_{2} \cdot H_{2}O \)  Mol. Wt. 212.2 (monohydrate)

Caffeine is 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione or its monohydrate.

Caffeine contains not less than 98.5 per cent and not more than 101.5 per cent of \( C_{8}H_{10}N_{4}O_{2} \), calculated on the dried basis.

**Description.** Silky white crystals, white glistening needles or a white crystalline powder; odourless; sublimes readily.

**Identification**

*Test A* may be omitted if tests *B* and *D* are carried out. Tests *B*, *C* and *D* may be omitted if test *A* is carried out.

**A.** Determine by infrared absorption spectrophotometry (2.4.6), after drying the substance under examination, at 100° for 1 hour. Compare the spectrum with that obtained with caffeine *RS* or with the reference spectrum of caffeine.

**B.** To 10 mg in a porcelain dish, add 1 ml of hydrochloric acid and 0.1 g of potassium chlorate and evaporate to dryness on a water-bath. Expose the residue to the vapours of dilute ammonia solution; a purple colour is produced which disappears on addition of a solution of a fixed alkali.

**C.** To a saturated solution add a few drops of tannic acid solution; a white precipitate is produced which is soluble in excess of the reagent.

**D.** To 5 ml of saturated solution add 1.5 ml of 0.05 M iodine, the solution remains clear. Add a few drops of dilute hydrochloric acid; a brown precipitate is formed which dissolves on neutralisation with sodium hydroxide solution.

**Tests**

**Appearance of solution.** A 1.0 per cent w/v solution is clear (2.4.1) and colourless (2.4.1).

**Acidity or alkalinity.** Dissolve 0.2 g in 10 ml of boiling water and cool. Add 0.1 ml of bromothymol blue solution. The solution is coloured green or yellow. Titrate with 0.02 M sodium hydroxide to a blue colour; not more than 0.1 ml is required.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 40 volumes of 1-butanol, 30 volumes of chloroform, 10 volumes of strong ammonia solution and 3 volumes of acetone.

**Test solution.** A 2 per cent w/v solution of the substance under examination in a mixture of 3 volumes of chloroform and 2 volumes of methanol.

**Reference solution.** A 0.01 per cent w/v solution of the substance under examination in a mixture of 3 volumes of chloroform and 2 volumes of methanol.

Apply to the plate 10 µl of each solution. After development, dry in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Arsenic** (2.3.10). Mix 3.3 g with 3 g of anhydrous sodium carbonate, add 10 ml of bromine solution and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite and dissolve the cooled residue in 16 ml of brominated hydrochloric acid and 45 ml of water. Remove the excess of bromine with 2 ml of stannous chloride solution \( \text{AsT} \). The resulting solution complies with the limit test for arsenic (3 ppm).

**Heavy metals** (2.3.13). Mix 2.0 gm with 5 ml of 0.1 M hydrochloric acid and 45 ml of water, warm gently until solution is complete and cool to room temperature. The solution complies with the limit test for heavy metals, Method A (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent (for the anhydrous form) and 8.5 per cent (for the monohydrate form), determined on 1.0 g by drying in an oven at 100° to 105° for 1 hour.

**Assay.** Weigh accurately about 0.18 g and dissolve with warming in 5 ml of anhydrous glacial acetic acid. For Caffeine Hydrate, use material previously dried at 100° to 105°. Cool, add 10 ml of acetic anhydride and 20 ml of toluene. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25).

1 ml 0.1 M perchloric acid is equivalent to 0.01942 g of \( C_{8}H_{10}N_{4}O_{2} \).

**Storage.** Store protected from light and moisture.

**Labelling.** The label states whether it is anhydrous or monohydrate.

**Calamine**

Prepared Calamine

Calamine is Zinc Oxide with a small proportion of ferric oxide. Calamine contains not less than 98.0 per cent and not more than 100.5 per cent of ZnO, calculated on the ignited basis.
Description. A fine, amorphous, impalpable, pink or reddish-brown powder.

Identification

A. Shake 1 g with 10 ml of dilute hydrochloric acid and filter; the filtrate gives the reactions of zinc salts (2.3.1).

B. To 1 g add 10 ml of dilute hydrochloric acid, heat to boiling and filter. To the filtrate add a few drops of ammonium thiocyanate solution; a reddish colour is produced.

Tests

Acid-insoluble substances. Not more than 1 per cent w/w, determined by the following method. Dissolve 1.0 g in 25 ml of warm dilute hydrochloric acid. If any insoluble residue remains, filter, wash with water, dry to constant weight at 105º, cool and weigh.

Alkaline substances. Digest 1.0 g with 20 ml of warm water, filter and add 2 drops of phenolphthalein solution to the filtrate. If a red colour is produced, not more than 0.2 ml of 0.05 M sulphuric acid is required to decolorise it.

Water-soluble dyes. Shake 1.0 g with 10 ml of water and filter; the filtrate is colourless.

Ethanol-soluble dyes. Shake 1.0 g with 10 ml of ethanol (90 per cent) and filter; the filtrate is colourless.

Arsenic (2.3.10). Dissolve 1.25 g in 15 ml of brominated hydrochloric acid AsT, add 45 ml of water and remove the excess of bromine with a few drops of stannous chloride solution AsT. The resulting solution complies with the limit test for arsenic (8 ppm).

Lead. Dissolve 2.0 g in a mixture of 20 ml of water and 5 ml of glacial acetic acid, filter and add 0.25 ml of potassium chromate solution; the solution remains clear for 5 minutes.

Calcium. Dissolve 0.5 g in a mixture of 10 ml of water and 2.5 ml of glacial acetic acid by warming on a water-bath, if necessary and filter. To 0.5 ml of the filtrate, add 15 ml of dilute ammonia solution and 2 ml of a 2.5 per cent w/v solution of ammonium oxalate and allow to stand for 2 minutes; the solution remains clear.

Soluble barium salts. To the remainder of the filtrate obtained in the test for Calcium add 2 ml of 1 M sulphuric acid and allow to stand for 5 minutes; the solution remains clear.

Chlorides (2.3.12). Dissolve 0.33 g in water with the addition of 1 ml of nitric acid and dilute to 30 ml with water. The resulting solution complies with the limit test for chlorides (750 ppm).

Sulphates (2.3.17). Dissolve 0.1 g in water with the addition of 3 ml of 2 M hydrochloric acid, filter and dilute to 60 ml with water. The resulting solution complies with the limit test for sulphates (0.6 per cent).

Assay. Weigh accurately about 1.5 g and digest with 50.0 ml of 0.5 M sulphuric acid, applying gentle heat until no further solution occurs. Filter and wash the residue with hot water until the last washing is neutral to litmus paper. To the combined filtrate and washings, add 2.5 g of ammonium chloride, cool and titrate with 1 M sodium hydroxide using methyl orange solution as indicator.

1 ml of 0.5 M sulphuric acid is equivalent to 0.04068 g of ZnO.

Storage. Store protected from light and moisture.

Aqueous Calamine Cream

Calamine 40 g
Zinc Oxide 30 g
Liquid Paraffin 200 g
Self-Emulsifying Glycerol Monostearate 50 g
Cetostearyl Alcohol 40 g
Cetomacrogol 1000 10 g
Phenoxyethanol 5 g
Purified Water, freshly boiled and cooled 625 g

Melt together the Cetostearyl Alcohol and Cetomacrogol 1000, stir until cold and dissolve this mixture and the Self-Emulsifying Glycerol Monostearate in the Liquid Paraffin at 60º. Add with rapid stirring to a solution of the Phenoxyethanol in 450 g of the Purified Water at the same temperature and stir until cold. Triturate the Calamine and the Zinc Oxide with the remainder of the Purified Water and incorporate in the cream with stirring. Aqueous Calamine Cream contains not less than 6.30 per cent and not more than 7.67 per cent w/w of ZnO.

Identification

The residue obtained in the Assay is yellow when hot and white when cool.

Tests

Other tests. Complies with the tests stated under Creams.

Assay. Weigh accurately about 4 g. Heat carefully, taking care to avoid spurring, until the liquid is completely evaporated and the solid is charred. Ignite the residue to constant weight at a temperature of not less than 900º.

Storage. Store at a temperature not exceeding 30º. Do not freeze.

Labelling. The label states (1) the concentrations of Calamine and Zinc Oxide in the preparation; (2) that the preparation is intended for external use only; (3) the storage conditions.
Calamine Lotion

Triturate the Calamine, the Zinc Oxide and the Bentonite with a solution of the Sodium Citrate in about 700 ml of Purified Water and add the Liquefied Phenol, the Glycerin and sufficient Purified Water to produce 1000 ml.

Identification

A. To 2 ml add 2 ml of periodic acid reagent, shake, centrifuge and add 0.5 ml of the supernatant liquid to 2 ml of ammoniacal silver nitrate solution in a test-tube; a silver mirror is produced on the walls of the tube.

B. Mix 2 ml with 50 ml of water, centrifuge and decant the supernatant liquid. Suspend the residue in 20 ml of water, add 1 ml of hydrochloric acid, mix and filter. 5 ml of the filtrate, after neutralisation by dropwise addition of 2 M sodium hydroxide, gives the reactions of zinc salts (2.3.1).

Tests

Microbial contamination (2.2.9). 1 g is free from Staphylococcus aureus and 10 g is free from Pseudomonas aeruginosa.

Storage. Store at a temperature not exceeding 30°.

Labelling. The label states (1) the concentration of Calamine in the preparation; (2) that the preparation is intended for external use only; (3) the storage conditions.

Calamine Ointment

Triturate the calamine with part of the White Soft Paraffin until smooth and gradually incorporate the remainder of the White Soft Paraffin.

Calamine Ointment contains not less than 7.8 per cent and not more than 9.4 per cent w/w of Zn.

Identification

The residue obtained in the Assay is yellow when hot and white when cool.

Tests

Other tests. Complies with the tests stated under Ointments.

Assay. Weigh accurately about 1.0 g. Heat gently until the base is completely volatilised or charred. Increase the heat until all the carbon is removed and ignite the residue until, after further ignition, two successive weighings do not differ by more than 0.2 per cent of the weight of the residue.

1 g of the residue is equivalent to 0.8034 g of Zn.

Storage. Store in well-closed containers, at a temperature not exceeding 30°.

Labelling. The label states (1) the concentration of Calamine in the preparation; (2) that the preparation is intended for external use only; (3) the storage conditions.

Calciferol Capsules

Calciferol Capsules contain Cholecalciferol or Ergocalciferol usually as a vegetable oil solution contained in soft gelatin capsules.

Calciferol Capsules contain not less than 90.0 per cent and not more than 125.0 per cent of the stated amount of cholecalciferol, C_27H_44O or ergocalciferol, C_{28}H_{44}O.

Identification

Extract a capsule with 5 ml of ethanol-free chloroform, filter and to 1 ml of the filtrate add 9 ml of antimony trichloride solution. The light absorption of the resulting solution shows an absorption maximum at about 500 nm (2.4.7).

Tests

Other tests. Comply with the tests stated under Capsules.

Assay. Carry out the following procedure in subdued light.

Empty the contents of 20 capsules, or more if required. Weigh accurately a quantity of the contents of the capsules containing about 6 mg of Cholecalciferol or Ergocalciferol, add 50 ml of ethanol (95 per cent), 14 ml of glycerin and 20 ml of a 50 per cent w/v solution of potassium hydroxide. Boil under a reflux condenser for 30 minutes, stirring occasionally, add 110 ml of water and allow to stand for 10 minutes with occasional stirring. Cool and add sufficient ethanol (95 per cent) to produce 250.0 ml. Shake 5.0 ml of the resulting solution with 25.0 ml of light petroleum (40° to 60°) for 3 minutes and evaporate 5.0-ml portions, accurately measured, in duplicate,
of the extract to dryness in a current of oxygen-free nitrogen. Dissolve each residue in 1.0 ml of ethanol-free chloroform, add rapidly 9.0 ml of antimony trichloride solution and measure the absorbance of each solution at the maximum at about 500 nm and 550 nm (2.4.7), 90 to 120 seconds after adding the reagent. Repeat the operation using 1.0 ml portions, in duplicate, of a solution containing a known amount of cholecalciferol RS or ergocalciferol RS as appropriate, in ethanol-free chloroform and beginning at the words “add rapidly 9.0 ml of...”. Calculate the content of cholecalciferol or ergocalciferol, in mg, from the difference between the absorbances at the maximum at about 500 nm and 550 nm. Calculate the per centage w/v of C_{27}H_{44}O or C_{28}H_{44}O taking 0.87 g as the value of the weight per ml of the injection.

Storage. Store in a single dose container protected from light at a temperature not exceeding 30°.

Calciferol Oral Solution

Calciferol Oral Drops; Calciferol Solution

Calciferol Oral Solution is a solution of Cholecalciferol or Ergocalciferol in a suitable vegetable oil and may be prepared by warming to 40° a 1 per cent w/v suspension of Cholecalciferol or Ergocalciferol in a suitable vegetable oil, such as Arachis Oil, carbon dioxide being bubbled through it to facilitate solution, and adding a sufficient quantity of the oil to produce a solution containing the stated amount of Cholecalciferol or Ergocalciferol.

Calciferol Oral Solution contains not less than 85.0 per cent and not more than 120.0 per cent of the stated amount of cholecalciferol, C_{27}H_{44}O or ergocalciferol, C_{28}H_{44}O.

Description. A pale yellow, oily liquid; odour, slight but not rancid.

Identification

To 1 ml of a 20 per cent v/v solution in ethanol-free chloroform add 9 ml of antimony trichloride solution. The light absorption of the resulting solution shows an absorption maximum at about 500 nm (2.4.7).

Tests

Other tests. Complies with the tests stated under Oral Liquids.

Assay. Carry out the following procedure in subdued light.

Weigh accurately about 15 g of the injection and dilute to 50.0 ml with dry 1,2-dichloroethane that has been purified by passing it through a column of silica gel. To 1.0 ml of this solution add rapidly 9.0 ml of antimony trichloride in 1,2-dichloroethane solution and measure the absorbance of the resulting solution at the maximum at about 500 nm and 550 nm (2.4.7), 90 to 120 seconds after adding the reagent. Repeat the operation using 1.0 ml of a 0.002 per cent w/v solution of cholecalciferol RS or ergocalciferol RS in dry, purified 1,2-dichloroethane beginning at the words “add rapidly 9.0 ml of...”. Calculate the content of C_{27}H_{44}O or C_{28}H_{44}O, in mg, from the difference between the absorbances at the maximum at about 500 nm and 550 nm.
20 per cent v/v solution of ether in hexane, using a flow rate of 1 to 2 ml per minute and collecting the fraction that contains the calciferol (identified conveniently by testing aliquots of successive 10 ml fractions with antimony trichloride solution). Evaporate the solvent under oxygen-free nitrogen at a temperature not exceeding 50° and dissolve the residue in 5.0 ml of ethanol-free chloroform. Using duplicate 1.0 ml portions of this solution add rapidly 9.0 ml of antimony trichloride solution and measure the absorbance of each solution at the maximum at about 500 nm and 550 nm, 90 to 120 seconds after adding the reagent (2.4.7). Repeat the operation using duplicate 1.0-ml portions of a solution containing a known amount of cholecalciferol RS or ergocalciferol RS in ethanol-free chloroform and beginning at the words “add rapidly 9.0 ml of antimony trichloride solution.....” Calculate the content of C27H44O or C28H44O, in mg, from the difference between the absorbances at the maximum at about 500 nm and 550 nm.

Determine the weight per ml of the oral solution (2.4.29) and calculate the content of C27H44O or C28H44O, weight in volume.

Storage. Store protected from light and moisture at a temperature not exceeding 30°.

Labelling. The label states the number of Units of antirachitic activity (vitamin D) per ml.

Calciferol Tablets

Calciferol Tablets contain Cholecalciferol or Ergocalciferol

Calciferol Tablets contain not less than 90.0 per cent and not more than 125.0 per cent of the stated amount of cholecalciferol, C27H44O or ergocalciferol, C28H44O.

Identification

Powder a tablet, extract with 5 ml of ethanol-free chloroform, filter and to 1 ml of the filtrate add 9 ml of antimony trichloride solution; a brownish-red colour is produced.

Tests

Uniformity of content. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

Carry out the following procedure as rapidly as possible in subdued light and protected from air.

Test solution. For tablets containing less than 250 µg, prepare the solution in the same manner but using 4 ml of water, 12 ml of dimethyl sulphoxide and 100 ml of hexane.

Reference solution (a). A 0.001 per cent w/v solution of cholecalciferol RS or ergocalciferol RS, as appropriate.

Reference solution (b). Dissolve 50.0 mg of cholecalciferol RS or ergocalciferol RS as appropriate in 10 ml of toluene without heating and dilute with the mobile phase to 100.0 ml; dilute 5.0 ml of this solution to 50.0 ml with the mobile phase. Reflux 5.0 ml of this solution, under nitrogen, using a water-bath, for 60 minutes to obtain a solution of cholecalciferol, precholecalciferol and trans-cholecalciferol. Cool and dilute the refluxed solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with porous silica or ceramic microparticles (3 to 10 µm) (such as Nucleosil 50-S 5 µm),
- mobile phase: a mixture of 997 volumes of hexane and 3 volumes of 1-pentanol,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 10 or 20 µl loop injector.

Inject a suitable volume of reference solution (b). Adjust the sensitivity so that the height of the peak due to cholecalciferol is more than 50 per cent of the full-scale deflection. Record the chromatograms after five more injections. The approximate relative retention times calculated with reference to cholecalciferol are 0.4 for precholecalciferol and 0.5 for trans-cholecalciferol. The resolution between precholecalciferol and trans-cholecalciferol should be not less than 1.0; if necessary adjust the proportions of the constituents and flow rate of the mobile phase to obtain the required resolution.

Inject reference solution (a). Adjust the sensitivity so that the height of the peak due to cholecalciferol or ergocalciferol is more than 50 per cent of the full-scale deflection.

Inject alternately the test solution and reference solution (a).

Calculate the content of cholecalciferol, C27H44O, or ergocalciferol, C28H44O in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Carry out the following procedure as rapidly as possible in subdued light and protected from air.

Weigh and powder 20 or more tablets as required. Weigh accurately a quantity of the powder containing about 6 mg of Ergocalciferol or Cholecalciferol, add 50 ml of ethanol (95 per cent), 14 ml of glycerin and 20 ml of a 50 per cent w/v solution of potassium hydroxide. Boil under a reflux condenser for 30 minutes, stirring occasionally, add 110 ml of water and allow to stand for 10 minutes with occasional stirring. Cool and add sufficient ethanol (95 per cent) to produce 250.0 ml.
Shake 5.0 ml of the resulting solution with 25.0 ml of light petroleum (40° to 60°) for 3 minutes and evaporate 5.0-ml portions, accurately measured, in duplicate, of the extract to dryness in a current of oxygen-free nitrogen. Dissolve each residue in 1.0 ml of ethanol-free chloroform, add rapidly 9.0 ml of antimony trichloride solution and measure the absorbance of each solution at the maximum at about 500 nm and 550 nm (2.4.7), 90 to 120 seconds after adding the reagent. Repeat the operations using 1.0-ml portions, in duplicate, of a solution containing a known amount of cholecalciferol RS or ergocalciferol RS as appropriate, in ethanol-free chloroform and beginning at the words “add rapidly 9.0 ml of.....”. Calculate the content of cholecalciferol or ergocalciferol, in mg, from the difference between the absorbances at the maximum at about 500 nm and 550 nm.

**Storage.** Store protected from light and moisture, at a temperature not exceeding 30°.

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**Calcium Carbonate**

Precipitated Chalk  
CaCO₃  
Mol. Wt. 100.1

Calcium Carbonate contains not less than 98.0 per cent and not more than 100.5 per cent of CaCO₃, calculated on the dried basis.

**Description.** A fine, white, microcrystalline powder.

**Identification**

A. Dissolve 5.0 g in 80 ml of 2 M acetic acid. When effervescence ceases, boil the solution for 2 minutes, allow to cool, dilute to 100 ml with 2 M acetic acid and filter, if necessary, through a sintered-glass filter reserving any residue for the test for Substances insoluble in acetic acid; 0.2 ml of the filtrate (solution A) gives reactions A and B of calcium salts (2.3.1).

B. Gives reaction A of carbonates (2.3.1).

**Tests**

**Substances insoluble in acetic acid.** Wash any residue obtained in Identification test A with four quantities, each of 5 ml, of hot water and dry at 100° for 1 hour; the residue weighs not more than 10 mg (0.2 per cent).

**Arsenic** (2.3.10). Dissolve 2.5 g in 15 ml of brominated hydrochloric acid and 45 ml of water and remove the excess of bromine with a few drops of stannous chloride solution AsT. The resulting solution complies with the limit test for arsenic (4 ppm).

**Heavy metals** (2.3.13). To 1.0 g add 5 ml of water, and 8 ml of dilute hydrochloric acid, the latter being added slowly, shake and evaporate to dryness on a water-bath. Dissolve the residue in 20 ml of water, filter, add to the filtrate 3 ml of dilute acetic acid and water to make 25 ml. The solution complies with the limit test for heavy metals, Method A (20 ppm).

**Barium.** Dissolve 0.6 g in 10 ml of 2 M acetic acid by boiling, cool and add 10 ml of calcium sulphate solution; the solution remains clear for not less than 15 minutes.

**Iron** (2.3.14). Dissolve 0.2 g in 5 ml water and 0.5 ml of iron-free hydrochloric acid, boil and dilute to 40 ml with water, the solution complies with the limit test for iron (200 ppm).

**Magnesium and alkali metals.** Dissolve 1.0 g in 10 ml of dilute hydrochloric acid, neutralise the solution by adding dilute ammonia solution, heat the solution to boiling and add 50 ml of hot ammonium oxalate solution. Cool, dilute to 100 ml with water and filter. To 50 ml of the filtrate add 1.5 ml of dilute sulphuric acid, evaporate to dryness on a water-bath, heat the residue to redness, allow to cool and weigh. The residue weighs not more than 5 mg (1.0 per cent).

**Chlorides** (2.3.12). 1.0 g dissolved in water by the addition of 3 ml of nitric acid complies with the limit test for chlorides (250 ppm).

**Sulphates** (2.3.17). Suspend 50.0 mg in 5 ml of water and add dropwise sufficient dilute hydrochloric acid to effect solution. Add 2 ml of dilute hydrochloric acid; the resulting solution complies with the limit test for sulphates (0.3 per cent).

**Loss on drying** (2.4.19). Not more than 2.0 per cent, determined on 1.0 g by drying in an oven at 200°.

**Assay.** Weigh accurately about 0.1 g and dissolve in 3 ml of dilute hydrochloric acid and 10 ml of water. Boil for 10 minutes, cool, dilute to 50 ml with water. Titrate with 0.05 M disodium edetate to within a few ml of the expected end-point, add 8 ml of sodium hydroxide solution and 0.1 g of calcion mixture and continue the titration until the colour of the solution changes from pink to a full blue colour.

1 ml of 0.05 M disodium edetate is equivalent to 0.005004 g of CaCO₃.

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**Calcium Chloride**

Calcium Chloride Dihydrate  
CaCl₂·2H₂O  
Mol. Wt. 147.1

Calcium Chloride contains not less than 97.0 per cent and not more than 103.0 per cent of CaCl₂·2H₂O.

**Description.** A white, crystalline powder or fragments or granules; odourless; hygroscopic.
Identification
A. Gives reactions A and B of calcium salts (2.3.1).
B. A 10 per cent w/v solution in carbon dioxide-free water prepared from distilled water (solution A) gives reaction A of chlorides (2.3.1).

Tests
Appearance of solution. Solution A is clear (2.4.1) and not more intensely coloured than reference solution YS6 (2.4.1).
Acidity or alkalinity. To 10 ml of a freshly prepared 10 per cent w/v solution add 2 drops of phenolphthalein solution. Titrate with 0.01 M hydrochloric acid or 0.01 M sodium hydroxide; not more than 0.2 ml is required.
Arsenic (2.3.10). Dissolve 3.33 g in 15 ml of brominated hydrochloric acid and 45 ml of water and remove the excess of bromine with a few drops of stannous chloride solution AsT. The resulting solution complies with the limit test for arsenic (3 ppm).
Aluminium and phosphate. To 10 ml of a 5.0 per cent w/v solution, add 2 drops of dilute hydrochloric acid and 1 drop of phenolphthalein solution. Add ammonium chloride-ammonium hydroxide solution dropwise until the solution is faintly pink, add a few drops in excess and heat the liquid to boiling; no turbidity or precipitate is produced.
Barium. To 10 ml of solution A add 1 ml of calcium sulphate solution. After not less than 15 minutes the solution is not more opalescent than a mixture of 10 ml of solution A and 1 ml of distilled water.
Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).
Iron (2.3.14). Dissolve 2.0 g in 0.5 ml of hydrochloric acid and 25 ml of water; the resulting solution complies with the limit test for iron (20 ppm).
Magnesium and alkali salts. Dissolve 1.0 g in 50 ml of water, add 0.5 g of ammonium chloride heat the solution to boiling and add 50 ml of hot ammonium oxalate solution. Cool, dilute to 100 ml with water and filter. To 50 ml of the filtrate add 1.5 ml of dilute sulphuric acid, evaporate to dryness on a water-bath, heat the residue to redness, allow to cool and weigh. The residue weighs not more than 5 mg (1.0 per cent).
Sulphates (2.3.17). 0.5 g dissolved in 15 ml of distilled water complies with the limit test for sulphates (300 ppm).
Assay. Weigh accurately about 0.15 g and dissolve in 50 ml of water. Titrate with 0.05 M disodium edetate to within a few ml of the expected end-point, add 8 ml of sodium hydroxide solution and 0.1 g of calcon mixture and continue the titration until the colour of the solution changes from pink to a full blue colour.

1 ml of 0.05 M disodium edetate is equivalent to 0.007351 g of CaCl₂.2H₂O.

Storage. Store protected from moisture.

Calcium Folinate
Leucovorin Calcium

C₂₀H₂₁CaN₇O₇ Mol. Wt. 511.5
Calcium Folinate is calcium N-[4-[2-amino-5-formyl-1,4,5,6,7,8-hexahydro-4-oxo-6-pteridinyl)methylaminobenzoyl]-L-glutamate.
Calcium Folinate contains not less than 95.0 per cent and not more than 105.0 per cent of C₂₀H₂₁CaN₇O₇, calculated on the anhydrous basis.
Description. A yellowish white or yellow powder; odourless.

Identification
Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with calcium folinate RS.

Tests
Heavy metals (2.3.13). 4.0 g complies with the limit test for heavy metals, Method B (5 ppm).
Water (2.3.43). Not more than 17.0 per cent determined on 0.5 g.

Determine by liquid chromatography (2.4.14).

Solvent mixture. Add 15 ml of a 25 per cent w/v solution of tetrabutylammonium hydroxide in methanol to 900 ml of water, adjust the pH to 7.5 ± 0.1 with 0.67 M sodium dihydrogen phosphate and dilute with water to 1000 ml.

Test solution. A 0.02 per cent w/v solution of the substance under examination in the solvent mixture.
Reference solution. A solution containing 0.0175 per cent w/v each of calcium folinate RS and folic acid RS in the solvent mixture.

Chromatographic system
- a stainless steel column 30 cm x 4 mm, packed with octadecylsilyl silica gel (3 to 10 µm),
- mobile phase: a mixture of 15 ml of a 25 per cent w/v solution of tetrabutylammonium hydroxide, 825 ml of water and 125 ml of acetonitrile, previously adjusted to pH 7.5 ± 0.1 with 0.67 M sodium dihydrogen phosphate, diluted with water to 1000 ml,
- flow rate. 1 to 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject the reference solution. The relative retention times for calcium folinate and folic acid are 1.0 and about 1.6 respectively. The test is not valid unless the relative standard deviation for replicate injections is not more than 3.6 per cent.

Assay. Use only freshly deionised water wherever water is specified throughout this procedure. Protect the solutions from unnecessary exposure to light and complete the Assay without prolonged interruption.

Determine by liquid chromatography (2.4.14).

Solvent mixture. Add 15 ml of a 25 per cent w/v solution of tetrabutylammonium hydroxide in methanol to 900 ml of water, adjust the pH to 7.5 ± 0.1 with 0.67 M sodium dihydrogen phosphate and dilute with water to 1000 ml.

Test solution. Transfer an accurately measured volume of the Injection containing about 9 mg of folinic acid to a 50-ml volumetric flask, dilute to volume with a solution prepared by adding 15 ml of a 25 per cent w/v solution of tetrabutylammonium hydroxide in methanol to 900 ml of water, adjusting the pH to 7.5 ± 0.1 with 0.67 M sodium dihydrogen phosphate and diluting with water to 1000 ml. Transfer 25.0 ml of this solution into a 60-ml separator, add 25 ml of dichloromethane, shake the mixture, allow the layers to separate and discard the dichloromethane extract. Repeat the extraction with two more quantities, each of 25ml, of dichloromethane, discarding the dichloromethane extracts. Filter the aqueous layer, discarding the first 5 ml of the filtrate, and collect the remaining filtrate in a glass-stoppered conical flask.

Reference solution. A solution containing 0.0175 per cent w/v each of calcium folinate RS and folic acid RS in the solvent mixture.

Calcium Folinate Injection

Leucovorin Calcium Injection

Calcium Folinate Injection is a sterile solution of Calcium Folinate in Water for Injection.

Calcium Folinate Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of folinic acid, C_{20}H_{23}N_{7}O_{7}.

Description. A clear, yellowish solution.

Identification

Transfer a volume containing about 6 mg of folinic acid to a glass-stoppered, 50-ml centrifuge tube, add about 40 ml of acetone, mix, centrifuge for a few minutes and decant the liquid phase. Repeat the washing with an additional 40 ml of acetone. Dry the precipitate obtained with a stream of dry nitrogen. The precipitate complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with calcium folinate RS.

Tests

pH (2.4.24). 6.5 to 8.5.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Storage. Store in single dose containers preferably of type I glass, protected from light.

Labelling. The label states the strength in terms of the equivalent amount of folinic acid.
Calcium Gluconate

Calcium Gluconate is calcium D-gluconate monohydrate.

Calcium Gluconate contains not less than 98.5 per cent and not more than 102.0 per cent of C\textsubscript{12}H\textsubscript{22}CaO\textsubscript{14},H\textsubscript{2}O.

Description. A white, crystalline powder or granules.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 50 volumes of ethanol (95 per cent), 30 volumes of water, 10 volumes of strong ammonia solution and 10 volumes of ethyl acetate.

Test solution. A 2 per cent w/v solution of the substance under examination in water; heating if necessary, to 60° in a water-bath to effect solution.

Reference solution. A 2 per cent w/v solution of calcium gluconate RS in water; heating if necessary, to 60° in a water-bath to effect solution.

Apply to the plate 5 µl of each solution. After development, dry the plate at 100° for 20 minutes, cool and spray with a 5 per cent w/v solution of potassium dichromate in a 40 per cent w/w solution of sulphuric acid. After 5 minutes the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. To 1 ml of a 3 per cent w/v solution add 0.05 ml of ferric chloride test solution; a yellow colour is produced.

C. A 2 per cent w/v solution gives reactions A and B of calcium salts (2.3.1).

Tests

Appearance of solution. A 2.0 per cent w/v solution at 60° is not more intensely coloured than reference solution YS6 (2.4.1). On cooling to room temperature the solution is not more opalescent than opalescence standard OS2 (2.4.1).

Acidity and alkalinity. Dissolve 0.5 g in 20 ml of water, add 0.1 ml of 0.01 M hydrochloric acid and 0.1 ml of phenolphthalein solution; no colour is produced. Add 0.3 ml of 0.01 M sodium hydroxide; a pink colour is produced.

Arsenic (2.3.10). Dissolve 5.0 g in 50 ml of water and 12 ml of stannated hydrochloric acid AsT. The resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals (2.3.13). 1.0 g dissolved in 4 ml of dilute hydrochloric acid and sufficient water to produce 25 ml complies with the limit test for heavy metals, Method A (20 ppm).

Chlorides (2.3.12). 1.0 g complies with the limit test for chlorides (250 ppm).

Sulphates (2.3.17). 1.0 g complies with the limit test for sulphates (150 ppm).

Sucrose and reducing sugars. To 10 ml of 5 per cent w/v solution in hot water add 2 ml of dilute hydrochloric acid and boil for 2 minutes. Cool, add 15 ml of sodium carbonate solution, allow to stand for 5 minutes and filter. Add 5 ml of the clear filtrate to 2 ml of potassium cupri-tartrate solution and boil for 2 minutes; no red precipitate is formed.

Assay. Weigh accurately about 0.5 g and dissolve in 50 ml of warm water; cool, add 5.0 ml of 0.05 M magnesium sulphate and 10 ml of strong ammonia solution and titrate with 0.05 M disodium edetate using mordant black II mixture as indicator. From the volume of 0.05 M disodium edetate required subtract the volume of the magnesium sulphate solution added. 1 ml of the remainder of 0.05 M disodium edetate is equivalent to 0.02242 g of C\textsubscript{12}H\textsubscript{22}CaO\textsubscript{14},H\textsubscript{2}O.

Calcium Gluconate Injection

Calcium Gluconate Injection is a sterile solution of Calcium Gluconate in Water for Injections. Not more than 5.0 per cent of the Calcium Gluconate may be replaced with a suitable calcium salt as a stabilising agent.

Calcium Gluconate Injection contains a quantity of calcium equivalent to not less than 8.5 per cent and not more than 9.4 per cent of the stated amount of calcium gluconate, C\textsubscript{12}H\textsubscript{22}O\textsubscript{14}Ca, H\textsubscript{2}O.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 50 volumes of ethanol (95 per cent), 30 volumes of water, 10 volumes of strong ammonia solution and 10 volumes of ethyl acetate.

Test solution. Dilute a suitable volume of the substance under examination to obtain a solution containing 2 per cent w/v of Calcium Gluconate.
Reference solution. A 2 per cent w/v solution of calcium gluconate RS in water; heating if necessary, to 60° in a water-bath to effect solution.

Apply to the plate 5 µl of each solution. After development, dry the plate at 100° for 20 minutes, cool and spray with a 5 per cent w/v solution of potassium dichromate in a 40 per cent w/w solution of sulphuric acid. After 5 minutes the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. To 1 ml add 0.05 ml of ferric chloride test solution; an intense yellow colour is produced.

C. Gives the reactions of calcium salts (2.3.1).

Tests

pH (2.4.24). 6.0 to 8.2.

Bacterial endotoxins (2.2.3). Not more than 0.17 Endotoxin Unit per mg of calcium gluconate.

Other tests. Complies with the tests stated under Parenteral preparations (Injections).

Assay. To an accurately measured volume equivalent to 0.5 g of Calcium Gluconate add 50 ml of water; cool, add 5.0 ml of 0.05 M magnesium sulphate and 10 ml of strong ammonia solution and titrate with 0.05 M disodium edetate using mordant black II mixture as indicator. From the volume of 0.05 M disodium edetate required subtract the volume of the magnesium sulphate solution added.

1 ml of the remainder of 0.05 M disodium edetate is equivalent to 0.002004 g of Ca.

Labelling. The label states (1) the strength as a percentage w/v of calcium gluconate equivalent to the total amount of calcium present; (2) that solutions containing visible solid particles must not be used; (3) the percentage of any added stabilising agent.

 Calcium Gluconate Tablets

Calcium Gluconate Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of calcium gluconate, C_{12}H_{22}O_{14}Ca.H_{2}O.

Identification

A warm filtered solution of the powdered tablets equivalent to a 10 per cent w/v solution of Calcium Gluconate complies with the following tests.

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 50 volumes of ethanol (95 per cent), 30 volumes of water, 10 volumes of strong ammonia solution and 10 volumes of ethyl acetate.

Test solution. A 2 per cent w/v solution of the substance under examination in water; heating if necessary, to 60° in a water-bath to effect solution.

Reference solution. A 2 per cent w/v solution of calcium gluconate RS in water; heating if necessary, to 60° in a water-bath to effect solution.

Apply to the plate 5 µl of each solution. After development, dry the plate at 100° for 20 minutes, cool and spray with a 5 per cent w/v solution of potassium dichromate in a 40 per cent w/w solution of sulphuric acid. After 5 minutes the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. To 1 ml of a 3 per cent w/v solution add 0.05 ml of ferric chloride test solution; a yellow colour is produced.

C. A 2 per cent w/v solution gives reactions A and B of calcium salts (2.3.1).

Tests

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.5 g of Calcium Gluconate and ignite, gently at first, until free from carbon. Cool, add 10 ml of water and sufficient dilute hydrochloric acid, dropwise, to effect complete solution of the residue. Neutralise with dilute ammonia solution, add 5.0 ml of 0.05 M magnesium sulphate and 10 ml of strong ammonia solution and titrate with 0.05 M disodium edetate using mordant black II mixture as indicator. From the volume of 0.05 M disodium edetate required subtract the volume of the magnesium sulphate solution added.

1 ml of the remainder of 0.05 M disodium edetate is equivalent to 0.02242 g of C_{12}H_{22}O_{14}Ca.H_{2}O.

 Calcium Lactate

Calcium Lactate is hydrated calcium (RS)-2-hydroxypropionate or mixtures of the calcium salts of (R)-, (S)- and (RS)-2-hydroxypropionic acid.
Calcium Lactate contains not less than 98.0 per cent and not more than 102.0 per cent of C₆H₁₀CaO₆, calculated on the dried basis.

**Description.** White granules or powder; odourless or with slight but not unpleasant odour. The pentahydrate is somewhat efflorescent.

**Identification**

A. A solution acidified with sulphuric acid and warmed with potassium permanganate develops the odour of acetaldehyde.

B. Gives the reactions of calcium salts and of lactates (2.3.1).

**Tests**

**Acidity or alkalinity.** To 10 ml of a 5.0 per cent w/v solution in carbon dioxide-free water add 0.1 ml of 0.1 M hydrochloric acid and 0.1 ml of phenolphthalein solution; no colour is developed. Add 0.6 ml of 0.1 M sodium hydroxide; a pink colour is produced.

**Arsenic** (2.3.10). Dissolve 5.0 g in 50 ml of water and 12 ml of stannated hydrochloric acid. The resulting solution complies with the limit test for arsenic (2 ppm).

**Heavy metals** (2.3.13). 1.0 g dissolved in 2.5 ml of dilute hydrochloric acid and sufficient water to produce 25 ml complies with the limit test for heavy metals, Method A (20 ppm).

**Iron** (2.3.14). 0.5 g complies with the limit test for Iron (80 ppm).

**Chlorides** (2.3.12). Dissolve 1.25 g in 10 ml water, add 2 ml of nitric acid and sufficient water to produce 50 ml; the resulting solution complies with the limit test for chlorides (200 ppm).

**Sulphates** (2.3.17). Dissolve 0.1 g in 10 ml of water, add 2 ml of hydrochloric acid and sufficient water to produce 15 ml; the resulting solution complies with the limit test for sulphates (0.15 per cent).

**Reducing sugars.** Dissolve 1 g in 10 ml of water, add 5 ml of potassium cupri-tartrate solution and boil; not more than a slight brick-red precipitate is produced.

**Loss on drying** (2.4.19). Not more than 30 per cent, determined on 0.5 g by drying in an oven at 120° for 4 hours.

**Assay.** Weigh accurately about 0.3 g, dissolve in 50 ml of water, add 5.0 ml of 0.05 M magnesium sulphate and 10 ml of strong ammonia solution and titrate with 0.05 M disodium edetate using mordant black II mixture as indicator. From the volume of 0.05 M disodium edetate required subtract the volume of the magnesium sulphate solution added.

1 ml of the remainder of 0.05 M disodium edetate is equivalent to 0.01091 g of C₆H₁₀CaO₆.

**Calcium Levulinate**

**Calcium Levulinate Tablets**

Calcium Lactate Tablets contain Calcium Lactate equivalent to not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of calcium lactate pentahydrate, C₆H₁₀CaO₆·5H₂O.

**Identification**

A. Extract a quantity of the powdered tablets with water, filter and acidify the filtrate with sulphuric acid, add potassium permanganate and warm; the odour of acetaldehyde is produced.

B. The powdered tablets, when moistened with hydrochloric acid and introduced on a platinum wire into the flame of a bunsen burner, gives a brick-red colour to the flame.

**Tests**

**Disintegration** (2.5.1). 30 minutes.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.3 g of calcium lactate pentahydrate, dissolve as completely as possible in 50 ml of water, add 5.0 ml of 0.05 M magnesium sulphate and 10 ml of strong ammonia solution and titrate with 0.05 M disodium edetate using mordant black II mixture as indicator. From the volume of 0.05 M disodium edetate required subtract the volume of the magnesium sulphate solution added.

1 ml of the remainder of 0.05 M disodium edetate is equivalent to 0.01542 g of C₆H₁₀CaO₆·5H₂O.

**Storage.** Store protected from moisture.

**Calcium Levulinate**

Calcium Levulinate is calcium di(4-oxopentanoate) dihydrate.

Calcium Levulinate contains not less than 97.5 per cent and not more than 100.5 per cent of C₁₀H₁₄CaO₆, calculated on the dried basis.
Description. A white, crystalline or amorphous powder; odour, faint and suggestive of burnt sugar.

Identification

A. Dissolve 0.5 g in 5 ml of water, add 5 ml of 1 M sodium hydroxide and filter. To the filtrate add 5 ml of iodine solution; a precipitate of iodoform is produced.

B. Dissolve 0.1 g in 2 ml of water, add 5 ml of dinitrophenylhydrazine solution and allow the mixture to stand in an ice-bath for 1 hour. Collect the precipitate on a filter, wash well with cold water and dry at 105° for 1 hour; the hydrazone so obtained melts between 198° and 206° (2.4.21).

C. Gives the reactions of calcium salts (2.3.1).

D. Melting range (2.4.21) 119° to 125°.

Tests

pH (2.4.24). 7.0 to 8.5, determined in a 10.0 per cent w/v solution.

Arsenic (2.3.10). Dissolve 3.3 g in 50 ml of water and 12 ml of stannated hydrochloric acid. The resulting solution complies with the limit test for arsenic (3 ppm).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

Reducing sugars. Dissolve 0.5 g in 10 ml of water, add 2 ml of 3 M hydrochloric acid, boil for about 10 minutes and cool. Add 5 ml of sodium carbonate solution allow to stand for 5 minutes, dilute with water to 20 ml and filter. Add 5 ml of the clear filtrate to about 2 ml of potassium cupri-tartrate solution and boil for 1 minute; no red precipitate is formed immediately.

Loss on drying (2.4.19). 10.5 per cent to 12.0 per cent, determined on 0.5 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 5 hours.

Assay. Weigh accurately about 0.2 g, dissolve in 50 ml of water; add 5.0 ml of 0.05 M magnesium sulphate and 10 ml of strong ammonia solution and titrate with 0.05 M disodium edetate using mordant black II mixture as indicator. From the volume of 0.05 M disodium edetate required subtract the volume of the magnesium sulphate solution added.

1 ml of the remainder of 0.05 M disodium edetate is equivalent to 0.01351 g of C_{10}H_{14}CaO_{6}.2H_{2}O.

Calcium Levulinate Injection

Calcium Levulinate Injection is a sterile solution of Calcium Levulinate in Water for Injections.

Calcium Levulinate Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of calcium levulinate, C_{10}H_{14}CaO_{6}.2H_{2}O.
Identification
A. In the test for β-Alanine, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).
B. Boil 50 mg in 5 ml of 1 M sodium hydroxide for 1 minute, cool, and add 5 ml of 1 M hydrochloric acid and 2 drops of ferric chloride test solution; a strong yellow colour is produced.
C. To 50 mg in 2 ml of 1 M sodium hydroxide add 0.1 ml of copper sulphate solution; a blue colour is produced.
D. Gives reaction A of calcium salts (2.3.1).

Tests
Appearance of solution. A 5.0 per cent w/v solution in carbon dioxide-free water is clear, (2.4.1) and colourless (2.4.1).

pH (2.4.24). 6.8 to 8.0, determined in a 5.0 per cent w/v solution.

Specific optical rotation (2.4.22). +25.0° to +27.5°, determined at 20° in a 5.0 per cent w/v solution.

β-Alanine. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 65 volumes of ethanol and 35 volumes of water.

Test solution (a). A 4 per cent w/v solution of the substance under examination in water.

Test solution (b). A 0.4 per cent w/v solution of the substance under examination in water.

Reference solution (a). A 0.4 per cent w/v solution of calcium pantothenate RS in water.

Reference solution (b). A 0.02 per cent w/v solution of β-alanine in water.

Apply to the plate 5 μl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of air, spray with ethanolic ninhydrin solution and heat at 110° for 10 minutes. Any spot corresponding to β-alanine in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b).

Heavy metals (2.3.13). 1.0 g dissolved in 25 ml of water complies with the limit test for heavy metals, Method A (20 ppm).

Loss on drying (2.4.19). Not more than 3.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.18 g and dissolve in 50 ml of anhydrous glacial acetic acid. Titrate 0.1 M perchloric acid, determining the end point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02383 g of C₁₈H₃₂CaN₂O₁₀.

Storage. Store protected from moisture.

Dibasic Calcium Phosphate

Calcium Hydrogen Phosphate
CaHPO₄  Mol. Wt. 136.1 (anhydrous)
CaHPO₄·2H₂O Mol. Wt. 172.1 (dihydrate)

Dibasic Calcium Phosphate is anhydrous or contains two molecules of water of hydration.

Dibasic Calcium Phosphate contains not less than 98.0 per cent and not more than 105.0 per cent of CaHPO₄ (for anhydrous material) or of CaHPO₄·2H₂O (for the dihydrate).

Description. A white, crystalline powder; odourless.

Identification
A. Gives reaction B of calcium salts (2.3.1).
B. Dissolve 0.1 g in a mixture of 5 ml of 2 M nitric acid and 5 ml of water; the solution gives reaction C of phosphates (2.3.1).

Tests
Acid-insoluble substances. Heat 5.0 g with a mixture of 40 ml of water and 10 ml of hydrochloric acid and dilute to 100 ml with water. Filter, wash with hot water until the last washing is free from chloride and dry the residue at 105° for 1 hour (0.1 per cent).

Arsenic (2.3.10). Dissolve 1.0 g in 15 ml of brominated hydrochloric acid, add 45 ml of water and remove the excess of bromine with a few drops of stannous chloride solution AsT. The resulting solution complies with the limit test for arsenic (10 ppm).

Heavy metals (2.3.13). Dissolve 2.5 g in 20 ml of 2 M hydrochloric acid, filter if necessary, and add 6 M ammonia until a precipitate is formed. Add 2 M hydrochloric acid just enough to dissolve the precipitate and dilute to 50 ml with distilled water (solution A). 10 ml of this solution complies with the limit test for heavy metals, Method A (40 ppm).

Barium. To 10 ml of solution A add 0.5 ml of 1 M sulphuric acid, mix and set aside for 15 minutes. The solution is not more opalescent than a mixture of 10 ml of solution A and 0.5 ml of distilled water treated in the same manner.

Iron (2.3.14). 2.0 ml of solution A diluted to 10 ml with water complies with the limit test for Iron (400 ppm).

Carbonate. Suspend 1 g in 5 ml of water and add 2 ml of hydrochloric acid; no effervescence is produced.
Chlorides (2.3.12). Dissolve 0.2 g in water by the addition of 2 ml of nitric acid. The solution complies with the limit test for chlorides (0.125 per cent).

Sulphates (2.3.17). Dissolve 30.0 mg in 25 ml of water by the addition of 2 ml of hydrochloric acid. The solution complies with the limit test for sulphates (2.3.17) (0.5 per cent).

Nitrate. To 0.1 g add 10 ml of water, 10 ml of nitrogen-free sulphuric acid and 1 ml of indigo carmine solution and heat to boiling; the blue colour does not disappear.

Reducing substances. Shake 1.0 g with 5 ml of water and 5 ml of 3 M potassium permanganate for 1 minute. Add 0.1 ml of 0.005 M potassium permanganate and shake for 20 seconds. The slight pink colour is not less intense than that produced by treating 1 g of calcium carbonate in the same manner.

Proteinous impurities. Heat 0.5 g gently in a dry test-tube; no change in colour is observed and no unpleasant odour is emitted.

Monocalcium and tricalcium phosphates. Dissolve 2.0 g in 30.0 ml of 1 M hydrochloric acid, add 20 ml of water and 0.05 ml of methyl orange solution and titrate the excess of acid with 1 M sodium hydroxide. Not less than 14.0 ml and not more than 15.5 ml of 1 M hydrochloric acid (for anhydrous material) and not less than 11.0 ml and not more than 12.5 ml of 1 M hydrochloric acid (for the dihydrate) is required.

Loss on ignition (2.4.20). 6.5 to 8.5 per cent (for anhydrous material) and 24.5 to 26.5 per cent (for the dihydrate), determined on 1.0 g by igniting at 500°.

Assay. Weigh accurately about 0.3 g and dissolve in a mixture of 5 ml of water and 1 ml of 7 M hydrochloric acid, add 25.0 ml of 0.1 M disodium edetate and dilute to 200 ml with water. Neutralise with strong ammonia solution, add 10 ml of ammonia buffer pH 10.0 and 50 mg of mordant black 11 mixture and titrate the excess of disodium edetate with 0.1 M zinc sulphate.

1 ml of 0.1 M disodium edetate is equivalent to 0.01361 g of CaHPO₄ or 0.01721 g of CaHPO₄·2H₂O.

Identification
A. Gives reaction B of calcium salts (2.3.1).
B. Dissolve 0.1 g in a mixture of 5 ml of 2 M nitric acid and 5 ml of water; the solution gives reaction C of phosphates (2.3.1).

Tests
Acid-insoluble substances. Heat 5.0 g with a mixture of 40 ml of water and 10 ml of hydrochloric acid and dilute to 100 ml with water. Filter, wash with hot water until the last washing is free from chloride and dry the residue at 105° for 1 hour (0.3 per cent).

Water-soluble substances. Digest 2.0 g with 100 ml of water for 30 minutes on a water-bath, cool, add sufficient water to restore the original volume, stir well and filter. Evaporate 50 ml of the filtrate to dryness and dry the residue at 105° to constant weight (0.5 per cent).

Arsenic (2.3.10). Dissolve 2.0 g in a mixture of 15 ml of brominated hydrochloric acid, add 45 ml of water and remove the excess of bromine with a few drops of stannous chloride solution AsT. The resulting solution complies with the limit test for arsenic (5 ppm).

Heavy metals (2.3.13). Warm 1.0 g with 4 ml of dilute hydrochloric acid, add sufficient water to produce 50 ml and filter. 25 ml of this solution complies with the limit test for heavy metals, Method A (40 ppm).

Iron (2.3.14). Dissolve 0.2 g in a mixture of 5 ml of water and 0.5 ml of iron-free hydrochloric acid with the addition of 1 g of citric acid. Dilute the solution to 40 ml with water. The solution complies with the limit test for iron (200 ppm).

Carbonate. Suspend 1 g in 10 ml of water and add 2 ml of hydrochloric acid; no effervescence is produced.

Chlorides (2.3.12). Dissolve 0.25 g in 25 ml of water by the addition of 1 ml of nitric acid. The solution complies with the limit test for chlorides (0.1 per cent).

Sulphates (2.3.17). Dissolve 100.0 mg in water with the aid of 3 ml of 1 M hydrochloric acid and dilute to 60 ml with water. 15 ml of the resulting solution complies with the limit test for sulphates (0.6 per cent).

Proteinous impurities. Heat 0.5 g gently in a dry test-tube; no change in colour is observed and no unpleasant odour is emitted.

Loss on ignition (2.4.20). Not more than 8.0 per cent, determined on 1.0 g by igniting at 800° for 30 minutes.

Water (2.3.43). Not more than 2.5 per cent, determined on 1.0 g.

Assay. Weigh accurately about 1.0 g and dissolve in 10 ml of hydrochloric acid by heating on a water-bath, add 50 ml of
**Calcium Stearate**

Octadecanoic acid, calcium salt

Calcium Stearate, a compound of calcium with a mixture of solid organic acids obtained from fats and consists chiefly of variable proportions of calcium stearate and calcium palmitate.

Calcium Stearate contains the equivalent of not less than 9.0 per cent and not more than 10.5 per cent of calcium oxide (CaO).

**Identification**

A. Heat 1 g with a mixture of 25 ml of water and 5 ml of hydrochloric acid; fatty acids are liberated and appear as an oily layer floating on the surface of the liquid. The water layer gives the tests for calcium (2.3.1).

B. Mix 25 g with 200 ml of hot water; add 60 ml of 2 M sulphuric acid, and heat the mixture, with frequent stirring, until the separated fatty acid layer is clear. Wash the fatty acids with boiling water until free from sulphate, collect them in a small beaker, and warm on a steam bath until the water has separated and the fatty acids are clear. Allow the acids to cool, pour off the water layer, melt the acids, filter into a dry beaker, and dry at 105°C for 20 minutes; the fatty acids so obtained congeal at a temperature not below 54°C (2.4.10).

**Tests**

**Loss on drying** (2.4.19). Not more than 4.0 per cent, determined on 1.0 g by drying in an oven at 105°C.

**Heavy metals** (2.3.13). Place 2.5 g in a porcelain dish, place a 500 mg portion in a second dish to provide the control, and to each add 5 ml of a 1 in 4 solution of magnesium nitrate in alcohol. Cover the dishes with 7.5-cm short-stem funnels so that the stems are straight up. Heat on a hot plate at low heat for 30 minutes, then heat at medium heat for 30 minutes, and cool. Remove the funnels, add 2 ml of standard lead solution (20 ppm Pb) to the control, and heat each dish over a suitable burner until most of the carbon is burned off. Cool, add 10 ml of nitric acid, and transfer the solutions into 250 ml beakers. Add 5 ml of 70 per cent perchloric acid, cautiously evaporate to dryness, add 2 ml of hydrochloric acid to the residues, and wash down the insides of the beakers with water. Evaporate carefully to dryness again, swirling near the dry point to avoid spattering. Repeat the hydrochloric acid treatment, then cool, and dissolve the residues in about 10 ml of water. To each solution add 1 drop of phenolphthalein solution and add sodium hydroxide solution until the solutions just turn pink, then add 3 M hydrochloric acid until the solutions become colourless. Add 1 ml of 1 M acetic acid and a small amount of charcoal to each solution, and filter through filter paper into 50-ml Nessler cylinders. Wash with water, dilute with water to 40 ml, add 1.2 ml of thioacetamide reagent and 2 ml of pH 3.5 acetate buffer to each tube, and allow to stand for 5 minutes; the color of the test solution does not exceed that of the control (10 ppm Pb).

**Assay.** Boil about 1.2 g accurately weighed, with 50 ml of 1 M sulphuric acid for about 3 hours using a watch glass cover to avoid splattering, or until the separated fatty acid layer is clear, adding water, if necessary to maintain the original volume.

[Note-Stirring may be helpful in obtaining a clear layer and decreasing extraction time.] Cool, filter, and wash the filter and the flask thoroughly with water until the last washing is not acid to litmus. Neutralize the filtrate with 1 M sodium hydroxide to litmus. While stirring, preferably with a magnetic stirrer, titrate with 0.05 M disodium edetate as follows. Add about 30 ml from a 50-ml burette, then add 1 ml of 1 M sodium hydroxide and 300 mg of hydroxy napthol blue, and continue the titration to a blue end-point.

1 ml of 0.05 M disodium edetate is equivalent to 0.002804 g of calcium.

**Capreomycin Sulphate**

![Capreomycin Sulphate](attachment:image.png)

C<sub>25</sub>H<sub>46</sub>N<sub>14</sub>O<sub>12</sub>S  
Mol. Wt. 766.8

Capreomycin Sulphate is the disulphate salt of capreomycin, a polypeptide mixture produced by certain strains of *Streptomyces capreolus*.

It has a potency equivalent to not less than 700 µg and not more than 1050 µg of capreomycin per mg.

**Description.** A white or almost white powder.
Identification

A. When examined in the range 230 nm to 350 nm (2.4.7), a 0.004 per cent w/v solution in 0.1 M hydrochloric acid shows an absorption maximum only at 268 nm. Absorbance at 268 nm, about 1.2.

B. When examined in the range 230 nm to 350 nm (2.4.7), a 0.004 per cent w/v solution in 0.1 M sodium hydroxide shows an absorption maximum only at 287 nm. Absorbance at 287 nm, about 0.8.

C. It gives reaction A of sulphates (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution in water is clear (2.4.1), when examined immediately after preparation.

pH (2.4.24). 4.5 to 7.5, determined in a 3.0 per cent w/v solution.

Capreomycin content. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the injection containing about 25 mg of capreomycin in 100 ml of water.

Reference solution. A 0.025 per cent w/v solution of capreomycin sulphate RS in water.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica particles (5 µm) (such as Spherisorb CN),
- mobile phase: 55 volumes of the solution prepared by dissolving 0.5 g of ammonium bisulphate in 1000 ml of water, filter and 45 volumes of methanol,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 268 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the resolution between the two principal peaks is at least 1.5. In the chromatogram obtained with the test solution, the sum of the areas of the two principal peaks, due to capreomycins 1A and 1B, is not less than 90 per cent of the total areas of all the peaks.

Sulphated ash (2.3.18). Not more than 3.0 per cent.

Loss on drying (2.4.19). Not more than 10.0 per cent, determined on 0.1 g by drying in an oven for 4 hours at 100° at a pressure not exceeding 0.7 kPa.

Assay. Determine by the microbiological assay of antibiotics (2.2.10).

Capreomycin Sulphate intended for use in the manufacture of parenteral preparations complies with the following additional requirements.

Bacterial endotoxins (2.2.3). Not more than 0.35 Endotoxin Unit per mg of capreomycin.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture.

Capreomycin Injection

Capreomycin Injection is a sterile material consisting of Capreomycin Sulphate with or without auxiliary agents. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Capreomycin injection contains an amount of Capreomycin Sulphate equivalent to not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of capreomycin.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. When examined in the range 230 nm to 350 nm (2.4.7), a solution containing 0.004 per cent w/v of capreomycin in 0.1 M hydrochloric acid shows an absorption maximum only at 268 nm. Absorbance at 268 nm, about 1.2.

B. When examined in the range 230 nm to 350 nm (2.4.7), a solution containing 0.004 per cent w/v of capreomycin in 0.1 M sodium hydroxide shows an absorption maximum only at 287 nm. Absorbance at 287 nm, about 0.8.

Tests

Appearance of solution. A 10.0 per cent w/v solution in water is clear (2.4.1), when examined immediately after preparation.

pH (2.4.24). 4.5 to 7.5, determined in a 3.0 per cent w/v solution.

Capreomycin content. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the injection containing about 25 mg of capreomycin in 100 ml of water.

Reference solution. A 0.025 per cent w/v solution of capreomycin sulphate RS in water.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica particles (5 µm) (such as Spherisorb CN),
– mobile phase: a mixture of 55 volumes of a solution
prepared by dissolving 0.5 g of ammonium bisulphate
in 1000 ml of water; filtered and 45 volumes of methanol,
– flow rate. 1.5 ml per minute,
– spectrophotometer set at 268 nm,
– a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the
resolution between the two principal peaks is at least 1.5.

In the chromatogram obtained with the test solution, the sum
of the areas of the two principal peaks, due to capreomycins
1A and 1B, is not less than 90 per cent of the total areas of all
the peaks.

Loss on drying (2.4.19). Not more than 10.0 per cent, determined
on 0.1 g by drying in an oven at 100° at a pressure not exceeding
0.7 kPa for 4 hours.

Bacterial endotoxins (2.2.3). Not more than 0.35 Endotoxin
Unit per mg of capreomycin.

Assay. Determine by the microbiological assay of antibiotics
(2.2.10).

Storage. Store protected from moisture, at a temperature not
exceeding 25°.

Labeling. The label states the quantity of Capreomycin
Sulphate in terms of the equivalent amount of capreomycin.

Captopril

\[
\text{C}_{9}\text{H}_{15}\text{NO}_{3}\text{S} \quad \text{Mol. Wt. 217.3}
\]

Captopril is 1-[(2S)-3-mercapto-2-methylpropionyl]-L-proline.

Captopril contains not less than 97.5 per cent and not more
than 102.0 per cent of C₉H₁₅NO₃S, calculated on the dried
basis.

Description. A white to off-white, crystalline powder; odour,
characteristic, sulphide-like.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6).
Compare the spectrum with that obtained with captopril RS
or with the reference spectrum of captopril.

B. Determine by thin-layer chromatography (2.4.17), coating
the plate with silica gel G.

Test solution. A 0.4 per cent w/v solution of the substance
under examination in methanol.

Reference solution. A 0.4 per cent w/v solution of captopril
RS in methanol.

Apply to the plate, in the form of 1-cm bands, 50 µl of each
solution. Allow the mobile phase to rise 12 cm. Dry in air and
spray with a freshly prepared mixture of 1 volume of strong
ammonia solution and 6 volumes of a 0.04 per cent w/v solution
of 5,5’-dithiobis(2-nitrobenzoic acid) in methanol and allow
to stand for 5 minutes. The principal band in the chromatogram
obtained with the test solution corresponds to that in the
chromatogram obtained with the reference solution.

C. Melting range (2.4.21) 104° to 110°.

Tests

Specific optical rotation (2.4.22). –125° to –134°, determined
in a 1.0 per cent w/v solution in ethanol.

Heavy metals (2.3.13). 0.66 g complies with the limit test for
heavy metals, Method B (30 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined
on 1.0 g by drying in an oven at 60° at a pressure not exceeding
0.7 kPa.

Assay. Weigh accurately about 0.3 g, dissolve in 100 ml of
water in a stoppered-flask, add 10 ml of 1.8 M sulphuric acid
and 1 g of potassium iodide. Titrate with 0.025 M potassium
iodate using 3 ml of starch solution, added towards the end-
point, as indicator.

1 ml of 0.025 M potassium iodate is equivalent to 0.03308 g of
C₉H₁₅NO₃S.

Storage. Store protected from moisture.

Captopril Tablets

Captopril Tablets contain not less than 90.0 per cent and not
more than 110.0 per cent of the stated amount of captopril,
C₉H₁₅NO₃S.

Identification

Determine by thin-layer chromatography (2.4.17), coating the
plate with silica gel G.

Mobile phase. A mixture of 75 volumes of toluene, 25 volumes
of glacial acetic acid and 1 volume of methanol.

Test solution. Extract a quantity of the powdered tablets
containing 100 mg of Captopril with 25 ml of methanol and
centrifuge. Use the clear supernatant liquid.
Reference solution. A 0.4 per cent w/v solution of captopril RS in methanol.

Apply to the plate, in the form of 1-cm bands, 50 µl of each solution. Allow the mobile phase to rise 12 cm. Dry in air and spray with a freshly prepared mixture of 1 volume of strong ammonia solution and 6 volumes of a 0.04 per cent w/v solution of 5,5’-dithiobis(2-nitrobenzoic acid) in methanol and allow to stand for 5 minutes. The principal band in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus. No 2
Medium. 900 ml of 0.1 M hydrochloric acid.
Speed and time. 50 rpm and 20 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance (2.4.7) of the filtrate, suitably diluted if necessary, at the maximum at about 212 nm.

Calculate the content of C₉H₁₅NO₃S in the medium from the absorbance obtained from a solution of known concentration of captopril RS.

D. Not less than 80 per cent of the stated amount of C₉H₁₅NO₃S.

Other tests. Complies with the tests stated under Tablets.

Assay. Protect the solutions from exposure to air and use within 8 hours of preparation.

Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the finely powdered tablets containing about 25 mg of Captopril in 25 ml of the mobile phase with the aid of ultrasound for 15 minutes, centrifuge and use the clear supernatant liquid.

Reference solution. A 0.1 per cent w/v solution of captopril RS in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilil silica gel (3 to 10 µm),
- mobile phase: a mixture of 55 volumes of methanol and 45 volumes of water containing 0.05 volumes of phosphoric acid,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of C₉H₁₅NO₃S in the tablets.

Storage. Store protected from moisture.

Caramel

Burst Sugar

Caramel is a concentrated solution of the product obtained by heating Sucrose or Dextrose until the sweet taste is destroyed.

Description. A thick, free-flowing, dark brown liquid; odour, slight and characteristic.

Identification

To 20 ml of a 5 per cent w/v solution add 0.5 ml of phosphoric acid; no precipitate is produced.

Tests

pH (2.4.24). 3.0 to 5.5, determined in a 10.0 per cent w/v solution.

Weight per ml (2.4.29). Not less than 1.30 g.

Acid-stability. Dilute 50 ml of a 1 per cent w/v solution to 250 ml with water; add 5 ml of hydrochloric acid and heat gently to boiling under reflux. Allow to cool and set aside for 24 hours; the solution remains clear. Repeat the test on the same test solution but boil for 30 minutes; the solution remains clear for 24 hours.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Iron (2.3.14). Evaporate 0.4 g to dryness, add 0.2 ml of nitric acid, ignite and dissolve the residue in 1 ml of dilute nitric acid. The solution complies with the limit test for Iron (100 ppm).

Microbial contamination (2.2.9). 1 g is free from Escherichia coli and Salmonellae.

Sulphated ash (2.3.18). Not more than 2.0 per cent.

Storage. Store protected from moisture.

Carbamazepine

\[
\text{CONH}_2
\]

C₁₅H₁₂N₂O

Mol. Wt. 236.3

Carbamazepine is 5H-dibenz[b,f]azepine-5-carboxamide.
Carbamazepine contains not less than 97.0 per cent and not more than 103.0 per cent of C_{15}H_{12}N_{2}O, calculated on the dried basis.

Description. A white or yellowish-white, crystalline powder; almost odourless; exhibits polymorphism.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with carbamazepine RS.

B. In the Assay, the principal peak in the chromatogram obtained with test solution (b) corresponds to the peak in the chromatogram obtained with the reference solution (b).

Tests

Acidity or alkalinity. Stir 1.0 g with 20 ml of carbon dioxide-free water for 15 minutes and filter. Titrate 10 ml of the filtrate with 0.1 M sodium hydroxide using 0.05 ml of phenolphthalein solution as indicator; not more than 0.5 ml is required. Add 0.15 ml of a 0.05 per cent w/v solution of methyl red and titrate with 0.01 M hydrochloric acid until the colour changes to red; not more than 1.0 ml is required.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution (a). Dissolve 0.15 g of the substance under examination in methanol and dilute to 50 ml with the same solvent. Mix with the aid of ultrasound and dilute 10 ml of this solution to 20 ml with water.

Test solution (b). Dilute 10 ml of test solution (a) to 50 ml with a mixture of equal volumes of methanol and water.

Reference solution (a). Dissolve 7.5 mg of carbamazepine RS, 7.5 mg of 10,11-dihydrocarbamazepine RS and 7.5 mg of iminodibenzyl in methanol and dilute to 100 ml with the same solvent. Dilute 1.0 ml of this solution to 50 ml with a mixture of equal volumes of methanol and water.

Reference solution (b). Dissolve 0.15 g of carbamazepine RS in methanol and dilute to 50 ml with the same solvent. Dilute 5 ml of this solution to 50 ml with a mixture of equal volumes of methanol and water.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica particles (10 µm),
- mobile phase: a mixture of 3 volumes of tetrahydrofuran, 12 volumes of methanol and 85 volumes of water adding 0.2 ml of formic acid and 0.5 ml of triethylamine to 1000 ml of this solution,
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- a 20 µl loop injector.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to carbamazepine and 10,11-dihydrocarbamazepine is more than 1.7.

Inject test solution (a). Record the chromatograms for 6 times the retention time of carbamazepine (about 10 minutes). The areas of any peaks corresponding to 10,11-dihydrocarbamazepine and iminodibenzyl are not more than the areas of the corresponding peaks in the chromatogram obtained with reference solution (a) (0.1 per cent each). The area of any other secondary peak is not more than the area of the peak due to carbamazepine (0.1 per cent) and the sum of all the secondary peaks is not more than 5 times the area of the peak due to carbamazepine (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Chlorides (2.3.12). 1.5 g complies with the limit test for chlorides (165 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

Assay. Determine by liquid chromatography (2.4.14) as given under the test for Related substances using test solution (b) and reference solution (b).

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately test solution (b) and reference solution (b). Calculate the content of C_{15}H_{12}N_{2}O.

Storage. Store protected from moisture.

Carbamazepine Tablets

Carbamazepine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of carbamazepine, C_{15}H_{12}N_{2}O.

Identification

Boil a quantity of the powdered tablets containing 0.2 g of Carbamazepine with 15 ml of acetone, filter the hot solution, wash the filtrate with two 5 ml quantities of hot acetone, cool in ice, evaporate the combined filtrates to dryness. The residue complies with the following test.
Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with carbamazepine RS.

**Tests**

**Related substances.** Determine by liquid chromatography (2.4.14).

*Test solution.* Shake a quantity of the powdered tablets containing 0.3 g of Carbamazepine with 100 ml of methanol for 15 minutes. Dilute to 200 ml with water, mix and filter.

*Reference solution.* Dissolve 7.5 mg each of carbamazepine RS, 10,11-dihydrocarbamazepine and iminodibenzyl in methanol and dilute to 100 ml with the same solvent. Dilute 1 ml of the resulting solution to 50 ml with methanol (50 per cent).

**Chromatographic system**

- a stainless steel column 25 cm × 4.6 mm, packed with nitrile groups chemically bonded to porous silica particles (10 µm) (such as Nucleosil 10 CN),
- mobile phase: a mixture of 30 volumes of tetrahydrofuran, 120 volumes of methanol and 850 volumes of water; adding 0.2 ml of anhydrous formic acid and 0.5 ml of triethylamine to 1000 ml of the solution,
- flow rate. 2 ml per minute,
- spectrophotometer set at 230 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless in the chromatogram obtained the resolution between the peaks due to carbamazepine and 10,11-dihydrocarbamazepine is at least 1.7.

Inject the test solution and continue the chromatography for 6 times the retention time of carbamazepine which is about 10 minutes.

In the chromatogram obtained with the test solution, the areas of any peaks corresponding to 10,11-dihydrocarbamazepine and iminodibenzyl are not greater than the areas of the corresponding peaks in the chromatogram obtained with the reference solution (0.1 per cent). The area of any other secondary peak is not greater than the area of the peak due to carbamazepine (0.1 per cent) and the sum of the areas of any such peaks is not greater than 5 times the area of the peak due to carbamazepine (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the peak due to carbamazepine in the chromatogram obtained with the reference solution (0.05 per cent).

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14) as given under the test for Related substances using the following solutions.

*Test solution.* Shake a quantity of the powdered tablets containing about 0.3 g of Carbamazepine with 100.0 ml of methanol for 15 minutes. Dilute to 200.0 ml with water, mix, filter and further dilute 1 volume of the filtrate to 5 volumes with methanol (50 per cent).

*Reference solution.* A 0.03 per cent w/v solution of carbamazepine RS in methanol (50 per cent).

Inject alternately the test solution and the reference solution. Calculate the content of C\(_{15}\)H\(_{12}\)N\(_2\)O in the tablets.

**Storage.** Store protected from moisture.

---

**Carbenicillin Sodium**

**Carbenicillin Disodium**

\[
\text{C}_{17}\text{H}_{16}\text{N}_{2}\text{Na}_{2}\text{O}_{6}\text{S} \quad \text{Mol. Wt. 422.4}
\]

Carbenicillin Sodium is the disodium (6\text{R})-6-[[2\text{RS})-2-carboxylato-2-phenylacetamido]penicillinate.

Carbenicillin Sodium contains the equivalent of not less than 770 µg of carbenicillin per mg, calculated on the anhydrous basis.

**Description.** A white or slightly yellowish powder; odourless; hygroscopic.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carbenicillin sodium RS* or with the reference spectrum of carbenicillin.

B. Heat 0.5 g in a small sealed container on a water-bath for 3 minutes, remove the seal, and immediately replace by a cork fitted with a platinum loop carrying a drop of a solution freshly prepared by mixing 1 ml of a 0.5 per cent w/v solution of *sodium carbonate*, 1 ml of *phenolphthalein solution* and 10 ml of water; the reagent is decolourised within 2 minutes.

C. A 5 per cent w/v solution gives the reactions of sodium salts (2.3.1).
Tests

pH (2.4.24). 6.5 to 8.0, determined in a 10.0 per cent w/v solution.

Specific optical rotation (2.4.22). +182° to +196°, determined at 20° in a 1.0 per cent w/v solution.

Iodine-absorbing substances. Not more than 8.0 per cent, calculated on the anhydrous basis, determined by the following method. Weigh accurately about 0.13 g and dissolve in sufficient mixed phosphate buffer pH 7.0 to produce 25.0 ml. To 10.0 ml add 10 ml of mixed phosphate buffer pH 4.0 and 10.0 ml of 0.01 M iodine and titrate immediately with 0.01 M sodium thiosulphate using starch solution, added towards the end of the titration, as indicator. Repeat the operation without the substance under examination. The difference between the titration represents the amount of iodine-absorbing substances present.

1 ml of 0.01M sodium thiosulphate is equivalent to 0.000489 g of iodine-absorbing substances.

Bacterial endotoxins (2.2.3). Not more than 0.05 Endotoxin Unit per mg of carbenicillin.

Water (2.3.43). Not more than 6.0 per cent, determined on 0.15 g.

Assay. Determine by the microbiological assay of antibiotics, Method A (2.2.10) and express the result in µg of carbenicillin per mg.

Storage. Store in sterile containers, sealed so as to exclude micro-organisms, in a refrigerator (2° to 8°).

Carbenicillin Sodium Injection

Carbenicillin Injection; Carbenicillin Disodium Injection

Carbenicillin Sodium Injection is a sterile material consisting of Carbenicillin Sodium, with or without auxiliary substances. It is filled in sealed containers. The injection is constituted by dissolving the contents of a sealed container in the requisite amount of Water for Injections immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Carbenicillin Sodium Injection contains the equivalent of not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of carbenicillin, C₁₇H₁₈N₂O₆S.

Description. A white or almost white powder; odourless; hygroscopic.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with carbenicillin sodium RS or with the reference spectrum of carbenicillin.

B. Heat 0.5 g in a small sealed container on a water-bath for 3 minutes, remove the seal, and immediately replace by a cork fitted with a platinum loop carrying a drop of a solution freshly prepared by mixing 1 ml of a 0.5 per cent w/v solution of sodium carbonate, 1 ml of phenolphthalein solution and 10 ml of water; the reagent is decolourised within 2 minutes.

C. A 5 per cent w/v solution gives the reactions of sodium salts (2.3.1).

Tests

pH (2.4.24). 6.5 to 8.0, determined in a 10.0 per cent w/v solution.

Specific optical rotation (2.4.22). +182° to +196°, determined at 20° in a 1.0 per cent w/v solution.

Iodine-absorbing substances. Not more than 8.0 per cent, calculated on the anhydrous basis, determined by the following method. Weigh accurately about 0.13 g and dissolve in sufficient mixed phosphate buffer pH 7.0 to produce 25.0 ml. To 10.0 ml add 10 ml of mixed phosphate buffer pH 4.0 and 10.0 ml of 0.01 M iodine and titrate immediately with 0.01 M sodium thiosulphate using starch solution, added towards the end of the titration, as indicator. Repeat the operation without the substance under examination. The difference between the titration represents the amount of iodine-absorbing substances present.

1 ml of 0.01M sodium thiosulphate is equivalent to 0.000489 g of iodine-absorbing substances.

Bacterial endotoxins (2.2.3). Not more than 0.05 Endotoxin Unit per mg of carbenicillin.

Water (2.3.43). Not more than 6.0 per cent, determined on 0.15 g.

Assay. Determine the weight of the contents of 10 containers and determine by the microbiological assay of antibiotics, Method A (2.2.10) using the mixed contents of the 10 containers.

Labelling. The label states the quantity of Carbenicillin Sodium contained in the sealed container in terms of the equivalent amount of carbenicillin.
Carbenoxolone Sodium

\[
\text{C}_{34}\text{H}_{48}\text{Na}_{2}\text{O}_{7} \quad \text{Mol. Wt. 614.7}
\]

Carbenoxolone Sodium is disodium 3β-(3-carboxylatopropionyloxy)-11-oxo-olean-12-en-30-oate.

Carbenoxolone Sodium contains not less than 97.0 per cent and not more than 103.0 per cent of \( \text{C}_{34}\text{H}_{48}\text{Na}_{2}\text{O}_{7} \), calculated on the anhydrous basis.

**Description.** A white or pale cream powder; hygroscopic; irritant to nasal membranes.

**Identification**

A. Dissolve 0.1 g in 5 ml of water, just acidify with 2 M hydrochloric acid, stir well and filter. Wash the residue with water until the washings are no longer acidic and dry to constant weight at 105°. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with carbenoxolone sodium RS treated in the same manner or with the reference spectrum of carbenoxolone.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0025 per cent w/v solution in a mixture of equal volumes of methanol and 0.02 M sodium carbonate shows an absorption maximum only at about 256 nm; absorbance at about 256 nm, about 0.5.

C. Mix 5 mg with 50 mg of resorcinol and 2 ml of sulphuric acid (80 per cent). Heat at 200° for 10 minutes, cool, pour into 200 ml of water and add sufficient 5 M sodium hydroxide to make the mixture just alkaline; an intense green fluorescence is produced.

D. A 5 per cent w/v solution gives the reactions of sodium salts (2.3.1).

**Tests**

**pH** (2.4.24). 8.0 to 9.2, determined in a 10.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +132° to +140°, determined in a 1.0 per cent w/v solution in a mixture of equal volumes of methanol and 0.02 M sodium carbonate.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel F254 (such as precoated Merck silica gel 60 F254 plates).

**Mobile phase.** A mixture of 60 volumes of ethyl acetate, 20 volumes of methanol, 11 volumes of water and 1 volume of strong ammonia solution.

**Test solution.** A 1.5 per cent w/v solution of the substance under examination in methanol.

**Reference solution.** A 0.03 per cent w/v solution of the substance under examination in methanol.

**Water** (2.3.43). Not more than 4.0 per cent, determined on 0.6 g.

**Assay.** Weigh accurately about 1.0 g and dissolve in 30 ml of water. Add 30 ml of chloroform and 15 ml of a mixture of 10 volumes of 2 M hydrochloric acid and 90 volumes of water, shake and allow to separate. Add the chloroform layer to 40 ml of a 20 per cent w/v solution of sodium chloride, shake and allow to separate. Repeat the extraction with four quantities, each of 15 ml, of chloroform, combine the chloroform extracts and add sufficient chloroform to produce 100.0 ml. Evaporate 25.0 ml, dry the residue at 100° at a pressure of 2 kPa and dissolve in 10 ml of dimethylformamide. Titrate with 0.1 M tetrabutylammonium hydroxide using thymol blue solution as indicator. Carry out a blank titration.

1 ml of 0.1 M tetrabutylammonium hydroxide is equivalent to 0.03073 g of \( \text{C}_{34}\text{H}_{48}\text{Na}_{2}\text{O}_{7} \).

**Carbenoxolone Tablets**

Carbenoxolone Sodium Tablets

Carbenoxolone Sodium Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of carbenoxolone sodium, \( \text{C}_{34}\text{H}_{48}\text{Na}_{2}\text{O}_{7} \).

**Identification**

A. Shake a quantity of the powdered tablets containing 0.2 g of Carbenoxolone Sodium with 10 ml of methanol, filter and
evaporate to dryness. The residue complies with the following tests.

1. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0025 per cent w/v solution in a mixture of equal volumes of methanol and 0.02 M sodium carbonate shows an absorption maximum only at about 256 nm; absorbance at about 256 nm, about 0.5.

2. Mix 5 mg with 50 mg of resorcinol and 2 ml of sulphuric acid (80 per cent). Heat at 200° for 10 minutes, cool, pour into 200 ml of water and add sufficient 5 M sodium hydroxide to make the mixture just alkaline; an intense green fluorescence is produced.

B. A 5 per cent w/v solution of the residue obtained in test A gives the reactions of sodium salts (2.3.1).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel F254 (such as precoated Merck silica gel 60 F254 plates).

Mobile phase. A mixture of 60 volumes of ethyl acetate, 20 volumes of methanol, 11 volumes of water and 1 volume of strong ammonia solution.

Test solution. Triturate a quantity of the powdered tablets containing 0.1 g of Carbenoxolone Sodium with 20 ml of methanol, filter, evaporate the filtrate to low volume and add sufficient methanol to produce 10 ml.

Reference solution. Dilute 3 volumes of the test solution to 100 volumes with methanol.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Spray with a 1.5 per cent w/v solution of vanillin in sulphuric acid (60 per cent) and heat at 105° for 10 to 15 minutes. By both methods of visualisation, any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Other tests. Complies with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Triturate a quantity of the powdered tablets containing about 75 mg of Carbenoxolone Sodium with a small volume of methanol, filter and add sufficient methanol to produce 250.0 ml. To 10.0 ml add 10 ml of 0.02 M sodium carbonate and sufficient of a mixture of equal volumes of methanol and 0.02 M sodium carbonate to produce 100.0 ml and measure the absorbance of the resulting solution at the maximum at about 256 nm (2.4.7). Calculate the content of C₃₄H₄₈Na₂O₇ taking 199 as the specific absorbance at the maximum at about 256 nm.

Carbidopa

\[
\text{C}_{10}\text{H}_{14}\text{N}_{2}\text{O}_{4},\text{H}_2\text{O} \quad \text{Mol. Wt. 244.3}
\]

Carbidopa is (S)-3-(3,4-dihydroxyphenyl)-2-hydrazino-2-methylpropionic acid monohydrate.

Carbidopa contains not less than 98.5 per cent and not more than 101.0 per cent of C₁₀H₁₄N₂O₄, calculated with on the dried basis.

Description. A white to creamy white powder; odourless or practically odourless.

Identification

Tests A and C may be omitted if tests B, D and E are carried out. Tests B, D and E may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with carbidopa RS or with the reference spectrum of carbidopa.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.004 per cent w/v solution in a 1 per cent v/v solution of hydrochloric acid in methanol shows an absorption maximum only at about 282 nm; absorbance at about 282 nm, about 0.52.

C. Complies with the test for Specific optical rotation.

D. Shake vigorously about 5 mg with 10 ml of water for 1 minute and add 0.3 ml of ferric chloride solution; an intense green colour is produced, which quickly becomes reddish brown.

E. Suspend 20 mg in 5 ml of water and add 5 ml of cupric tartaric solution and heat; the colour of the solution changes to dark brown and a red precipitate is produced.

Tests

Appearance of solution. Dissolve 0.25 g in 25 ml of 1 M hydrochloric acid. The solution is clear (2.4.1) and not more intensely coloured than reference solution BYS6 or BS6 (2.4.1).

Specific optical rotation (2.4.22). -22.5° to -26.5°, determined in a solution prepared by dissolving 0.25 g in 25 ml of aluminium chloride solution.

Hydrazine. Determine by thin-layer chromatography (2.4.17), coating the plate with silanised silica gel G.

Mobile phase. A mixture of 2 volumes of methanol and 1 volume of water.
**Test solution.** Place 25 g of *strongly basic anion exchange resin* into each of two stoppered conical flasks, add 150 ml of *carbon dioxide-free water* to each flask and allow to stand for 30 minutes shaking occasionally. Decant the liquid from both flasks and repeat the process with further quantities, each of 150 ml, of *carbon dioxide-free water*. Separately transfer the resin portions into two 100-ml measuring cylinders, 3.5 to 4.5 cm in internal diameter, using 60 ml of *carbon dioxide-free water* for one portion (A) and 20 ml of *carbon dioxide-free water* for the other portion (B). Into each cylinder, insert a gas-inlet tube, 2 to 3 mm in internal diameter at the end and reaching almost to the bottom of the cylinder, and pass a rapid current of *nitrogen for chromatography* through each mixture so that homogeneous suspensions are produced.

After 30 minutes, without interrupting the gas flow, add 1 ml of a solution prepared by dissolving 0.5 g of the substance under examination in sufficient $0.1 \text{ M hydrochloric acid}$ to produce 2 ml to cylinder A. After 1 minute stop the gas flow to cylinder A and transfer the contents, through a moistened filter paper, into cylinder B. After 1 minute, stop the gas flow to cylinder B and immediately pour the solution through a moistened filter paper into a freshly prepared mixture of 1 ml of a 20 per cent w/v solution of *sodium metabisulphite* and then with two quantities, each of 50 ml, of *water* and use the toluene layer.

**Reference solution.** Prepare at the same time and in the same manner but using 1 ml of a 0.002 per cent w/v solution of *hydrazine sulphate* in $2 \text{ M hydrochloric acid}$ in place of 1 ml of the solution of the substance under examination.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with the test solution showing a yellow fluorescence is not more intense than the corresponding spot in the chromatogram obtained with the reference solution.

**Methyldopa and 3-O-methylcarbidopa.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 0.1 g in sufficient $0.1 \text{ M hydrochloric acid}$ to produce 10 ml.

**Reference solution (a).** Dissolve 5 mg of *methyldopa RS* and 5 mg of *3-O-methylcarbidopa RS* in sufficient $0.1 \text{ M hydrochloric acid}$ to produce 100 ml.

**Reference solution (b).** Dissolve 5 mg of *carbidopa RS* and 5 mg of *methyldopa RS* in sufficient $0.1 \text{ M hydrochloric acid}$ to produce 10 ml.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilyl silica gel (5 µm),
- mobile phase: a mixture of 98 volumes of a 1.4 per cent w/v solution of *potassium dihydrogen phosphate* and 2 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 282 nm,
- a 20 µl loop injector.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to methyldopa and carbidopa is greater than 4.0.

Inject the test solution and reference solution (a) in the chromatogram obtained with the test solution, the areas of any peaks corresponding to methylodopa and 3-O-methylcarbidopa are not greater than the areas of the corresponding peaks in the chromatogram obtained with reference solution (a).

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). 6.9 to 7.9 per cent, determined on 1.0 g by drying in an oven at $105^\circ$.

**Assay.** Weigh accurately about 0.15 g and dissolve in 75 ml of *anhydrous glacial acetic acid* with the aid of gentle heat. Titrate with $0.1 \text{ M perchloric acid}$, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of $0.1 \text{ M perchloric acid}$ is equivalent to 0.02262 g of C$_{10}$H$_{14}$N$_2$O$_4$.

**Storage.** Store protected from light.

**Carbimazole**

![Carbimazole](image)

C$_{7}$H$_{10}$N$_2$O$_2$S  
Mol. Wt. 186.2

Carbimazole is ethyl 3-methyl-2-thioxo-4-imidazoline-1-carboxylate.

Carbimazole contains not less than 98.0 per cent and not more than 102.0 per cent of C$_{7}$H$_{10}$N$_2$O$_2$S, calculated on the dried basis.
Description. A white or creamy-white, crystalline powder; odour, characteristic.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with carbimazole RS or with the reference spectrum of carbimazole.

B. In the test for Thiamizole and other related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. To a solution prepared by dissolving about 10 mg in a mixture of 50 ml of water and 0.05 ml of dilute hydrochloric acid, add 1 ml of potassium iodobismuthate solution; a red precipitate is produced.

Tests

Thiamazole and other related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 80 volumes of dichloromethane and 20 volumes of acetone.

Test solution (a). 1 per cent w/v solution of the substance under examination in dichloromethane.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with dichloromethane.

Reference solution (a). Dilute 1 ml of test solution (b) to 20 ml with dichloromethane.

Reference solution (b). A 0.1 per cent w/v solution of carbimazole RS in dichloromethane.

Reference solution (c). Dissolve 5 mg of thiamazole in sufficient dichloromethane to produce 100 ml.

Apply to the plate 10 µl of each solution. After development, allow the plate to dry in air for 30 minutes and examine in ultraviolet light at 254 nm. In the chromatogram obtained with test solution (a), any spot corresponding to thiamazole is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.5 per cent); any spot other than the principal spot and the spot corresponding to thiamazole is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over phosphorus pentoxide at a pressure not exceeding 0.7 kPa for 24 hours.

Assay. Weigh accurately about 50 mg and dissolve in sufficient water to produce 500.0 ml. To 10.0 ml of the solution add 10 ml of 1 M hydrochloric acid and sufficient water to produce 100.0 ml and measure the absorbance of the resulting solution at the maximum at about 291 nm (2.4.7). Calculate the content of C7H10N2O2S taking 557 as the specific absorbance at 291 nm.

Storage. Store protected from light.

Carbimazole Tablets

Carbimazole Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of carbimazole, C7H10N2O2S. The tablets may be coated.

Identification

A. Shake a quantity of the powdered tablets containing 50 mg of Carbimazole with two quantities, each of 5 ml of chloroform. Combine the chloroform extracts, filter and evaporate the filtrate to dryness. Dry the residue at 60° at a pressure not exceeding 0.7 kPa for 30 minutes. The residue complies with the following test.

B. To a small quantity of the powdered tablets add 1 drop of dilute potassium iodobismuthate solution; a scarlet colour is produced.

Tests

Thiamazole and other related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 80 volumes of dichloromethane and 20 volumes of acetone.

Test solution (a). Shake a quantity of the powdered tablets containing 10 mg of Carbimazole with 2 ml of chloroform for 5 minutes and filter.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with dichloromethane.

Reference solution (a). Dilute 1 ml of test solution (b) to 20 ml with dichloromethane.

Reference solution (b). A 0.1 per cent w/v solution of carbimazole RS in dichloromethane.

Reference solution (c). Dissolve 5 mg of thiamazole in sufficient dichloromethane to produce 100 ml.

Apply to the plate 10 µl of each solution. After development, allow the plate to dry in air for 30 minutes and examine in ultraviolet light at 254 nm. In the chromatogram obtained with...
test solution (a), any spot corresponding to thiamazole is not more intense than the spot in the chromatogram obtained with reference solution (b) (1 per cent); any spot other than the principal spot and the spot corresponding to thiamazole is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

**Uniformity of content.** Complies with the test stated under Tablets.

Test solution. Powder one tablet, add 300 ml of water warmed to a temperature not exceeding 35°, shake for a few minutes and add sufficient water to produce 500.0 ml. Mix well, filter, dilute further, if necessary with water. Complete the Assay beginning at the words “Measure the absorbance....”.

**Other tests.** Complies with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 40 mg of Carbimazole, add 300 ml of water warmed to a temperature not exceeding 35°, shake for a few minutes and add sufficient water to produce 500.0 ml. Mix well and filter. Dilute 5.0 ml of the filtrate to 50.0 ml with water and mix well. Measure the absorbance of the resulting solution at the maximum at about 291 nm (2.4.7). Calculate the content of C$_7$H$_{10}$N$_2$O$_2$S taking 557 as the specific absorbance at the maximum at about 291 nm.

**Storage.** Store protected from light and moisture at a temperature not exceeding 30°.

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**Carbomers**

Carbomers are high molecular mass polymers of acrylic acid cross-linked with polyalkenyl ethers of sugars or polyalcohols.

Carbomers contains not less than 56.0 per cent and not more than 68.0 per cent of carboxylic acid (-COOH) groups, calculated on the dried basis.

**Description.** A white, fluffy powder, hygroscopic.

**Identification**

**Test A may be omitted if tests B, C, D and E are carried out.**

**Tests B, C, D may be omitted if tests A and E are carried out.**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with carbomers RS.

B. Adjust a 1 per cent w/v dispersion to about pH 7.5 with 1 M sodium hydroxide. A highly viscous gel is formed.

C. Add 2 ml of a 10 per cent w/v solution of calcium chloride with continuous stirring to 10 ml of the gel obtained in test B. A white precipitate is immediately produced.

D. Add 0.5 ml of thymol blue solution to 10 ml of a 1 per cent w/v dispersion. An orange colour is produced. Add 0.5 ml of cresol red solution to 10 ml of a 1 per cent w/v dispersion. A yellow colour is produced.

E. It complies with the test for viscosity (2.4.28).

**Tests**

**Apparent viscosity.** The nominal apparent viscosity is in the range 300 mPa s to 115 000 mPa s. For a product with a nominal apparent viscosity of 20 000 mPa s or greater, the apparent viscosity is 70.0 per cent to 130.0 per cent of the value stated on the label; for a product with a nominal apparent viscosity less than 20 000 mPa s, the apparent viscosity is 50.0 per cent to 150.0 per cent of the value stated on the label.

Dry the substance under examination in vacuum at 80° for 1 hour. Carefully add 2.5 g of the previously dried substance under examination to 500 ml of water in a 1000 ml beaker while stirring continuously at 1000 ± 50 rpm, with the stirrer shaft set at an angle of 60° to one side of the beaker. Add the previously dried substance over a period of 45 to 90 seconds, at a uniform rate, ensuring that loose aggregates of powder are broken up and continue stirring at 1000 ± 50 rpm for 15 minutes. Remove the stirrer, and place the beaker containing the dispersion in a water-bath at 25 ± 0.2° for 30 minutes. Insert the stirrer to a depth necessary to ensure that air is not drawn into the dispersion, and while stirring at 300 ± 25 rpm, titrate with a glass-calomel electrode system to pH 7.3 to 7.8 by adding a 18 per cent w/v solution of sodium hydroxide below the surface, determining the end-point potentiometrically (2.4.25). The total volume of the 18 per cent w/v solution of sodium hydroxide used is about 6.2 ml. Allow 2-3 minutes before the final pH determination. If the final pH exceeds 7.8, discard the preparation, and prepare another using a smaller amount of sodium hydroxide for titration. Return the neutralised preparation to the water-bath at 25° for 1 hour, then perform the viscosity determination without delay to avoid slight viscosity changes that occur 75 minutes after neutralisation. Determine the viscosity (2.4.28) with a rotating viscometer with a spindle rotating at 20 rpm, using a spindle suitable for the expected apparent viscosity.

**Free acrylic acid.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 0.125 g of the substance under examination in 25 ml of a 2.5 per cent w/v solution of aluminium potassium sulphate. Heat the suspension at 50° for 20 minutes with shaking. Then shake the suspension at room temperature for 60 minutes. Centrifuge and use the clear supernatant solution.

**Reference solution.** Dissolve 62.5 mg of acrylic acid RS in 100 ml of a 2.5 per cent w/v solution of aluminium potassium sulphate. Dilute 1.0 ml of this solution to 50.0 ml with 2.5 per cent w/v solution of aluminium potassium sulphate.

240
Chromatographic system
- a stainless steel column 12 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. dissolve 0.136 g in 100 ml of potassium dihydrogen phosphate, adjust to pH 2.5 using dilute phosphoric acid,
  B. equal volumes of a solution of 0.136 g of potassium dihydrogen phosphate in 100 ml of water and acetonitrile,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 205 nm,
- a 20 µl loop injector.

<table>
<thead>
<tr>
<th>Time (in min.)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 8</td>
<td>100 → 0</td>
<td>0</td>
<td>isocratic</td>
</tr>
<tr>
<td>9 – 20</td>
<td>0 → 100</td>
<td>100</td>
<td>linear gradient</td>
</tr>
<tr>
<td>21– 30</td>
<td>100 → 0</td>
<td>0</td>
<td>isocratic</td>
</tr>
</tbody>
</table>

Inject alternately the test solution and the reference solution. The retention time for acrylic acid is about 6.0 minutes. The area of the peak in the chromatogram obtained with the test solution is not more than half the mean area of the peak corresponding to benzene in the chromatograms obtained with the reference solution (0.25 per cent).

Benzene. Determine by gas chromatography (2.4.13).

Diluent. Dissolve 0.1 g of benzene in 100 ml of dimethyl sulfoxide. Further dilute 1.0 ml of the solution to 100.0 ml with water. Further dilute 1.0 ml of this solution to 100.0 ml with water.

Test solution. Weigh 50.0 mg of the substance under examination, add 5.0 ml of water and 1.0 ml of dimethyl sulfoxide.

Reference solution. Weigh 50.0 mg of the substance under examination, add 4.0 ml of water, 1.0 ml of dimethyl sulfoxide and 1.0 ml of the diluent.

Close the vials with a tight rubber membrane stopper coated with polytetrafluoroethylene and secure with an aluminium crimped cap. Shake to obtain a homogeneous dispersion.

Chromatographic system
- a glass column 1.5 m x 4 mm, packed with porous polymer beads (80 to 100 mesh),
- temperature : column 130°,
- inlet port and detector at 240°,
- flow rate. 30 ml per minute of the carrier gas.

Stratic head-space conditions which may be used:
- equilibration time 60 minutes,
- transfer line temperature 90°.

Inject 1 ml of the gaseous phase of the test solution and 1 ml of the gaseous phase of the reference solution; repeat these injections twice more. Maximum relative standard deviation of the differences in area between the analyte peaks obtained from the 3 replicate pair injections of the reference solution and the test solution is 15 per cent. The test is not valid unless the relative standard deviation for replicate injections is not more than 15 per cent.

The mean area of the peak corresponding to benzene in the chromatograms obtained with the test solution is not more than half the mean area of the peak corresponding to benzene in the chromatograms obtained with the reference solution (2 ppm).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method C (20 ppm). Use 2 ml of lead standard solution (10 ppm Pb).

Sulphated ash (2.3.18). Not more than 4.0 per cent, determined on 1.0 g.

Loss on drying (2.4.19). Not more than 3.0 per cent, determined on 1.0 g by drying in vacuum at 80° for 60 minutes.

Assay. Weigh accurately about 0.12 g, add 50 ml of water slowly with stirring and heating at 60° for 15 minutes. Stop heating, add 150 ml of water and continue stirring for 30 minutes. Add 2 g of potassium chloride and titrate with 0.2 M sodium hydroxide determining the end-point potentiometrically (2.4.25).

1 ml of 0.2 M sodium hydroxide is equivalent to 0.009 g of carboxylic acid (-COOH) groups.

Storage. Store protected from moisture.

Labelling. The label states the nominal apparent viscosity.

Carboprost Tromethamine

\[ C_{21}H_{36}O_5C_4H_11NO_3 \]  Mol. Wt. 489.65

Carboprost Tromethamine contains not less than 95.0 per cent and not more than 105.0 per cent of C_{21}H_{36}O_{5}, C_{6}H_{11}NO_{3}, calculated on the dried basis.

_Great care should be taken to prevent inhaling particles of Carboprost Tromethamine and exposing the skin to it._

**Description.** A white powder.

**Identification**

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with _carboprost tromethamine RS_. Examine the substances as mulls.

**Tests**

**Specific optical rotation.** (2.4.22) + 18.0° to + 24.0°, determined in a 1.0 per cent w/v solution in ethanol (95 per cent).

**15R-Epimer and 5-trans isomer.** Determine by liquid chromatography (2.4.14).

Follow the method described under Assay but using 25 µl loop injector. The usual order of elution is guaiphenesin, the 2-naphthacyl ester of 15R-epimer, the 2-naphthacyl ester of carboprost and the 2-naphthacyl ester of the 5-trans isomer with retention times of about 7, 8, 11 and 13 minutes respectively. Measure the peak areas for the four components and calculate the contents of the 15R-epimer and 5-trans isomer. The percentages of 15R-epimer (as tromethamine salt) and 5-trans isomer are not more than 2.0 per cent and 4.0 per cent respectively.

**Loss on drying.** Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 50° for 16 hours at a pressure not exceeding 0.7 kPa.

**Assay.** Determine by liquid chromatography (2.4.14).

_**Test Solution.** Weigh accurately about 5 mg of the substance under examination, transfer to a stoppered 50-ml centrifuge tube. Add 20.0 ml of _dichloromethane_ and 2 ml of citrate buffer prepared by dissolving 10.5 g of _citric acid monohydrate_ in about 75 ml of _water_, adjusting the pH of the solution to 4.0 by addition of _sodium hydroxide solution_ slowly and diluting to 100 ml with _water_. Shake the stoppered tube for about 10 minutes and centrifuge. Transfer 4.0 ml of the lower dichloromethane layer to a suitable vial and evaporate the solvent with the aid of a stream of nitrogen. To the dried material add 100 µl of a freshly prepared 2 per cent w/v solution of _α-bromo-2'-acetonaphthone_ in _acetonitrile_ and swirl to wash down the sides of the vial. Add 50 ml of a freshly prepared 1 per cent v/v solution of _diisopropylethylamine_ in _acetonitrile_, swirl again and place the vial at a temperature of 30° to 35° for not less than 15 minutes. Evaporate the acetonitrile from the vial with the aid of a stream of nitrogen, add 2.0 ml of a 0.7 per cent w/v solution of guaiphenesin (internal standard) in the mobile phase, mix and filter the resulting solution through a fine porosity filter._

_**Reference solution.** Prepare in the same manner but using _carboprost tromethamine RS_ in place of the substance under examination._

**Chromatographic system**

- stainless steel column 30 cm x 4 mm, packed with porous silica particles (3 to 10 µm),
- mobile phase: a mixture of 7 ml of _1,3-butanediol_, 0.5 ml of _water_ and 992 ml of _dichloromethane_,
- flow rate. 1.5 ml per minute,
- spectrophotometer set 254 nm,
- a 10 µl loop injector.

Inject the reference solution. The retention times for guaiphenesin and the 2-naphthacyl ester of carboprost are about 7 minutes and 11 minutes respectively. The test is not valid unless the resolution between these two peaks is greater than 4.0 and the relative standard deviation for 4 replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of C_{21}H_{36}O_{5}, C_{6}H_{11}NO_{3}.

**Storage.** Store in a refrigerator (2° to 8°).

**Carboprost Tromethamine Injection**

Carboprost Tromethamine Injection is a sterile solution of Carboprost Tromethamine in Water for Injections. It may contain Benzyl alcohol, Sodium Chloride and Tromethamine.

Carboprost Tromethamine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of carboprost, C_{21}H_{36}O_{5}.

**Description.** A colourless solution.

**Identification**

Extract a volume of the injection containing 2.5 mg of Carboprost Tromethamine with 1.5 to 2 times its volume of _chloroform_. Discard the chloroform layer and acidify the aqueous layer with 3 to 5 drops of _hydrochloric acid_. Extract the acidified solution with an equivalent volume of _chloroform_. Filter the chloroform layer through a pledget of cotton and concentrate the filtrate to a volume of less than 1 ml. To the resulting solution add 150 mg to 180 mg of _potassium bromide_ IR and mix well. Dry the potassium bromide mixture in vacuum overnight and prepare a disc from the dried mixture.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with _carboprost tromethamine RS_ treated in the same manner.
Tests

**pH** (2.4.24). 7.0 to 8.0.

**Bacterial endotoxins.** Not more than 714.3 Endotoxin Units per mg of carboprost tromethamine.

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** Determine by liquid chromatography (2.4.14).

Test solution. Transfer a volume of the injection containing 500 µg of carboprost to a stoppered 50-ml centrifuge tube. Add 20.0 ml of dichloromethane and 1.0 ml of citrate buffer prepared by dissolving 10.5 g of citric acid monohydrate in about 75 ml of water, adjusting the pH of the solution to 4.0 by addition of sodium hydroxide solution slowly and diluting to 100.0 ml with water. Shake the stoppered tube for about 10 minutes and centrifuge. Transfer 8.0 ml of the lower dichloromethane layer to a suitable vial and evaporate the solution with the aid of a stream of nitrogen (The residue may not evaporate to dryness because of the presence of benzyl alcohol). Add 100 µl of a freshly prepared 2 per cent w/v solution of α-bromo-2'-acetonaphthone in acetonitrile and swirl to wash down the sides of the vial. Add 50 µl of a freshly prepared 1 per cent v/v solution of disisopropylethylamine in acetonitrile, swirl again and place the vial at a temperature of 30° to 35° for not less than 15 minutes. Evaporate the acetonitrile from the vial with the aid of a stream of nitrogen, add 1.0 ml of a 0.3 per cent w/v solution of guaiphenesin (internal standard) in the mobile phase, mix and filter the resulting solution through a fine porosity filter.

Reference solution. Prepare an aqueous solution containing about 0.332 mg of carboprost tromethamine RS and 9 mg of benzyl alcohol per ml. Transfer 2.0 ml of the resulting solution to a stoppered 50-ml centrifuge tube and proceed as given under the test solution beginning at the words “Add 20.0 ml of dichloromethane....”

Chromatographic system
- a stainless steel column 30 cm x 4 mm, packed with porous silica particles (3 to 10 µm),
- mobile phase: a mixture of 7 ml of 1,3-butandiol, 0.5 ml of water and 992 ml of dichloromethane,
- flow rate. 1.5 ml per minute,
- spectrophotometer set 254 nm,
- a 10 µl loop injector.

Inject the reference solution. The retention times for guaiphenesin and the 2-naphthacyl ester of carboprost are about 7 minutes and 11 minutes respectively. The test is not valid unless the resolution between these two peaks is greater than 4.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the quantity, in µg, of carboprost C₂₁H₃₆O₅ per ml of the injection from the ratios of the peak response of the 2-naphthacyl ester of carboprost and the internal standard obtained with the test solution, the ratios of the peak response of the 2-naphthacyl ester of carboprost and the internal standard obtained with the reference solution and the concentration, in µg per ml, of carboprost in carboprost tromethamine RS in the reference solution.

**Storage.** Store in a refrigerator (2° to 8°).

**Labelling.** The label states the strength in terms of the equivalent amount of carboprost in a suitable dose-volume.

Sodium Carboxymethyl Cellulose

Carmellose Sodium

Sodium Carboxymethylcellulose is the sodium salt of a partially-substituted poly(carboxymethyl) ether of cellulose.

Sodium Carboxymethylcellulose contains not less than 6.5 per cent and not more than 10.8 per cent of sodium, Na, calculated on the dried basis.

**Description.** A white or almost white, granular powder; odourless or almost odourless; hygroscopic.

**Identification.**

A. Sprinkle a quantity containing 1.0 g of the dried substance on to 90 ml of carbon dioxide-free water at 40° to 50°, stir vigorously until a colloidal solution is produced, cool and dilute to 100 ml with carbon dioxide-free water (solution A). To 10 ml of solution A add 1 ml of copper sulphate solution; a blue, cotton-like precipitate is produced.

B. Boil 5 ml of solution A for a few minutes; no precipitate is produced.

C. Solution A gives the reactions of sodium salts (2.3.1).

**Tests**

**Appearance of solution.** Solution A is not more opalescent than opalescence standard OS4 (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

**pH** (2.4.24). 6.0 to 8.0, determined in solution A.

**Apparent viscosity.** 75 to 140 per cent of the declared value, determined by the following method. To 50 ml of water heated to 90° add, with stirring, a quantity containing 2 g of the dried substance under examination or, for a product of low viscosity, use the quantity required to give the concentration on the label. Allow to cool, dilute to 100 ml with water and continue
stirring until solution is complete. Determine the viscosity by Method C (2.4.28), at 20° using a shear rate of 10 s⁻¹. If necessary, use rates slightly below and slightly above 10 s⁻¹ and interpolate.

**Arsenic** (2.3.10). Place 5.0 g in a dry Kjeldahl flask, add 20 ml of *nitric acid*, and warm cautiously until the reaction commences. Allow the reaction to subside without further heating, then add a mixture of 20 ml of *nitric acid* and 5 ml of *sulphuric acid* and heat until brown fumes cease to be evolved. Add 0.5 ml of *perchloric acid* (60 per cent), heat until white fumes appear, and if the liquid is still dark add further small quantities of *nitric acid* and heat until the liquid becomes pale yellow. Heat again until the white fumes appear and continue heating for a further 15 minutes. Add 0.5 ml of *perchloric acid* (60 per cent) and continue heating for a few minutes. Allow the solution to cool add 10 ml of *water*, and heat until white fumes appear. Repeat the heating with a further 5 ml of *water*, cool and add 40 ml of *water* and 10 ml of *stannated hydrochloric acid* AsT. The resulting solution complies with the limit test for arsenic (1 ppm). Prepare the standard using 0.5 ml of *arsenic standard solution* (10 ppm As).

**Heavy metals** (2.3.13). To the residue obtained in the test for Sulphated ash add 1 ml of *hydrochloric acid*, evaporate to dryness on a water-bath and dissolve the residue in 20 ml of *water*. 12 ml of the solution complies with the limit test for heavy metals, Method D (20 ppm). Prepare the standard using lead standard solution (1 ppm Pb).

**Chlorides** (2.3.12). 10 ml of solution A complies with the limit test for chlorides (0.25 per cent).

**Sulphated ash** (2.3.18). 20.0 to 33.3 per cent, calculated on the dried basis, determined on 1.0 g dispersed in a mixture of equal volumes of *sulphuric acid* and *water*.

**Loss on drying** (2.4.19). Not more than 10.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.2 g and disperse in 80 ml of *anhydrous glacial acetic acid*. Heat on a water-bath for 2 hours, cool. Titrate with 0.1 M *perchloric acid* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M *perchloric acid* is equivalent to 0.002299 g of Na.

**Storage.** Store protected from light.

**Labelling.** The label states (1) the apparent viscosity in millipascal seconds of a 2 per cent w/v solution or, where the viscosity is low, the concentration of the solution to be used and the apparent viscosity in mPa s; (2) that the contents are not intended for use in the manufacture of an injectable preparation.

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**Carnauba Wax**

Carnauba Wax is obtained from the leaves of *Copernicia cerifera* Mart. (Fam. Palmae) after purification to remove foreign matter.

**Description.** A pale yellow to light brown coarse powder, flakes or lumps of hard brittle wax; odour, characteristic and free from rancidity.

**Identification**

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

**Mobile phase.** A mixture of 98 volumes of *chloroform* and 2 volumes of *ethyl acetate*.

**Test solution.** Dissolve 0.1 g of the substance under examination, with warming, in 5 ml of *chloroform* and use the warm solution.

**Reference solution.** Dissolve 5 mg of (+)-menthol, 5 µl of *menthyl acetate* and 5 mg of *thymol* in 10 ml of *toluene*.

Apply separately to the plate, as bands 20 mm x 3 mm, 30 µl of test solution and 10 µl of reference solution.

After development, dry the plate in air and spray with a freshly prepared 20 per cent w/v solution of *phosphomolybdic acid* in ethanol (95 per cent) and heat at 105° for 15 minutes. The chromatogram obtained with the reference solution shows in the lower part a dark blue band due to menthol, a reddish band above it due to thymol and a dark blue band in the upper part due to menthyl acetate. The chromatogram obtained with the test solution shows a large blue band due to triacontanol (melissyl alcohol) at an Rf value between those of the bands due to menthol and thymol in the chromatogram obtained with the reference solution and blue bands at Rf values between those of the bands due to menthyl acetate and thymol in the chromatogram obtained with the reference solution. In addition, the chromatogram obtained with the test solution shows further bands at higher Rf values than menthyl acetate, that with the highest Rf value being very pronounced, and a number of faint bands below that due to triacontanol; a band on the line of application is blue.

**Tests**

**Melting range** (2.4.21). 78° to 88°, determined by Method II.

**Heavy metals** (2.3.13). 0.5 g complies with the limit test for heavy metals, Method B (40 ppm).

**Acid value.** Not more than 12.0, determined by the following method. Weigh accurately about 2.0 g (w) in a flask fitted with a reflux condenser, add 40 ml of *xylene* and heat until the substance has dissolved. Add 20 ml of *ethanol* (95 per cent) and titrate the hot solution with 0.5 M *ethanolic potassium perchlorate*.
hydroxide, using phenolphthalein solution as indicator, until a pink colour persists for at least 10 seconds \((n_1, \text{ml})\). Repeat the operation without the substance under examination \((n_2, \text{ml})\). Calculate the acid value from the expression \(28.05\frac{(n_2-n_1)}{w}\).

**Saponification value.** Between 78 and 95, determined by the following method. To the titrated solution from the determination of the Acid value, add 20.0 ml of 0.5 M ethanolic potassium hydroxide and boil under a reflux condenser for 3 hours. Titrate the hot solution immediately with 0.5 M hydrochloric acid, using 1 ml of phenolphthalein solution as indicator, until the red colour is discharged. Reheat the solution to boiling and continue the titration, if necessary, until the red colour no longer reappears on heating \((n_3, \text{ml})\). Repeat the operation without the substance under examination \((n_4, \text{ml})\). Calculate the saponification value from the expression 
\[ a + \frac{28.05(n_4-n_3)}{w} \]
where \(a\) is the acid value.

**Sulphated ash** (2.3.18). Not more than 0.25 per cent, determined on 2.0 g.

**Storage.** Store protected from light and moisture.

---

**Cefaclor**

\[
\text{C}_{15}\text{H}_{14}\text{ClN}_{3}\text{O}_{4}\text{S},\text{H}_{2}\text{O} \quad \text{Mol. Wt 385.8}
\]

Cefaclor is \((6R,7R)-7-[[((2R)-2-\text{amino-2-phenylacetyl})\text{amino}]-3-\text{chboro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.}

Cefaclor contains not less than 96.0 per cent and not more than 102.0 per cent of \(\text{C}_{15}\text{H}_{14}\text{ClN}_{3}\text{O}_{4}\text{S}\), calculated on the anhydrous basis.

**Description.** A white or slightly yellow powder.

**Identification**

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cefaclor RS or with the reference spectrum of cefaclor.

**Tests**

**pH** (2.4.24). 3.0 to 4.5, determined in a suspension, prepared by dispersing 0.25 g in 10 ml of carbon dioxide-free water.

**Specific optical rotation** (2.4.22). +101° to +111°, determined in 1.0 per cent w/v solution in a 1.0 per cent w/v solution of hydrochloric acid.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Solvent mixture.** A 0.27 per cent w/v solution of sodium dihydrogen phosphate, adjusted to pH 2.5 with phosphoric acid.

**Test solution.** Dissolve 50 mg of the substance under examination in 10 ml of the solvent mixture.

**Reference solution (a)** A solution containing 0.0025 per cent w/v cefaclor RS and 0.005 per cent w/v delta-3-cefaclor RS in the solvent mixture.

**Reference solution (b)** Dilute 1 ml of the test solution to 100 ml with the solvent mixture.

**Chromatographic system**

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase A. a 0.78 per cent w/v solution of sodium dihydrogen phosphate adjusted to pH 4.0 with phosphoric acid,
- B. mix 450 ml of acetonitrile with 550 ml of mobile phase A,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 220 nm,
- a 20 µl loop injector.

Increase the concentration of mobile phase B continuously and linearly by 0.67 per cent v/v per minute for 30 minutes (25 per cent v/v). Then increase the concentration of mobile phase B continuously and linearly by 5 per cent v/v per minute for 15 minutes (100 per cent v/v). Finally elute with mobile phase B for 10 minutes.

Equilibrate the column with a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A for at least 15 minutes between each analysis. Inject the solutions. At the end of the programme change the composition of the mobile phase to a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A to re-equilibrate the column.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to cefaclor and delta-3-cefaclor is not less than 2.0 and the tailing factor of the cefaclor peak is not more than 1.2. If necessary, adjust the acetonitrile content of the mobile phase.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any peak, other than the principal peak and any peaks due to the mobile phase, is not greater than 0.5 times the area of the
principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of the areas of all such peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b).

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Water** (2.3.43). 3.0 to 6.5 per cent, determined on 0.2 g.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 15 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

**Reference solution (a).** A 0.03 per cent w/v solution of cefaclor RS in the mobile phase.

**Reference solution (b).** A solution containing 0.03 per cent w/v each of cefaclor RS and delta-3-cefaclor RS in the mobile phase.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture prepared by adding 220 volumes of methanol to a mixture of 780 volumes of water, 10 volumes of triethylamine and 1 g of sodium pentanesulphonate, adjusted to pH 2.5 with orthophosphoric acid,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 265 nm,
- a 20 µl loop injector.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cefaclor and delta-3-cefaclor is not less than 2.5. Adjust the concentration of methanol in the mobile phase, if necessary. The test is not valid unless the tailing factor of the cefaclor peak is not more than 1.5.

Inject reference solution (a) 6 times. The test is not valid unless the relative standard deviation of the peak area of cefaclor is not more than 1.0 per cent.

Inject alternately the test solution and reference solution (a).

Calculate the content of C_{15}H_{14}ClN_{3}O_{4}S.

**Storage.** Store protected from moisture.

**Cefaclor Capsules**

Cefaclor Capsules contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of cefaclor, C_{15}H_{14}ClN_{3}O_{4}S.

**Identification**

A. Shake a quantity of the contents of the capsules containing 0.3 g of anhydrous cefaclor with 100 ml of water, filter and dilute 1 ml of the filtrate to 100 ml with water.

When examined in the range 190 nm to 310 nm (24.7), the resulting solution shows an absorption maximum only at about 264 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

**Tests**

**Dissolution** (2.5.2).

Apparatus. No 1

Medium. 900 ml of water.

Speed and time. 50 rpm and 45 minute.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 264 nm (2.4.7). Calculate the content of C_{15}H_{14}ClN_{3}O_{4}S in the medium from the absorbance obtained from a solution of known concentration of cefaclor RS in the same medium.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Solvent mixture.** A 0.27 per cent w/v solution of sodium dihydrogen orthophosphate, adjusted pH to 2.5, if necessary, with orthophosphoric acid.

**Test solution.** Shake a quantity of the contents of the capsules containing 0.5 g of anhydrous cefaclor with 200 ml of the solvent mixture, dilute to 250 ml with the solvent mixture and filter.

**Reference solution (a).** A 0.002 per cent w/v solution of cefaclor RS in the solvent mixture.

**Reference solution (b).** A solution containing 0.0025 per cent w/v of cefaclor RS and 0.005 per cent w/v of delta-3-cefaclor RS in the solvent mixture.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm) (such as Spherisorb ODS-2),
- mobile phase: A. a 0.78 per cent w/v solution of sodium dihydrogen orthophosphate adjusted to pH 4.0 with orthophosphoric acid,
- B. a mixture of 450 volumes of acetonitrile and 550 volumes of mobile phase A.
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) | Comments
---|---|---|---
0 – 30 | 95 – 75 | 5 – 25 | linear gradient
30 – 45 | 75 – 0 | 25 – 100 | 
45 – 55 | 0 | 100 | isocratic
55 – 70 | 0 – 95 | 100 – 5 | re-equilibration

Equilibrate the column with a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A for at least 15 minutes.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cefaclor and delta-3-cefaclor is not less than 2.0. If necessary, adjust the proportion of acetonitrile in the mobile phase.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution the area of any secondary peak is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Shake a quantity of the contents of capsules containing about 75 mg of anhydrous cefaclor with 200 ml of the mobile phase, dilute to 250.0 ml with the mobile phase and filter.

**Reference solution (a).** A 0.03 per cent w/v solution of cefaclor RS in the mobile phase.

**Reference solution (b).** A solution containing 0.03 per cent w/v each of cefaclor RS and delta-3-cefaclor RS in the mobile phase.

**Chromatographic system.**
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilylexsilica gel (5 µm) (such as Beckman Ultrasphere ODS and Supelcosil LC-18-DB),
- mobile phase: a solution prepared by dissolving 1 g of sodium pentanesulphonate in a mixture of 780 volumes of water and 10 volumes of triethylamine, adjusting the pH to 2.5 with orthophosphoric acid adding 220 volumes of methanol and mixing,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 265 nm,
- a 20 µl loop injector.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cefaclor and delta-3-cefaclor is not less than 2.5.

Inject alternately the test solution and reference solution (a). Calculate the content of C₁₅H₁₄ClN₃O₄S in the capsules.

**Storage.** Store protected from moisture.

**Labelling.** The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous cefaclor.

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**Cefaclor Oral Suspension**

Cefaclor Oral Suspension is a mixture consisting of Cefaclor with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of Water just before use.

Cefaclor Oral Suspension contains not less than 80.0 per cent and not more than 120.0 per cent of the stated amount of cefaclor, C₁₅H₁₄ClN₃O₄S.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of cefaclor, C₁₅H₁₄ClN₃O₄S.

**Storage.** Store protected from moisture at a temperature not exceeding 30º.

The constituted suspension complies with the tests stated under Oral liquids and with the following tests.

**Identification**

A. Shake a quantity of the oral suspension containing 0.3 g of anhydrous cefaclor with 500 ml of water and filter.

When examined in the range 190 nm to 310 nm (2.4.7), the filtrate shows an absorption maximum only at about 264 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

**Tests**

**Related substances.** Determine by liquid chromatography (2.4.14).

**Solvent mixture.** A 0.27 per cent w/v solution of sodium dihydrogen orthophosphate, adjusted to pH 2.5 with orthophosphoric acid.
**Test solution.** Shake a quantity of the oral suspension containing about 0.25 g of anhydrous cefaclor with 200 ml of the solvent mixture, dilute to 250 ml with the solvent mixture and filter.

**Reference solution (a).** A 0.001 per cent w/v solution of cefaclor RS in the solvent mixture.

**Reference solution (b).** A solution containing 0.0025 per cent w/v of cefaclor RS and 0.005 per cent w/v of delta-3-cefaclor RS in the solvent mixture.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm) (such as Spherisorb ODS-2),
- mobile phase: A. a 0.78 per cent w/v solution of sodium dihydrogen orthophosphate, adjusted to pH 4.0 with orthophosphoric acid,
  B. a mixture of 450 volumes of acetonitrile and 550 volumes of mobile phase A,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 220 nm,
- a 20 µl loop injector.

<table>
<thead>
<tr>
<th>Time (in min.)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 30</td>
<td>95 – 75</td>
<td>5 – 25</td>
<td>linear gradient</td>
</tr>
<tr>
<td>30 – 45</td>
<td>75 – 0</td>
<td>25 – 100</td>
<td>linear gradient</td>
</tr>
<tr>
<td>45 – 55</td>
<td>0</td>
<td>100</td>
<td>isocratic</td>
</tr>
<tr>
<td>55 – 70</td>
<td>0 – 95</td>
<td>100 – 5</td>
<td>re-equilibration</td>
</tr>
</tbody>
</table>

Equilibrate the column with a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A for at least 15 minutes.

Inject reference solution (b). The test is not valid unless resolution between the peaks due to cefaclor and delta-3-cefaclor is not less than 2.5 and the tailing factor of the peak due to cefaclor is not more than 1.5.

Inject alternately the test solution and reference solution (a).

Determine the weight per ml of the oral suspension (2.4.29) and calculate the content of C₁₅H₁₄ClN₃O₄S, weight in volume.

Repeat the procedure using a portion of the constituted suspension that has been stored at the temperature and for the period stated on the label.

**Storage.** Store at the temperature and use within the period stated on the label.

**Labelling.** The label states the quantity in terms of the equivalent amount of anhydrous cefaclor.

**Cefaclor Sustained-release Tablets**

Cefaclor Sustained-release Tablets are prolonged-release tablets containing Cefaclor. The appropriate release of the active ingredient is demonstrated by a suitable dissolution test.

Cefaclor Tablets contains not less than 90.0 per cent and not more than 105.0 per cent of the stated amount of cefaclor, C₁₅H₁₄ClN₃O₄S.
Identification
A. Shake a quantity of the powdered tablets containing 0.3 g of anhydrous cefaclor with 100 ml of water, filter and dilute 1 ml of the filtrate to 100 ml with water.

When examined in the range 190 nm to 310 nm (2.4.7), the resulting solution shows an absorption maximum only at about 264 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests
Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A 0.27 per cent w/v solution of sodium dihydrogen orthophosphate, adjusted pH to 2.5, if necessary, with orthophosphoric acid.

Test solution. Shake a quantity of the powdered tablets containing 0.75 g of anhydrous cefaclor with 200 ml of the solvent mixture, dilute to 250 ml with the solvent mixture and filter.

Reference solution (a). A 0.003 per cent w/v solution of cefaclor RS in the solvent mixture.

Reference solution (b). A solution containing 0.0025 per cent w/v of cefaclor RS and 0.005 per cent w/v of delta-3-cefaclor RS in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm) (such as Spherisorb ODS-2),
- mobile phase: A. a 0.78 per cent w/v solution of sodium dihydrogen orthophosphate adjusted to pH 4.0 with orthophosphoric acid

B. a mixture of 450 volumes of acetonitrile and 550 volumes of mobile phase A.
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 220 nm,
- a 20 µl loop injector.

Time (in min.) Mobile Mobile Mobile Comment
phase A phase B phase A
0 – 30 95 – 75 5 – 25 linear gradient
30 – 45 75 – 0 25 – 100 linear gradient
45 – 55 0 100 isocratic
55 – 70 0 – 95 100 – 5 re-equilibration

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cefaclor and delta-3-cefaclor is not less than 2.5.

Inject the test solution and reference solution (a). In the chromatogram obtained with test solution the area of any secondary peak is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent) and the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 75 mg of anhydrous cefaclor, disperse in the mobile phase, shake, dilute to 250.0 ml with the mobile phase and filter.

Reference solution (a). A 0.03 per cent w/v solution of cefaclor RS in the mobile phase.

Reference solution (b). A solution containing 0.03 per cent w/v each of cefaclor RS and delta-3-cefaclor RS in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm) (such as Beckman Ultrasphere ODS and Supelcosil LC-18-DB),
- mobile phase: a solution prepared by dissolving 1 g of sodium pentanesulphonate in a mixture of 780 volumes of water and 10 volumes of triethylamine, adjusting the pH to 2.5 with orthophosphoric acid, adding 220 volumes of methanol and mixing,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 265 nm,
- a 20 µl loop injector.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cefaclor and delta-3-cefaclor is not less than 2.5.

Inject the test solution and reference solution (a). Calculate the content of C15H14ClN3O4S in the tablets.

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the equivalent amount of anhydrous cefaclor.
Cefadroxil

C\(_{16}\)H\(_{17}\)N\(_3\)O\(_5\)S.H\(_2\)O  Mol. Wt. 381.4
Cefadroxil is 7-[(R)-2-amino-2-(4-hydroxyphenyl)acetamido]-3-methyl-3-cephem-4-carboxylic acid monohydrate.

Cefadroxil contains not less than 95.0 per cent and not more than 101.0 per cent of C\(_{16}\)H\(_{17}\)N\(_3\)O\(_5\)S, calculated on the anhydrous basis.

Description. A white to off-white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cefadroxil RS or with the reference spectrum of cefadroxil.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel H and impregnating the dry plate by placing it in a tank containing a shallow layer of about 1 cm of a mixture of 95 volumes of n-hexane and 5 volumes of 1-tetradecane, allowing the solvent to ascend to the top, removing the plate and allowing the solvent to evaporate.

Mobile phase. A mixture of 60 volumes of 0.1 M citric acid, 40 volumes of 0.1 M disodium hydrogen phosphate and 1.5 volumes of a 6.66 per cent w/v solution of ninhydrin in acetone.

Test solution. A 0.2 per cent w/v solution of the substance under examination in water.

Reference solution (a). A 0.2 per cent w/v solution of cefadroxil RS in water.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 2 µl of each solution. After development, dry the plate in air, spray with a 0.2 per cent w/v solution of ninhydrin in ethanol, dry at 110° for 10 minutes and examine. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single compact spot.

Tests

pH (2.4.24). 4.0 to 6.0, determined in a 5.0 per cent w/v suspension.

Specific optical rotation (2.4.22). +165° to +178°, determined in a 1.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 56 volumes of ethyl acetate, 20 volumes of ethanol (95 per cent), 20 volumes of water and 4 volumes of formic acid.

Solvent mixture. 75 volumes of ethanol (95 per cent), 22 volumes of water and 3 volumes 2.4 M hydrochloric acid.

Test solution. A 2.5 per cent w/v solution of the substance under examination in the solvent mixture.

Reference solution (a). Dilute 1 ml of the test solution to 100 ml with the solvent mixture.

Reference solution (b). A solution containing 0.025 per cent w/v each of 7-aminodesacetoxycephasporanic acid RS and D-a-4-hydroxyphenylglycine RS.

Reference solution (c). A mixture of equal volumes of the test solution and reference solution (b).

Apply to the plate 2 µl of the test solution, reference solution (a) and reference solution (b) and 4 µl of solution (c). Allow the mobile phase to rise 12 cm. Dry the plate in air, and spray with a 3.0 per cent w/v solution of ninhydrin in a 4.55 per cent w/v solution of sodium metabisulphite. Any secondary spot in the chromatogram obtained with the test solution is not more intense than any of the spots in the chromatograms obtained with reference solution (a) and reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (c) exhibits three distinct spots.

N,N-Dimethylaniline (2.3.21). Not more than 20 ppm, determined by Method B.

Water (2.3.43). 4.2 to 6.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. A freshly prepared 0.1 per cent w/v solution of the substance under examination in phosphate buffer pH 5.0.

Reference solution. A freshly prepared 0.1 per cent w/v solution of cefadroxil RS in phosphate buffer pH 5.0.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octadecysilyl silica gel (3 to 10 µm),
- mobile phase: a mixture of 96 volumes of phosphate buffer pH 5.0 and 4 volumes of acetonitrile,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.
Inject alternately the test solution and the reference solution.

Calculate the content of C_{16}H_{17}N_{3}O_{5}S.

Storage. Store protected from moisture at a temperature not exceeding 30°C.

Cefadroxil Capsules

Cefadroxil Capsules contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of anhydrous cefadroxil, C_{16}H_{17}N_{3}O_{5}S.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel H and impregnating the dry plate by placing it in a tank containing a shallow layer of about 1 cm of a mixture of 95 volumes of n-hexane and 5 volumes of 1-tetradecane, allowing the solvent to ascend to the top, removing the plate and allowing the solvent to evaporate.

Mobile phase. A mixture of 60 volumes of 0.1 M citric acid, 40 volumes of 0.1 M disodium hydrogen phosphate and 1.5 volumes of a 6.66 per cent w/v solution of ninhydrin in acetone.

Test solution. Shake a quantity of the contents of a capsule with sufficient water to produce a solution containing 0.2 per cent w/v of Cefadroxil.

Reference solution (a). A 0.2 per cent w/v solution of cefadroxil RS in water.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 20 µl of each solution. After development, dry the plate in air, spray with a 0.2 per cent w/v solution of ninhydrin in ethanol, dry at 110°C for 10 minutes and examine. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single compact spot.

Tests

Water (2.3.43). Not more than 7.0 per cent, determined on 0.5 g of the mixed contents of 20 capsules.

Dissolution (2.5.2).

Apparatus. No 1
Medium. 900 ml of water.
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance (2.4.7) of the filtrate, suitably diluted if necessary, at the maximum at about 263 nm.

Calculate the content of C_{16}H_{17}N_{3}O_{5}S in the medium from the absorbance obtained from a solution of known concentration of cefadroxil RS.

D. Not less than 75 per cent of the stated amount of C_{16}H_{17}N_{3}O_{5}S.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Prepare the following solutions freshly.

Test solution. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 0.2 g of Cefadroxil, add sufficient phosphate buffer pH 5.0, shake for 30 minutes, dilute to 200.0 ml with the same solvent and filter.

Reference solution. A 0.1 per cent w/v solution of cefadroxil RS in phosphate buffer pH 5.0.

Chromatographic system
– a stainless steel column 25 cm x 4 mm, packed with octadecysilyl silica gel (5 µm),
– mobile phase: a mixture of 96 volumes of phosphate buffer pH 5.0 and 4 volumes of acetonitrile,
– flow rate. 1.5 ml per minute,
– spectrophotometer set at 230 nm,
– a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of C_{16}H_{17}N_{3}O_{5}S in the capsules.

Storage. Store protected from moisture at a temperature not exceeding 30°C.

Labelling. The label states the strength in terms of anhydrous cefadroxil.

Cefadroxil Oral Suspension

Cefadroxil Mixture

Cefadroxil Oral Suspension is a mixture of Cefadroxil with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of water just before use.

Cefadroxil Oral Suspension contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of C_{16}H_{17}N_{3}O_{5}S.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be
expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of cefadroxil.

**Identification**

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H* and impregnating the dry plate by placing it in a tank containing a shallow layer of about 1 cm of a mixture of 95 volumes of *n-hexane* and 5 volumes of *l-tetradecane*, allowing the solvent to ascend to the top, removing the plate and allowing the solvent to evaporate.

**Mobile phase**. A mixture of 60 volumes of 0.1 M *citric acid*, 40 volumes of 0.1 M *disodium hydrogen phosphate* and 1.5 volumes of a 6.66 per cent w/v solution of *ninhydrin* in *acetone*.

**Test solution**. Dilute a suitable quantity of the freshly prepared suspension with water to obtain a solution containing 0.2 per cent w/v of cefadroxil. Filter the solution.

**Reference solution (a)**. A 0.2 per cent w/v solution of cefadroxil *RS* in water.

**Reference solution (b)**. A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 20 µl of each solution After development, dry the plate in air, spray with a 0.2 per cent w/v solution of *ninhydrin* in *ethanol*, dry at 110° for 10 minutes and examine. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single compact spot.

**Water** (2.3.43). Not more than 2.0 per cent, determined on 1.0 g, using a mixture of 2 volumes of *carbon tetrachloride*, 2 volumes of *chloroform* and 1 volume of *methanol* in place of *methanol* in the titration vessel.

The constituted suspension complies with the tests stated under Oral liquids and with the following tests.

**Tests**

**pH** (2.4.24). 4.5 to 6.0.

**Assay**. Determine by liquid chromatography (2.4.14).

**Test solution**. Transfer an accurately weighed quantity of the suspension containing about 0.1g of cefadroxil to a 100-ml volumetric flask, add phosphate buffer pH 5.0, shake for 30 minutes, dilute to 100.0 ml with the same solvent and filter.

**Reference solution**. A 0.1 per cent w/v solution of cefadroxil *RS* in phosphate buffer pH 5.0.

**Chromatographic system**

- a stainless steel column 25 cm x 4 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 96 volumes of phosphate buffer pH 5.0 and 4 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Determine the weight per ml (2.4.29) of the suspension and calculate the content of C₁₆H₁₇N₃O₅S, weight in volume. Repeat the procedure using a portion of the suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

**Labelling**. The label states the quantity of active ingredient in terms of anhydrous cefadroxil.

**Cefadroxil Tablets**

Cefadroxil Tablets contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of anhydrous cefadroxil, C₁₆H₁₇N₃O₅S. The tablets may be coated.

**Identification**

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H* and impregnating the dry plate by placing it in a tank containing a shallow layer of about 1 cm of a mixture of 95 volumes of *n-hexane* and 5 volumes of *l-tetradecane*, allowing the solvent to ascend to the top, removing the plate and allowing the solvent to evaporate.

**Mobile phase**. A mixture of 60 volumes of 0.1 M *citric acid*, 40 volumes of 0.1 M *disodium hydrogen phosphate* and 1.5 volumes of a 6.66 per cent w/v solution of *ninhydrin* in *acetone*.

**Test solution**. Shake a quantity of the powdered tablets with sufficient water to produce a solution containing 0.2 per cent w/v of cefadroxil. Filter the solution.

**Reference solution (a)**. A 0.2 per cent w/v solution of cefadroxil *RS* in water.

**Reference solution (b)**. A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 20 µl of each solution After development, dry the plate in air, spray with a 0.2 per cent w/v solution of...
ninhydrin in ethanol, dry at 110° for 10 minutes and examine. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single compact spot.

Tests

Dissolution (2.5.2).

Apparatus. No 1
Medium. 900 ml of water
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted if necessary, at the maximum at about 263 nm (2.4.7). Calculate the content of C16H17N3O5S in the medium from the absorbance obtained from a solution of known concentration of cefadroxil RS.

D. Not less than 75 per cent of the stated amount of C16H17N3O5S.

Other tests. Comply with the tests stated under Tablets.

Water (2.3.43). Not more than 8.0 per cent, determined on 0.5 g of the powdered tablets.

Assay. Determine by liquid chromatography (2.4.14).

Prepare the following solutions freshly.

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.2 g of cefadroxil, dissolve in phosphate buffer pH 5.0 by shaking for 30 minutes and dilute to 200.0 ml the same solvent. Filter the solution.

Reference solution. A 0.1 per cent w/v solution of cefadroxil RS in phosphate buffer pH 5.0.

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecysil silica gel (3 to 10 µm),
- mobile phase: a mixture of 96 volumes of phosphate buffer pH 5.0 and 4 volumes of acetonitrile,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the content of C16H17N3O5S in the tablets.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of anhydrous cefadroxil.
Chromatographic system
- a stainless steel column 30 cm x 4 mm, packed with octadecylsilyl silica gel (3 to 10 µm),
- mobile phase: a mixture of 9 volumes of phosphate buffer pH 3.6 and 1 volume of acetonitrile,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the relative retention times of salicylic acid and cefazolin are 0.7 and 1.0 respectively.

Inject alternately the test solution and the reference solution. Calculate the content of C₁₄H₁₄N₈O₄S₃.

Cefazolin Sodium intended for use in the manufacture of parenteral preparations complies with the following additional requirements.

Bacterial endotoxins (2.2.3). Not more than 0.15 Endotoxin Unit per mg of cefazolin.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store in sterile containers, sealed so as to exclude micro-organisms protected from moisture at a temperature not exceeding 30°.

Labelling. The label states the quantity of Cefazolin Sodium contained in the sealed container in terms of the equivalent amount of cefazolin.

Cefazolin Sodium Injection

Cefazolin Injection; Cephazolin Sodium Injection; Cephazolin Injection

Cefazolin Sodium Injection is a sterile material consisting of Cefazolin Sodium with or without excipients. It is filled in sealed containers.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cefazolin Sodium Injection contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of cefazolin, C₁₄H₁₄N₈O₄S.

Description. A white to off-white, crystalline powder; odourless.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. Determine by infra-red absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cefazoline sodium RS or with the reference spectrum of cefazolin sodium.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 4.0 to 6.0, determined in a 10.0 per cent w/v solution.

Specific optical rotation (2.4.22). -10.0° to -24.0°, determined in a 5.5 per cent w/v solution in 0.1 M sodium bicarbonate.

Bacterial endotoxins (2.2.3). Not more than 0.15 Endotoxin Unit per mg of cefazolin.

Water (2.3.43). Not more than 6.0 per cent, determined on 0.15 g.

Assay. Determine by liquid chromatography (2.4.14).

Solution A. Prepare by dissolving 0.75 g of salicylic acid (internal standard) in 5 ml of methanol and diluting to 100.0 ml with mixed phosphate buffer pH 7.0.

Test solution. Determine the weight of the contents of 10 containers. Weigh accurately a quantity of the mixed contents of the 10 containers, dissolve in the mixed phosphate buffer pH 7.0 and dilute to obtain a solution containing 0.1 per cent w/v of cefazolin. To 5.0 ml of this solution add 5.0 ml of solution A and add sufficient volume of mixed phosphate buffer pH 7.0 to produce 100.0 ml and mix.

Reference solution. A 0.1 per cent w/v solution of cefazolin sodium RS in mixed phosphate buffer pH 7.0. To 5.0 ml of this solution add 5.0 ml of solution A and add sufficient volume of mixed phosphate buffer pH 7.0 to produce 100.0 ml and mix.

Chromatographic system
- a stainless steel column 30 cm x 4 mm, packed with octadecylsilyl silica gel (3 to 10 µm),
- mobile phase: a mixture of 9 volumes of phosphate buffer pH 3.6 and 1 volume of acetonitrile,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the relative retention times of salicylic acid and cefazolin are 0.7 and 1.0 respectively.
Inject alternately the test solution and the reference solution. Calculate the content of C$_{25}$H$_{26}$N$_9$NaO$_8$S$_2$ in the injection.

Storage. Store protected from moisture at a temperature not exceeding 30°. The constituted solution should be stored protected from light and used within 24 hours when stored at a temperature not exceeding 30° or within 4 days when stored between 2° and 8°.

Labelling. The label states the quantity of Cefazolin Sodium contained in the sealed container in terms of the equivalent amount of cefazolin.

**Cefoperazone Sodium**

![Cefoperazone Sodium structure](image)

C$_{25}$H$_{26}$N$_9$NaO$_8$S$_2$  Mol Wt. 667.7

Cefoperazone sodium is sodium salt of 7-D-(-)-α-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido)-α-(4-hydroxyphenyl) acetamido-3-[(1-methyl-1H-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylic acid. Cefoperazone Sodium contains not less than 95.0 per cent and not more than 102.0 per cent of C$_{25}$H$_{26}$N$_9$NaO$_8$S$_2$ calculated on the anhydrous basis.

**Description.** A white or almost white crystalline powder.

**Identification**

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Gives the reactions of sodium salts (2.3.1).

**Tests**

pH (2.4.24). 4.5 to 6.5, determined in a 25.0 per cent w/v solution.

Water (2.3.43). Not more than 5.0 per cent, determined on 0.5 g.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 10 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

**Reference solution.** A 0.01 per cent w/v solution of cefoperazone dihydrate RS in the mobile phase.

**Chromatographic system**

- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 884 volumes of water, 110 volumes of acetonitrile, 3.5 volumes of a 6 per cent w/v solution of acetic acid and 2.5 volumes of a solution prepared by dissolving 14 ml of triethylamine and 5.7 ml of glacial acetic acid in 100 ml of water, and mixed,
- flow rate, 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 10 µl loop injector.

Inject alternately the test solution and the reference solution. Calculate the content of C$_{25}$H$_{26}$N$_9$NaO$_8$S$_2$ by multiplying the content of cefoperazone by 1.034.

Cefoperazone Sodium intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

**Bacterial endotoxins** (2.2.3). Not more than 0.20 Endotoxin Unit per mg of cefoperazone sodium.

Cefoperazone Sodium intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

**Sterility** (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture.

Labelling. The label states whether it is intended for use in the manufacture of parenteral preparations.

**Cefoperazone Injection**

Cefoperazone Sodium Injection

Cefoperazone Injection is a sterile material consisting of Cefoperazone Sodium with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).
Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cefoperazone Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of cefoperazone, C$_{25}$H$_{27}$N$_{9}$O$_{8}$S$_{2}$.

Description. A white or almost white powder.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. It gives the reactions of sodium salts (2.3.1).

Tests

pH (2.4.24). 4.5 to 6.5, determined in a 25.0 per cent w/v solution.

Bacterial endotoxins (2.2.3). Not more than 0.20 Endotoxin Unit per mg of cefoperazone.

Water (2.3.43). Not more than 5.0 per cent, except that where it is in the freeze-dried form, the limit is not more than 2.0 per cent.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Determine the weight of the contents of 10 containers. Weigh accurately a quantity of the mixed contents of 10 containers containing about 25 mg of cefoperazone, dissolve in the mobile phase and dilute to 250.0 ml with the mobile phase.

Reference solution. A 0.01 per cent w/v solution of cefoperazone dihydrate RS in the mobile phase.

Chromatographic system

- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 884 volumes of water, 110 volumes of acetonitrile, 3.5 volumes of a 6 per cent w/v solution of acetic acid and 2.5 volumes of a solution prepared by dissolving 14 ml of triethylamine and 5.7 ml of glacial acetic acid in 100 ml of water, and mixed,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 5000, the symmetry factor is at most 1.6 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the content of C$_{25}$H$_{27}$N$_{9}$O$_{8}$S$_{2}$ in the injection.

Storage. Store protected from light at a temperature not exceeding 30º.

Labelling. The label states the quantity of Cefoperazone Sodium contained in the sealed container in terms of the equivalent amount of cefoperazone.

Cefotaxime Sodium

\[
\text{C}_{16}\text{H}_{16}\text{N}_{5}\text{NaO}_{7}\text{S}_{2} \quad \text{Mol. Wt. 477.4}
\]

Cefotaxime Sodium is sodium (7R)-3-acetoxymethyl-7-[(Z)-2-(2-aminothiazol-yl)-2-(methoxyimino)acetamido]-3-cepham-4-carboxylate.

Cefotaxime Sodium contains the equivalent of not less than 85.5 per cent and not more than 96.4 per cent of cefotaxime, C$_{16}$H$_{17}$N$_{5}$O$_{7}$S$_{2}$, calculated on the anhydrous basis.

Description. An off-white to pale yellow, crystalline powder.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Gives the reactions of sodium salts (2.3.1).

Tests

pH (2.4.24). 4.5 to 6.5, determined in a 10.0 per cent w/v solution.

Water (2.3.43). Not more than 6.0 per cent, determined on 0.15 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. A 0.01 per cent w/v solution of the substance under examination in water.

Reference solution. A 0.01 per cent w/v solution of cefotaxime sodium RS in water.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecysilyl silica gel (3 to 10 µm),
– mobile phase: a solution prepared by dissolving 60 mg of potassium dihydrogen phosphate and 1.2 g of disodium hydrogen phosphate in 1000 ml of water and mixing with 120 ml of methanol,
– flow rate. 1.5 ml per minute,
– spectrophotometer set at 254 nm,
– a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the content of C16H17N5O7S2.

Cefotaxime Sodium intended for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.20 Endotoxin Unit per mg of cefotaxime.

Cefotaxime Sodium intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture in tamper-evident containers.

Labelling. The label states whether or not the contents are intended for use in the manufacture of parenteral preparations.

Cefotaxime Sodium Injection

Cefotaxime Injection

Cefotaxime Sodium Injection is a sterile material consisting of Cefotaxime Sodium with or without excipients. It is filled in sealed containers.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cefotaxime Sodium Injection contains a quantity of Cefotaxime Sodium equivalent to not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of cefotaxime, C16H17N5O7S2.

Description. An off-white to pale yellow, crystalline powder.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Gives the reactions of sodium salts (2.3.1).

Tests

pH (2.4.24). 4.5 to 6.5, determined in a 10.0 per cent w/v solution.

Bacterial endotoxins (2.2.3). Not more than 0.20 Endotoxin Unit per mg of cefotaxime.

Water (2.3.43). Not more than 6.0 per cent, determined on 0.15 g.

Assay

Determine by liquid chromatography (2.4.14).

Test solution. Determine the weight of the contents of 10 containers. Weigh accurately a quantity of the mixed contents of the 10 containers dissolve in water and dilute to obtain a solution containing 0.01 per cent w/v of cefotaxime.

Reference solution. A 0.01 per cent w/v solution of cefotaxime sodium RS in water.

Chromatographic system

– a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilyl silica gel (3 to 10 µm),
– mobile phase: a solution prepared by dissolving 60 mg of potassium dihydrogen phosphate and 1.2 g of disodium hydrogen phosphate in 1000 ml of water and mixing with 120 ml of methanol,
– flow rate. 1.5 ml per minute,
– spectrophotometer set at 254 nm,
– a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the content of C16H17N5O7S2 in the injection.

Storage. Store protected from light at a temperature not exceeding 30°.
Ceftazidime

Ceftazidime is pentahydrate of the inner salt of (7R)-7-[(Z)-2-(2-aminothiazol-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-(1-pyridinomethyl)-3-cepham-4-carboxylate.

Ceftazidime contains not less than 95.0 per cent and not more than 102.0 per cent of C_{22}H_{22}N_{6}O_{7}S_{2}, calculated on the dried basis.

Description. A white to cream-coloured, crystalline powder.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 3.0 to 4.0, determined in a 0.5 per cent w/v solution.

Pyridine. Not more than 0.4 per cent.

Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity containing 0.5 g of ceftazidime and dissolve in sufficient mixed phosphate buffer pH 7.0 to produce 100.0 ml.

Reference solution. Weigh accurately about 200 mg of pyridine and dissolve in sufficient water to produce 100.0 ml. Immediately prior to chromatography add to 2.0 ml of the resulting solution sufficient mixed phosphate buffer pH 7.0 to produce 200.0 ml and mix well.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (3 to 10 µm),
- mobile phase: a mixture of 10 volumes of a 2.88 per cent w/v solution of ammonium dihydrogen phosphate previously adjusted to pH 7.0 with dilute ammonia solution, 30 volumes of acetonitrile and 60 volumes of water,
- column temperature. 38° to 42°,
- flow rate. 1.6 ml per minute,
- spectrophotometer set at 254 nm,
- a 10 µl loop injector.

Inject the reference solution. Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained is at least 50 per cent of the full scale deflection of the recorder.

Inject the reference solution six times. The test is not valid unless the relative standard deviation of the area of the principal peak in replicate injections is not more than 3.0 per cent.

Inject alternately the test solution and the reference solution. Record the chromatograms and measure the areas of the pyridine peaks.

Calculate the content of pyridine.

Loss on drying (2.4.19). 13.0 to 15.0 per cent, determined on 0.3 g by drying in an oven over phosphorus pentoxide at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 30 mg of the substance under examination in 2.5 ml of phosphate buffer pH 7.0, dilute to 25.0 ml with water and mix. Protect this solution from light. Immediately before chromatography, dilute 5.0 ml of this solution to 50.0 ml with water.

Reference solution. Treat 30 mg ceftazidime RS in a similar manner.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 100 ml of phosphate buffer pH 7.0 and 20 ml of acetonitrile diluted to 1000 ml with water,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the content of C_{22}H_{22}N_{6}O_{7}S_{2}.

Ceftazidime intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.10 Endotoxin Unit per mg.

Ceftazidime intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.
Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light and moisture.

Ceftazidime For Injection

Ceftazidime for Injection is a sterile mixture of sterile Ceftazidime and Sodium Carbonate.

Ceftazidime for Injection contains not less than 90.0 per cent and not more than 105.0 per cent of ceftazidime, C22H22N6O7S2, calculated on the dried and sodium carbonate-free basis.

Description. A white or almost white powder.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Gives the reactions of sodium salts and reaction A of carbonates (2.3.1).

Tests

pH (2.4.24). 5.0 to 7.5, determined in a solution containing 100 mg of anhydrous ceftazidime per ml.

Pyridine. Not more than 0.4 per cent.

Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity containing about 0.5 g of ceftazidime and dissolve in sufficient mixed phosphate buffer pH 7.0 to produce 100.0 ml.

Reference solution. Weigh accurately about 200 mg of pyridine and dissolve in sufficient water to produce 100.0 ml. Immediately prior to chromatography add to 2.0 ml of the resulting solution sufficient mixed phosphate buffer pH 7.0 to produce 200.0 ml and mix well.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (3 to 10 µm),
- mobile phase: a mixture of 10 volumes of a 2.88 per cent w/v solution of ammonium dihydrogen phosphate previously adjusted to pH 7.0 with dilute ammonia solution, 30 volumes of acetonitrile and 60 volumes of water,
- flow rate. 1.6 ml per minute,
- spectrophotometer set at 254 nm,
- a 10 µl loop injector.

Inject the reference solution six times. The test is not valid unless the relative standard deviation of the area of the principal peak in replicate injections is not more than 3.0 per cent.

Inject alternately the test solution and the reference solution. Measure the areas of the pyridine peaks.

Calculate the content of pyridine.

Sodium carbonate. Weigh accurately a quantity containing about 50 mg of anhydrous ceftazidime and dissolve in sufficient water to produce 100.0 ml. Dilute the resulting solution appropriately with water and determine by Method A for flame photometry (2.4.4), measuring at 589 nm or by Method A for atomic absorption spectrophotometry (2.4.2), using sodium solution FP, suitably diluted with water for the reference solutions.

1 g of Na is equivalent to 2.305 g of Na2CO3.

Bacterial endotoxins (2.2.3). Not more than 0.10 Endotoxin Unit per mg of ceftazidime.

Sterility (2.2.11). Complies with the tests for sterility.

Loss on drying (2.4.19). Not more than 13.5 per cent, determined on 0.3 g by drying at 25° for 4 hours at a pressure not exceeding 0.7 kPa and continuing the drying by heating in an oven at 100° for 3 hours at a pressure not exceeding 0.7 kPa.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity containing about 50 mg of anhydrous ceftazidime dissolve in water and dilute to 50.0 ml with the same solvent. Protect this solution from light. Immediately before chromatography, dilute 5.0 ml to 50.0 ml with water.

Reference solution. Dissolve about 29 mg ceftazidime RS in 2.5 ml of mixed phosphate buffer pH 7.0 and dilute to 25.0 ml with water. Protect this solution from light. Immediately before chromatography, dilute 5.0 ml to 50.0 ml with water.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 100 ml of phosphate buffer pH 7.0 and 20 ml of acetonitrile diluted to 1000 ml with water,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of C22H22N6O7S2.
Storage. Store in sterile containers, sealed so as to exclude microorganisms, protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of ceftazidime.

Ceftazidime Injection

Ceftazidime Injection is a sterile material consisting of Ceftazidime for Injection with or without auxiliary substances. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of Water for Injections immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Ceftazidime Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of ceftazidime, C22H22N6O7S2.

Description. A white or almost white crystalline powder.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Gives the reactions of sodium salts and reaction A of carbonates (2.3.1).

Tests

pH (2.4.24). 5.0 to 7.5, determined in a solution containing 100 mg of ceftazidime per ml.

Pyridine. Not more than 0.4 per cent.

Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity containing about 0.5 g of ceftazidime and dissolve in sufficient mixed phosphate buffer pH 7.0 to produce 100.0 ml.

Reference solution. Weigh accurately about 200 mg of pyridine and dissolve in sufficient water to produce 100.0 ml. Immediately prior to chromatography add to 2.0 ml of the resulting solution sufficient mixed phosphate buffer pH 7.0 to produce 200.0 ml and mix well.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 10 volumes of a 2.88 per cent w/v solution of ammonium dihydrogen phosphate previously adjusted to pH 7.0 with dilute ammonia solution, 30 volumes of acetonitrile and 60 volumes of water,
- flow rate. 1.6 ml per minute,
- spectrophotometer set at 254 nm,
- a 10 µl loop injector.

Inject the reference solution and adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained is at least 50 per cent of the full-scale deflection of the recorder.

Inject the reference solution six times. The test is not valid unless the relative standard deviation of the area of the principal peak in replicate injections is not more than 3.0 per cent.

Inject alternately the test solution and the reference solution. Measure the areas of the pyridine peaks.

Calculate the content of pyridine.

Bacterial endotoxins (2.2.3). Not more than 0.10 Endotoxin Unit per mg of ceftazidime.

Sterility (2.2.11). Complies with the test for sterility.

Loss on drying (2.4.19). Not more than 13.5 per cent, determined on 0.3 g by drying at 25° for 4 hours at a pressure not exceeding 0.7 kPa and continuing the drying by heating in an oven at 100° for 3 hours at a pressure not exceeding 0.7 kPa.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Determine the weight of the contents of 10 containers. Weigh accurately a quantity of the mixed contents of the 10 containers containing about 50 mg of ceftazidime, dissolve in water and dilute to 50.0 ml with the same solvent. Protect this solution from light. Immediately before chromatography, dilute 5.0 ml to 50.0 ml with water.

Reference solution. Dissolve about 29 mg ceftazidime RS in 2.5 ml of mixed phosphate buffer pH 7.0 and dilute to 25.0 ml with water. Protect this solution from light. Immediately before chromatography, dilute 5.0 ml to 50.0 ml with water.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 100 ml of phosphate buffer pH 7.0 and 20 ml of acetonitrile diluted to 1000 ml with water.
flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the content of $C_{22}H_{22}N_6O_7S_2$ in the injection.

Storage. Store in sterile containers, sealed so as to exclude microorganisms, protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of ceftazidime.

Ceftriaxone Sodium

$C_{18}H_{16}N_8Na_2O_7S_3\cdot\frac{3}{2}H_2O$  Mol. Wt. 662.0

Ceftriaxone sodium is disodium (6R,7R)-7-[[Z][2-aminothiazol-4-yl](methoxyimino)acetyl]amino]-3-[[2-methyl-6-oxido-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl]sulphanyl]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.

Ceftriaxone sodium contains not less than 96.0 per cent and not more than 102.0 per cent of $C_{18}H_{16}N_8Na_2O_7S_3$, calculated on the anhydrous basis.

Description. A white or yellowish, crystalline powder, slightly hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ceftriaxone sodium RS or with the reference spectrum of ceftriaxone sodium.

B. Gives reaction A of sodium salts (2.3.1).

Tests

Appearance of solution. Dissolve 2.4 g in 20 ml of carbon dioxide-free water (Solution A). Dilute 2 ml of solution A to 20 ml with water; the resulting solution is clear (2.4.1) and not more intensely coloured than reference solution BYS5 or YS5 (2.4.1).

pH (2.4.24). 6.0 to 8.0, determined in solution A.

Specific optical rotation (2.4.22). -155.0° to -170.0°, determined in a 1.0 per cent w/v solution in water.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 30.0 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a). A 0.03 per cent w/v solution of ceftriaxone sodium RS in the mobile phase.

Reference solution (b). A solution containing 0.005 per cent w/v each of ceftriaxone sodium RS and ceftriaxone sodium E-isomer RS in the mobile phase.

Reference solution (c). Dilute 1.0 ml of the reference solution (a) to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilica silica (5 µm),
- mobile phase: 2.0 g of tetradeclammonium bromide and 2.0 g of tetraheptylammonium bromide in a mixture of 440 ml of water, 55 ml of 0.067 M mixed phosphate buffer solution pH 7.0, 5.0 ml of a buffer solution prepared by dissolving 20.17 g of citric acid in 800 ml of water, adjusting the pH to 5.0 with strong sodium hydroxide solution and diluting to 1000.0 ml with water, and 500 ml of acetonitrile,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject the test solution and reference solutions (b) and (c). Continue the chromatography for twice the retention time of the ceftriaxone peak. The test is not valid unless the resolution between the peaks due to ceftriaxone and ceftriaxone sodium E-isomer is at least 3.0; the area of any peak other than the principal peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1 per cent); the sum of the areas of all such peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (c) (4 per cent). Ignore any peak with an area 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Water (2.3.43). 8.0 per cent to 11.0 per cent, determined on 0.1 g.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances.
Inject alternately the test solution and reference solution (a).

Calculate the content of $\text{C}_{18}\text{H}_{18}\text{N}_{8}\text{O}_{7}\text{S}_{3}$.

Ceftriaxone sodium intended for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins complies with the following additional requirement.

**Bacterial endotoxins (2.2.3): Not more than 0.20 Endotoxin Unit per mg of ceftriaxone sodium.**

**Storage.** Store protected from light and moisture.

**Labelling.** The label states, where applicable, that the substance is free from bacterial endotoxins.

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**Ceftriaxone Injection**

Ceftriaxone Injection is a sterile material consisting of Ceftriaxone Sodium with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

**Storage.** The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Ceftriaxone Injection contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of ceftriaxone, $\text{C}_{18}\text{H}_{18}\text{N}_{8}\text{O}_{7}\text{S}_{3}$.

**Description.** A white or almost white powder.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ceftiraxone sodium RS* or with the reference spectrum of ceftriaxone sodium.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution (a).

C. It gives the reaction A of sodium salts (2.3.1).

**Tests**

**Appearance of solution.** A 1.2 per cent w/v solution in carbon dioxide-free water is clear (2.4.1) and not more intensely coloured than reference solution BYS5 or YS5 (2.4.1).

**pH** (2.4.24). 6.0 to 8.0, determined in a 10.0 per cent w/v solution.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve the substance under examination containing about 30 mg of ceftriaxone in 100 ml of the mobile phase and filter.

**Reference solution (a).** A 0.03 per cent w/v solution of ceftriaxone sodium RS in the mobile phase.

**Reference solution (b).** A solution containing 0.005 per cent w/v each of *ceftiraxone sodium RS* and *ceftiraxone sodium E-isomer RS* in the mobile phase.

**Reference solution (c).** Dilute 1.0 ml of the reference solution (a) to 100.0 ml with the mobile phase.

**Chromatographic system**

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Lichrosphere RP-18),
- mobile phase: dissolve 2 g of tetradecylammonium bromide and 2 g of tetraheptylammonium bromide in a mixture of 440 ml of water, 55 ml of 0.067 M mixed phosphate buffer pH 7.0, 5 ml of a buffer prepared by dissolving 20.17 g of citric acid in 800 ml of water, adjusting the pH to 5.0 with 10 M sodium hydroxide and diluting to 1000 ml with water, and 500 ml of acetonitrile,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is at least 3.0.

Inject the test solution and reference solution (c). Run the chromatogram at least twice the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent) and the sum of the areas of all the secondary peaks is not greater than 5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (5.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

**Bacterial endotoxins (2.2.3).** Not more than 0.2 Endotoxin Unit per mg of ceftriaxone.
Water (2.3.43). Not more than 11.0 per cent, determined on 0.1 g.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject alternately the test solution and reference solution (a).

Calculate the content of C_{18}H_{18}N_{8}O_{7}S_{3} in the injection.

Storage. Store protected from light at a temperature not exceeding 30º.

Labelling. The label on the sealed container states the quantity of Ceftriaxone Sodium contained in it in terms of the equivalent amount of ceftriaxone.

Cefuroxime Axetil

\[
\text{C}_{20}\text{H}_{22}\text{N}_{4}\text{O}_{10}\text{S} \quad \text{Mol. Wt. 510.5}
\]

Cefuroxime Axetil is a mixture of the 2 diastereoisomers of (1RS)-1-(acetyloxy)ethyl (6R,7R)-3-[(carbamoyloxy)methyl]-7-[[Z]-2-(furan-2-yl)-2-(methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.

Cefuroxime Axetil contains not less than 79.8 per cent and not more than 84.8 per cent of cefuroxime, C_{16}H_{16}N_{4}O_{8}S, calculated on the anhydrous and acetone-free basis.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cefuroxime axetil RS or with the reference spectrum of cefuroxime axetil.

B. In the Assay, the principal peaks in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (d).

Tests

Diastereoisomer ratio. Determine by liquid chromatography (2.4.14).

Use the chromatographic system, the test solution, and reference solutions (a), (b), (c) and (d) described under Assay.

In the chromatogram obtained with the test solution, the ratio of the peak due to cefuroxime axetil diastereoisomer A to the sum of the peaks due to cefuroxime axetil diastereoisomers A and B is between 0.48 and 0.55 by the normalisation procedure.

Related substances. Determine by liquid chromatography (2.4.14).

Use the chromatographic system, the test solution, and reference solutions (a), (b), (c) and (d) described under Assay.

The percentage sum of the peak corresponding to the E-isomers located by comparison with the chromatogram obtained with reference solution (c) is not more than 1.0 per cent, the percentage sum of the peak corresponding to the D^{3}-isomers located by comparison with the chromatogram obtained with reference solution (b) is not greater than 1.5 per cent and the area of any other secondary peak is not more than 0.5 per cent and the sum of all the secondary peaks found is not more than 3.0 per cent.

Acetone (5.4). Not more than 1.1 per cent.

Water (2.3.43). Not more than 1.5 per cent, determined on 0.4 g.

Assay. Determine by liquid chromatography (2.4.14).

NOTE – Prepare the solutions immediately before use.

Test solution. Dissolve 10.0 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b). Warm 5 ml of the test solution, to 60º for one hour to generate the D^{3}-isomers.

Reference solution (c) Expose 5 ml of the test solution to ultraviolet light at 254 nm for 24 hours to generate E-isomers.

Reference solution (d). A 0.02 per cent w/v solution of cefuroxime axetil RS in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with trimethylsilyl silica gel (5 µm),
- mobile phase: a mixture of 38 volumes of methanol and 62 volumes of a 2.3 per cent solution of ammonium dihydrogen phosphate,
- flow rate. 1 ml per minute,
- spectrophotometer set at 278 nm,
- a 20 µl loop injector.

Inject reference solution (d). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution, and reference solutions (a), (b) and (c). The retention times relative to cefuroxime axetil
diastereoisomer A (second peak) are approximately 0.9 for cefuroxime axetil diastereoisomer B, 1.2 for the cefuroxime axetil D\textsuperscript{1}-isomers and 1.7 and 2.1 for the E-isomers. The test is not valid unless in the chromatogram obtained with reference solution (d), the resolution between the peaks corresponding to cefuroxime axetil diastereoisomers A and B is at least 1.5. In the chromatogram obtained with reference solution (b), the resolution between the peaks corresponding to cefuroxime axetil diastereoisomer A and cefuroxime axetil D\textsuperscript{3}-isomer is at least 1.5.

Calculate the content of C\textsubscript{16}H\textsubscript{16}N\textsubscript{4}O\textsubscript{8}S as the sum of areas of the two diastereoisomer peaks.

1 mg of C\textsubscript{20}H\textsubscript{22}N\textsubscript{4}O\textsubscript{10}S is equivalent to 0.8313 mg of C\textsubscript{16}H\textsubscript{16}N\textsubscript{4}O\textsubscript{8}S.

Storage. Store protected from light and moisture.

Cefuroxime Axetil Tablets

Cefuroxime Axetil Tablets contain Cefuroxime Axetil. They may be coated.

Cefuroxime Axetil Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cefuroxime, C\textsubscript{16}H\textsubscript{16}N\textsubscript{4}O\textsubscript{8}S.

Identification

A. Extract a quantity of the powdered tablets containing 0.1 g of cefuroxime with 5 ml of dichloromethane, filter and evaporate the filtrate to dryness.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cefuroxime axetil RS or with the reference spectrum of cefuroxime axetil.

B. In the Assay, the principal peaks in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (c).

Tests

Dissolution (2.5.2).

Apparatus. No. 1

Medium. 900 ml of 0.1 M hydrochloric acid

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly. Dilute the filtrate, if necessary, with the dissolution medium.

Measure the absorbance of the resulting solution at the maximum at about 278 nm (2.4.7). Calculate the content of C\textsubscript{16}H\textsubscript{16}N\textsubscript{4}O\textsubscript{8}S in the medium from the absorbance obtained from a solution of known concentration of cefuroxime axetil RS.

D. Not less than 70 per cent of the stated amount of C\textsubscript{16}H\textsubscript{16}N\textsubscript{4}O\textsubscript{8}S.

Related substances. Determine by liquid chromatography (2.4.14).

Use the chromatographic system, the test solution and reference solutions (a), (b), and (c) described under Assay.

In the chromatogram obtained with the test solution the sum of the areas of the peaks corresponding to the E-isomers in the chromatogram obtained with reference solution (b) is not more than 1.5 per cent by normalisation, the sum of the areas of any peaks corresponding to the D\textsuperscript{1}-isomers in the chromatogram obtained with reference solution (a) is not more than 2.0 per cent by normalisation and the area of any other secondary peak is not more than 1.0 per cent by normalisation.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

NOTE – Prepare the solutions immediately before use.

Test solution. Disperse 10 tablets in 0.2 M ammonium dihydrogen orthophosphate with the pH previously adjusted to 2.4 with orthophosphoric acid, using 10 ml per g of the stated content of cefuroxime. Immediately add sufficient methanol to produce a solution containing the equivalent of 0.5 per cent w/v of cefuroxime and shake vigorously. Filter and dilute a quantity of the filtrate with sufficient of the mobile phase to produce a solution containing 0.025 per cent w/v of cefuroxime.

Reference solution (a). Warm a quantity of the test solution at 60º for one hour or until sufficient impurities (D\textsuperscript{3}-isomers) have been generated.

Reference solution (b). Expose a quantity of the test solution to ultraviolet light at 254 nm for 24 hours or until sufficient impurities (E-isomers) have been generated.

Reference solution (c). A 0.03 per cent w/v solution of cefuroxime axetil RS in the mobile phase.

Chromatographic system

– a stainless steel column 25 cm × 4.6 mm, packed with particles of silica (5 µm) the surface of which has been modified by chemically-bonded trimethylsilyl groups (such as Hypersil SAS),

– mobile phase: a mixture of 38 volumes of methanol and 62 volumes of 0.2 M ammonium dihydrogen orthophosphate, adjusted, if necessary, so that the resolution between the peaks corresponding to the cefuroxime axetil diastereoisomers A and B in reference solution (c) and between the peaks corresponding to cefuroxime axetil diastereoisomer A and the cefuroxime axetil D\textsuperscript{1}-isomer in reference solution (a) is in each case not less than 1.5,

– flow rate. 1.2 ml per minute,

– spectrophotometer set at 278 nm,

– a 20 µl loop injector.
Inject reference solution (c). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution, reference solutions (a), (b) and (c). The retention time relative to cefuroxime axetil diastereoisomer A are approximately 0.9 for cefuroxime axetil diastereoisomer B, 1.2 for the cefuroxime axetil D3-isomers and 1.7 and 2.1 for the E-isomers.

Calculate the content of C16H16N4O8S as the sum of the areas corresponding to diastereoisomers A and B. 1 mg of C20H22N4O10S is equivalent to 0.8313 mg of C16H16N4O8S.

**Labelling.** The quantity of active ingredient is stated in terms of the equivalent amount of cefuroxime.

### Cefuroxime Sodium

![Cefuroxime Sodium structure](image)

C16H15N4NaO8S Mol. Wt. 446.4

Cefuroxime Sodium is sodium (7R)-3-carbamoyloxymethyl-7-[(Z)-furan-2-yl-2-methoxyiminoacetamido]-3-cephem-4-carboxylate.

Cefuroxime Sodium contains not less than 90.0 per cent and not more than 105.0 per cent of cefuroxime, C16H16N4O8S, calculated on the anhydrous basis.

**Description.** A white or faintly yellow powder.

**Identification**

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Gives the reactions of sodium salts (2.3.1).

**Tests**

**pH** (2.4.24). 6.0 to 8.5, determined in a 10.0 per cent w/v solution.

**Water** (2.3.43). Not more than 3.5 per cent, determined on 0.15 g.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh accurately a quantity of the substance under examination containing 25 mg of cefuroxime and dissolve in sufficient water to produce 25.0 ml. Immediately transfer 5.0 ml of the resulting solution to a 100-ml volumetric flask, add 20.0 ml of a 0.15 per cent w/v solution of orcinol (internal standard) in water; dilute to volume with water and mix.

**Reference solution.** Treat a quantity of cefuroxime sodium RS equivalent to 25 mg of cefuroxime in a similar manner.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with heptylsilane chemically bonded to totally porous silica particles (5 µm),
- mobile phase: a mixture of 100 volumes of acetate buffer pH 3.4 and 10 volumes of acetonitrile,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the content of C16H16N4O8S.

Cefuroxime Sodium intended for use in the manufacture of parenteral preparations complies with the following additional requirements.

**Bacterial endotoxins** (2.2.3). Not more than 0.10 Endotoxin Unit per mg of cefuroxime.

**Sterility** (2.2.11). Complies with the test for sterility, using the membrane filtration method.

**Storage.** Store protected from moisture. If it is intended for use in the manufacture of parenteral preparations, it should be sterile and sealed so as to exclude micro-organisms.

**Labelling.** The label states whether or not the contents are intended for use in the manufacture of injectable preparations.

### Cefuroxime Injection

Cefuroxime Sodium Injection

Cefuroxime Injection is a sterile material consisting of Cefuroxime Sodium, with or without auxiliary substances. It is filled in a sealed container.

The injection is constituted by dissolving the contents of a sealed container in the requisite amount of Water for Injections immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).
Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cefuroxime Injection contains a quantity of Cefuroxime Sodium equivalent to not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of cefuroxime, C₁₆H₁₆N₄O₈S.

Description. A white or faintly yellow powder.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification
A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.
B. Gives the reactions of sodium salts (2.3.1).

Tests
pH (2.4.24). 6.0 to 8.5, determined in a 10.0 per cent w/v solution.
Bacterial endotoxins (2.2.3). Not more than 0.1 Endotoxin Unit per mg of cefuroxime.

Water (2.3.43). Not more than 3.5 per cent, determined on 0.15 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Determine the weight of the contents of 10 containers. Weigh accurately a quantity of the mixed contents of the 10 containers containing about 25 mg of cefuroxime and dissolve in sufficient water to produce 25.0 ml. Immediately transfer 5.0 ml of the resulting solution to a 100-ml volumetric flask, add 20.0 ml of a 0.15 per cent w/v solution of orcinol (internal standard) in water, dilute to volume with water and mix.

Reference solution. Treat a quantity of cefuroxime sodium RS equivalent to 25 mg of cefuroxime in a similar manner.

Chromatographic system
− a stainless steel column 15 cm x 4.6 mm, packed with hexylsilane chemically bonded to totally porous silica particles (5 μm),
− mobile phase: a mixture of 100 volumes of acetate buffer pH 3.4 and 10 volumes of acetonitrile,
− flow rate. 2 ml per minute,
− spectrophotometer set at 254 nm,
− a 10 μl loop injector.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the content of C₁₆H₁₆N₄O₈S in the injection.

Storage. Store in tightly-closed containers protected from moisture at a temperature not exceeding 30°.

Labelling. The label on the sealed container states the quantity of Cefuroxime Sodium contained in it in terms of the equivalent amount of cefuroxime.

Cellulose Acetate Phthalate

Cellacephate; Cellacefate

Cellulose Acetate Phthalate is a cellulose, some of the hydroxyl groups of which are esterified by acetyl groups and others by hydrogen phthaloyl groups.

Cellulose Acetate Phthalate contains not less than 17.0 per cent and not more than 26.0 per cent of acetyl groups, C₂H₃O and not less than 30.0 per cent and not more than 40.0 per cent of hydrogen phthaloyl groups, C₈H₅O₃ both calculated on the dried, acid-free basis.

Description. A white, free-flowing powder or colourless flakes; odourless or with a faint odour of acetic acid; hygroscopic.

Identification
A. To about 10 mg add 1 ml of ethanol (95 per cent) and 1 ml of sulphuric acid and warm; ethyl acetate, recognisable by its characteristic odour, is evolved.
B. To about 10 mg contained in a small test-tube add 10 mg of resorcinol, mix, add 0.5 ml of sulphuric acid and heat in a liquid paraffin bath at 160° for 3 minutes. Cool and pour the solution into a mixture of 25 ml of sodium hydroxide solution and 200 ml of water; the solution shows a vivid green fluorescence.

Tests
Viscosity (2.4.28). 50 mm²s⁻¹ to 90 mm²s⁻¹, determined in the following manner. Weigh accurately about 15 g, previously dried at 105° for 2 hours, and dissolve in 85 g of a mixture of 249 parts of dry acetone and 1 part of water. Determine at 25° the viscosity of the resulting solution by Method A, using a size D viscometer.

Appearance of a film. Dissolve 3.0 g in 17 ml of acetone with a water content of 0.35 to 0.45 per cent w/w. Allow 1 ml of this solution to flow over a glass plate and dry; a thin, colourless, transparent and glossy film is produced.

Free acid. Not more than 3.0 per cent, calculated as phthalic acid, C₈H₆O₄, on the anhydrous basis and determined in the
following manner. Weigh accurately 1.0 g, in fine powder, shake for 5 minutes with 100 ml of boiled water and filter. Wash the flask and the filter with two quantities, each of 10 ml, of water. Combine the filtrate and washings, add 5 drops of phenolphthalein solution and titrate with 0.1 M sodium hydroxide until a faint pink colour is obtained.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.0083 g of phthalic acid.

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 5.0 per cent, using 0.5 g dissolved in 20 ml of a mixture of equal volumes of anhydrous methanol and chloroform.

Assay. For acetyl groups — Weigh accurately about 0.1 g and heat on a water-bath for 30 minutes with 25.0 ml of 0.1 M sodium hydroxide under reflux. Cool, add 5 drops of phenolphthalein solution and titrate with 0.1 M hydrochloric acid until the colour is discharged. Carry out a blank titration. Calculate the acetyl groups, C₂H₃O, from the expression

\[ 0.43c/w - (0.578p + 0.518s), \]

where, c = volume, in ml, of 0.1 M hydrochloric acid consumed
w = weight, in g, of the sample, calculated with reference to anhydrous substance
p = percentage of hydrogen phthaloyl groups as determined in Assay for hydrogen phthaloyl groups
s = percentage of free acid.

For hydrogen phthaloyl groups — Weigh accurately about 0.4 g (calculated on the anhydrous basis) and dissolve without heating in 20 ml of 2-methoxyethanol, previously neutralised in the presence of 5 drops of phenolphthalein solution. Titrate with 0.1 M sodium hydroxide until a faint pink colour is produced. Calculate the hydrogen phthaloyl groups, C₈H₅O₃, from the expression

\[ 1.49b/w - 1.795s \]

where, b = volume, in ml, of 0.1 M sodium hydroxide consumed
w = weight, in g, of the sample, calculated with reference to the anhydrous substance
s = percentage of free acid.

Storage. Store protected from moisture at a temperature between 8° and 15°.

Cephalixin

\[ C_{16}H_{17}N_{3}O_{4}S \cdot H_{2}O \]

Mol. Wt. 365.4

Cephalixin is (7R)-3-methyl-7-(α-D-phenylglycylamino)-3-cephem-4-carboxylic acid monohydrate.

Cephalixin contains not less than 95.0 per cent and not more than 101.0 per cent of C₁₆H₁₇N₃O₄S, calculated on the anhydrous basis.

Description. A white or almost white, crystalline powder; odour, characteristic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cephalixin RS or with the reference spectrum of cephalixin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 4.0 to 5.5, determined in a 0.5 per cent w/v solution.

Specific optical rotation (2.4.22). +149° to +158°, determined in a 0.5 per cent w/v solution in phthalate buffer solution pH 4.4 and in a 2-dm tube.

Light absorption (2.4.7). Dissolve 50 mg in sufficient water to produce 100.0 ml. Absorbance of the solution at about 330 nm, not greater than 0.05 (2.4.7). Dilute 2 ml to 50.0 ml. When examined between 230 nm and 360 nm the solution shows an absorption maximum at about 262 nm; absorbance at about 262 nm, 0.44 to 0.49.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in mobile phase A and dilute to 50 ml with the same solvent.

Reference solution (a). Dissolve 10 mg of D-phenylglycine in mobile phase A and dilute to 10 ml with the same solvent.

Reference solution (b). Dissolve 10 mg of 7-aminodesacetoxycephalosporanic acid RS in phosphate buffer solution pH 7.0 and dilute to 10 ml with the same solvent.
Reference solution (c). Dilute 1 ml of reference solution (a) and 1 ml of reference solution (b) to 100 ml with mobile phase A.

Reference solution (d). Dissolve 10 mg of dimethylformamide and 10 mg of dimethylacetamide in mobile phase A and dilute to 10 ml with the same solvent. Dilute 1 ml to 100 ml with mobile phase A.

Reference solution (e). Dilute 1 ml of reference solution (c) to 20 ml with mobile phase A.

Reference solution (f). Dissolve 10 mg of cefotaxime sodium RS in mobile phase A and dilute to 10 ml with the same solvent. To 1 ml of the solution add 1 ml of the test solution and dilute to 100 ml with mobile phase A.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: A. a mixture of phosphate buffer solution pH 5.0, B. methanol,
- flow rate. 1.5 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 220 nm,
- a 20 µl loop injector.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 1</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>1 – 20</td>
<td>98 → 70</td>
<td>2 → 30</td>
</tr>
<tr>
<td>20 – 23</td>
<td>70 → 98</td>
<td>30 → 2</td>
</tr>
<tr>
<td>23 – 30</td>
<td>98</td>
<td>2</td>
</tr>
</tbody>
</table>

Inject reference solutions (c) and (f). In the chromatogram obtained with reference solution (c) the resolution between the peaks due to D-phenylglycine and 7-aminodesacetoxycephalosporanic acid is not less than 2.0 and in the chromatogram obtained with reference solution (f) the resolution between the peaks due to cephalexin and cefotaxime is not less than 1.5.

Inject the test solution and reference solutions (c), (d) and (e). In the chromatogram obtained with the test solution any peak corresponding to D-phenylglycine is not more than the area of the second peak in the chromatogram obtained with reference solution (c) (1.0 per cent). Ignore any peaks due to dimethylformamide and dimethylacetamide. The area of any other secondary peak is not more than the area of the first peak in the chromatogram obtained with reference solution (c) (1.0 per cent). The sum of the secondary peaks is not more than the three times the area of the first peak in the chromatogram obtained with reference solution (c). Ignore any peak with an area less than the second peak in the chromatogram obtained with reference solution (e) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). 4.0 to 8.0 per cent, determined on 0.3 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in water and dilute to 100.0 ml with the same solvent.

Reference solution (a). Dissolve 50 mg of cephalexin monohydrate RS in water and dilute to 100.0 ml with the same solvent.

Reference solution (b). Dissolve 10 mg of cefradine RS in 20 ml of reference solution (a) and dilute to 100 ml with water.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 2 volumes of methanol, 5 volumes of acetonitrile, 10 volumes of a 13.6 g per litre solution of potassium dihydrogen phosphate and 83 volumes of water,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject reference solution (b). In the chromatogram obtained, the resolution between the peaks due to cephalexin and cefradine is not less than 4.0.

Inject alternately the test solution and reference solution (a). Calculate the content of C_{16}H_{17}N_{3}O_{4}S.

Storage. Store protected from light at a temperature not exceeding 30°.

Cephalexin Capsules

Cephalexin Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous cephalexin, C_{16}H_{17}N_{3}O_{4}S.

Identification

Shake a quantity of the contents of the capsules containing 0.5 g of anhydrous cephalexin with 1 ml of water and 1.4 ml of 1 M hydrochloric acid, filter and wash the filter with 1 ml of water. Add slowly to the filtrate a saturated solution of sodium acetate until precipitation occurs. Add 5 ml of methanol, filter and wash the precipitate with two quantities, each of 1 ml, of methanol. The residue after drying at a pressure not exceeding 0.7 kPa complies with the following tests.
A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cephalxin RS or with the reference spectrum of cephalxin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel HF (such as Analtech plates). Impregnate the plate by development with a 5 per cent v/v solution of n-tetradecane in hexane. Allow the solvent to evaporate and carry out the chromatography in the same direction as the impregnation.

Mobile phase. A mixture of 3 volumes of acetone, 80 volumes of a 7.2 per cent w/v solution of disodium hydrogen orthophosphate and 120 volumes of a 2.1 per cent w/v solution of citric acid.

Test solution. Shake a quantity of the contents of the capsules containing about 0.25 g of anhydrous cephalxin with 10 ml of 2 M hydrochloric acid, filter and use the filtrate.

Reference solution (a). Dilute 1 volume of the test solution to 100 volumes with 2 M hydrochloric acid.

Reference solution (b). A 0.025 per cent w/v solution of 7-aminodesacetoxycephalosporanic acid RS in 2 M hydrochloric acid.

Reference solution (c). A 0.025 per cent w/v solution of DL-phenylglycine in 2 M hydrochloric acid.

Reference solution (d). A solution containing 2.5 per cent w/v of cephalxin RS and 0.025 per cent w/v each of 7-aminodesacetoxycephalosporanic acid RS and DL-phenylglycine in 2 M hydrochloric acid.

Apply to the plate 5 µl of each of the following solutions. After development, dry the plate at 90° for 3 minutes, spray the hot plate with a 0.1 per cent w/v solution of ninhydrin in the mobile phase, heat the plate at 90° for 15 minutes and allow to cool. In the chromatogram obtained with the test solution any spot corresponding to 7-aminodesacetoxycephalosporanic acid is not more intense than the spot in the chromatogram obtained with reference solution (b) (1 per cent), any spot corresponding to DL-phenylglycine is not more intense than the spot in the chromatogram obtained with reference solution (c) (1 per cent) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a) (1 per cent). The test is not valid unless the chromatogram obtained with reference solution (d) shows three clearly separated spots.

Dissolution (2.5.2).

Apparatus. No 1

Medium. 900 ml of water freshly prepared by distillation. Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 0.8 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with water. Measure the absorbance of the resulting solution at the maximum at about 261 nm (2.4.7). Calculate the content of C_{16}H_{17}N_{3}O_{4}S taking 235 as the specific absorbance at 261 nm.

D. Not less than 75 per cent of the stated amount of C_{16}H_{17}N_{3}O_{4}S.

Other tests. Complies with the tests stated under Capsules.

Water (2.3.43). Not more than 10.0 per cent, determined on 0.3 g of the contents of the capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the powdered mixed contents of 20 capsules containing about 0.25 g of anhydrous cephalxin with 100.0 ml of water for 30 minutes, add sufficient of water to produce 250.0 ml and filter. Dilute 25.0 ml of the filtrate to 50.0 ml with water.

Reference solution (a). A 0.05 per cent w/v solution of cephalxin RS in water.

Reference solution (b). A solution containing 0.01 per cent w/v each of cephalxin RS and cephradine RS in water.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecyldsilyl silica gel (5 µm) (such as Nucleosil C 18),
- mobile phase: a mixture of 2 volumes of methanol, 5 volumes of acetonitrile, 10 volumes of a 13.6 g per litre solution of potassium dihydrogen phosphate and 83 volumes of water,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject reference solution (b). Adjust the sensitivity so that the height of the peaks in the chromatogram obtained is at least half the full-scale deflection on the recorder. The test is not valid unless the resolution between the peaks corresponding to cephalxin and cephradine is at least 4.0.

Inject reference solution (a) six times. The relative standard deviation is not more than 1.0 per cent.

Inject alternately the test solution and reference solution (a). Calculate the content of C_{16}H_{17}N_{3}O_{4}S in the capsules.

Storage. Store protected from moisture at a temperature not exceeding 30°.
Labelling. The label states the strength in terms of the equivalent amount of anhydrous cephalixin.

Cephalexin Oral Suspension

Cephalexin Dry Syrup; Cephalexin Mixture

Cephalexin Oral Suspension is a mixture of Cephalexin with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of water just before use.

Cephalexin Oral Suspension contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of $C_{16}H_{17}N_{3}O_{4}S$.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of cephalixin, $C_{16}H_{17}N_{3}O_{4}S$.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution (a).

B. Weigh a quantity containing 0.1 g of anhydrous cephalixin, shake with 20 ml of methanol, filter and evaporate the filtrate to dryness using a rotary evaporator. Dissolve the residue in the minimum volume of a 1 per cent v/v solution of glacial acetic acid, decolourise if necessary by the addition of sufficient decolourising charcoal, shake and filter. To 0.25 ml of the resulting solution add 0.1 ml of a 1 per cent w/v solution of cupric sulphate and 0.05 ml of 2 M sodium hydroxide; an olive-green colour is produced.

The constituted suspension complies with the tests stated under Oral liquids and with the following tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Shake an accurately weighed quantity of the oral suspension containing about 0.25 g of anhydrous cephalixin with 100.0 ml of water for 30 minutes, add sufficient of water to produce 250.0 ml and filter. Dilute 25.0 ml of the filtrate to 50.0 ml with water.

Reference solution (a). A 0.05 per cent w/v solution of cephalixin RS in water.

Reference solution (b). A solution containing 0.01 per cent w/v each of cephalixin RS and cephradine RS in water.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 μm) (such as Nucleosil C 18),
- mobile phase: a mixture of 2 volumes of methanol, 5 volumes of acetonitrile, 10 volumes of a 13.6 g per litre solution of potassium dihydrogen phosphate and 83 volumes of water,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 μl loop injector.

Inject reference solution (b). Adjust the sensitivity so that the height of the peaks in the chromatogram obtained is at least half the full-scale deflection on the recorder. The test is not valid unless the resolution between the peaks corresponding to cephalixin and cephradine is at least 4.0.

Inject reference solution (a) six times. The relative standard deviation is not more than 1.0 per cent.

Inject alternately the test solution and reference solution (a). Determine the weight per ml (2.4.29) of the suspension and calculate the content of $C_{16}H_{17}N_{3}O_{4}S$, weight in volume.

Repeat the procedure using a portion of the suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of anhydrous cephalixin.

Cephalexin Tablets

Cephalexin Tablets contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of anhydrous cephalixin, $C_{16}H_{17}N_{3}O_{4}S$. The tablets may be coated.

Identification

Remove any coating. Shake a quantity of the powdered tablet cores containing 0.5 g of anhydrous cephalixin with 1 ml of water and 1.4 ml of 1 M hydrochloric acid, add 0.1 g of decolourising charcoal, shake, filter and wash the filter with 1 ml of water. Add slowly to the filtrate a saturated solution of sodium acetate until precipitation occurs. Add 5 ml of methanol, filter and wash the precipitate with two quantities, each of 1 ml, of methanol. The residue, after drying at a pressure not exceeding 0.7 kPa, complies with the following test.
A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cephalixin RS or with the reference spectrum of cephalixin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution (a).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel HF (such as Analtech plates). Impregnate the plate by development with a 5 per cent v/v solution of n-tetradecane in hexane. Allow the solvent to evaporate and carry out the chromatography in the same direction as the impregnation.

Mobile phase. A mixture of 3 volumes of acetone, 80 volumes of a 7.2 per cent w/v solution of disodium hydrogen orthophosphate and 120 volumes of a 2.1 per cent w/v solution of citric acid.

Test solution. Shake a quantity of the powered tablets containing 0.25 g of anhydrous cephalixin with 10 ml of 2 M hydrochloric acid, filter and use the filtrate.

Reference solution (a). Dilute 1 volume of the solution to 100 volumes with 2 M hydrochloric acid.

Reference solution (b). A 0.025 per cent w/v solution of 7-amino desacetoxycephalosporanic acid RS in 2 M hydrochloric acid.

Reference solution (c). A 0.025 per cent w/v solution of DL-phenylglycine in 2 M hydrochloric acid.

Reference solution (d). A solution containing 2.5 per cent w/v of cephalixin RS and 0.025 per cent w/v each of 7-amino desacetoxy cephalosporanic acid RS and DL-phenylglycine in 2 M hydrochloric acid.

Apply separately to the plate 5 µl of each solution. After development, dry the plate at 90° for 3 minutes, spray the hot plate with development, dry the plate at 90° for 3 minutes, spray the hot

Dissolution (2.5.2).

Apparatus. No 1

Medium. 900 ml of water freshly prepared by distillation Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with water: Measure the absorbance of the resulting solution at the maximum at about 261 nm (2.4.7). Calculate the content of C_{16}H_{17}N_{3}O_{4}S taking 235 as the specific absorbance at 261 nm.

D. Not less than 75 per cent of the stated amount of C_{16}H_{17}N_{3}O_{4}S.

Tests

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing about 0.25 g of anhydrous cephalixin with 100.0 ml of water for 30 minutes, add sufficient of water to produce 250.0 ml and filter. Dilute 25.0 ml of the filtrate to 50.0 ml with water.

Reference solution (a). A 0.05 per cent w/v solution of cephalixin RS in water.

Reference solution (b). A solution containing 0.01 per cent w/v each of cephalixin RS and cephradine RS in water.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilica gel (5 µm) (such as Nucleosil C 18),
- mobile phase: a mixture of 2 volumes of methanol, 5 volumes of acetonitrile, 10 volumes of a 13.6 g per litre solution of potassium dihydrogen phosphate and 83 volumes of water,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject reference solution (b). Adjust the sensitivity so that the height of the peaks in the chromatogram obtained is at least half the full-scale deflection on the recorder. The test is not valid unless the resolution between the peaks corresponding to cephalixin and cephradine is at least 4.0.

Inject reference solution (a) six times. The relative standard deviation is not more than 1.0 per cent.

Inject alternately the test solution and reference solution (a).

Calculate the content of C_{16}H_{17}N_{3}O_{4}S in the tablets.

Storage. Store protected from light and moisture at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of anhydrous cephalixin.
Cephaloridine

![Chemical Structure of Cephaloridine](image)

C_{19}H_{17}N_{3}O_{4}S_{2}  
Mol. Wt. 415.5

Cephaloridine is (7R)-3-(1-pyridiniomethyl)-7-[(2-thienyl)-acetamido]-3-cephem-4-carboxylate (α-form or δ-form).

Cephaloridine contains not less than 96.0 per cent and not more than 102.0 per cent of C_{19}H_{17}N_{3}O_{4}S_{2}, calculated on the anhydrous basis.

**Description.** A white or almost white, crystalline powder; odour, slight and resembling that of pyridine.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cephaloridine (α-form) RS or cephaloridine (δ-form) RS or with the reference spectrum of cephaloridine (α-form) or cephaloridine (δ-form).

B. Mix 20 mg with a few drops of an 80 per cent v/v solution of chloramine containing 1 per cent v/v of nitric acid; a bluish-green colour is produced.

C. To a 0.5 per cent w/v solution add 1 ml of chlorammine solution and 2 ml of 0.1 M sodium hydroxide; a dull red colour is produced which persists for 1 minute.

D. Gives the reactions of penicillins and cephalosporins (2.3.1).

**Tests**

**Appearance of solution** (2.4.1). Solution A is clear.

**pH** (2.4.24). 4.0 to 6.0, determined in a 10.0 per cent w/v solution (solution A) prepared by dissolving in carbon dioxide-free water, warming to 30° and cooling to 20°.

**Specific optical rotation** (2.4.22). +46.0° to +50.0°, determined at 25° in a 1.0 per cent w/v solution.

**Light absorption.** When examined in the range 230 nm to 360 nm (2.4.7) a 0.0012 per cent w/v solution shows absorption maxima at about 240 nm and 255 nm; absorbance at the maximum at about 240 nm, 0.43 to 0.48. The ratio of the absorbance at the maximum at about 240 nm to that at about 255 nm is not more than 1.10.

**Pyridine.** Dissolve about 25 mg in 10 ml of water and add 2.5 ml of a buffer solution prepared by adjusting a 5 per cent w/v solution of disodium hydrogen phosphate to pH 6.0 with phosphoric acid and adding 1 per cent v/v of aniline. Add 1.25 ml of a solution prepared by decolorising with a 0.5 per cent v/v solution of bromine with potassium cyanide solution, shaking and allowing to stand for 2 minutes, and sufficient water to produce 25 ml and allow to stand for 25 minutes. Measure the absorbance of the resulting solution at the maximum at about 462 nm, using as the blank a solution prepared in a similar manner but omitting the substance under examination (2.4.7). The absorbance is not more than that of a solution prepared by treating 2.5 ml of a 0.005 per cent w/v solution of pyridine in a similar manner.

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Water** (2.3.43). Not more than 0.5 per cent w/w (α-form) and not more than 3.0 per cent w/w (δ-form), determined on 0.25 g. Use as the solvent a mixture of equal volumes of dehydrated methanol and dehydrated pyridine in place of methanol.

**Assay.** Weigh accurately about 60 mg and dissolve in sufficient water to produce 50.0 ml. Transfer 10.0 ml to a stoppered flask, add 5 ml of 1 M sodium hydroxide and allow to stand for 20 minutes. Add 20 ml of a buffer solution containing 4.0 to 6.0, determined in a 10.0 per cent w/v solution of sodium acetate and 44.2 per cent v/v of glacial acetic acid, 5 ml of 1 M hydrochloric acid and 25.0 ml of 0.01 M iodine, close the flask with a wet stopper and allow to stand for 3 hours in a water-bath at 30°, protected from light. Titrate the excess of iodine with 0.02 M sodium thiosulphate using starch solution, added towards the end of the titration, as indicator. To a further 10.0 ml of the solution add 20 ml of the buffer solution and 25.0 ml of 0.01 M iodine, allow to stand for 3 hours in a water-bath at 30°, protected from light. Titrate the excess of iodine with 0.02 M sodium thiosulphate using starch solution, added towards the end of the titration, as indicator. The difference between the titrations represents the volume of 0.01 M iodine equivalent to the cephaloridine present. Calculate the content of C_{19}H_{17}N_{3}O_{4}S_{2} from the difference obtained by simultaneously carrying out the Assay using cephaloridine (δ-form) RS instead of the substance under examination.

Cephaloridine intended for use in the manufacture of parenteral preparations complies with the following additional tests.

**Pyrogens.** Complies with the test for pyrogens (2.2.8), using not less than 50 mg per kg of the rabbit’s weight, dissolved in 1 ml of water for injection.

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage.** Store protected from light and moisture in a refrigerator (8° to 15°). If the material is intended for use in the manufacture of parenteral preparations, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

**Labelling.** The label states (1) whether the contents are Cephaloridine (α-form) or Cephaloridine (δ-form); (2) whether or not it is intended for use in the manufacture of injectable preparations.
Cephaloridine Injection

Cephaloridine Injection is a sterile material consisting of Cephaloridine with or without auxiliary substances. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of Water for Injections immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cephaloridine Injection contains not less than 90.0 per cent and not more than 105.0 per cent of the stated amount of anhydrous cephaloridine, C₁₉H₁₇N₃O₄S₂.

Description. A white or almost white, crystalline powder; odour, slight and resembling that of pyridine.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cephaloridine (α-form) RS or cephaloridine (δ-form) RS or with the reference spectrum of cephaloridine (α-form) or cephaloridine (δ-form).

B. Mix 20 mg with a few drops of an 80 per cent v/v solution of sulphuric acid containing 1 per cent v/v of nitric acid; a bluish-green colour is produced.

C. To a 0.5 per cent w/v solution add 1 ml of chloramine solution and 2 ml of 0.1 M sodium hydroxide; a dull red colour is produced which persists for 1 minute.

D. Gives the reactions of penicillins and cephalosporins (2.3.1).

Tests

Appearance of solution (2.4.1). Solution A is clear.

pH (2.4.24). 4.0 to 6.0, determined in a 10.0 per cent w/v solution (solution A) prepared by dissolving in carbon dioxide-free water, warming to 30° and cooling to 20°.

Specific optical rotation (2.4.22). +46.0° to +50.0°, determined at 25° in a 1.0 per cent w/v solution.

Light absorption. When examined in the range 230 nm to 360 nm (2.4.7) a 0.0012 per cent w/v solution shows absorption maxima at about 240 nm and 255 nm; absorbance at the maximum at about 240 nm, 0.43 to 0.48. The ratio of the absorbance at the maximum at about 240 nm to that at about 255 nm is not more than 1.10.

Pyridine. Dissolve about 25 mg in 10 ml of water and add 2.5 ml of a buffer solution prepared by adjusting a 5 per cent w/v solution of disodium hydrogen phosphate to pH 6.0 with phosphoric acid and adding 1 per cent v/v of aniline. Add 1.25 ml of a solution prepared by decolorising a 0.5 per cent v/v solution of bromine with potassium cyanide solution, shaking and allowing to stand for 2 minutes, and sufficient water to produce 25 ml and allow to stand for 25 minutes. Measure the absorbance of the resulting solution at the maximum at about 462 nm, using as the blank a solution prepared in a similar manner but omitting the substance under examination (2.4.7). The absorbance is not more than that of a solution prepared by treating 2.5 ml of a 0.005 per cent w/v solution of pyridine in a similar manner.

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 0.5 per cent w/w (α-form) and not more than 3.0 per cent w/w (δ-form), determined on 0.25 g. Use as the solvent a mixture of equal volumes of dehydrated methanol and dehydrated pyridine in place of methanol.

Assay. Determine the weight of the contents of 10 containers. Weigh accurately a quantity of the mixed contents of the 10 containers containing about 60 mg of cephaloridine and dissolve in sufficient water to produce 50.0 ml. Transfer 10.0 ml to a stoppered flask, add 5 ml of 1 M sodium hydroxide and allow to stand for 20 minutes. Add 20 ml of a buffer solution containing 35.0 per cent w/v of sodium acetate and 42.4 per cent v/v of glacial acetic acid, 5 ml of 1 M hydrochloric acid and 25.0 ml of 0.01 M iodine, close the flask with a wet stopper and allow to stand for 3 hours in a water-bath at 30°, protected from light. Titrate the excess of iodine with 0.02 M sodium thiosulphate using starch solution, added towards the end of the titration, as indicator. To a further 10.0 ml of the solution add 20 ml of the buffer solution and 25.0 ml of 0.01 M iodine, allow to stand for 3 hours in a water-bath at 30°, protected from light. Titrate the excess of iodine with 0.02 M sodium thiosulphate using starch solution, added towards the end of the titration, as indicator. The difference between the titrations represents the volume of 0.01 M iodine equivalent to the cephaloridine present. Calculate the content of C₁₉H₁₇N₃O₄S₂ from the difference obtained by simultaneously carrying out the Assay using cephaloridine (δ-form) RS instead of the substance under examination.

Cephaloridine intended for use in the manufacture of parenteral preparations complies with the following additional tests.

Pyrogens. Complies with the test for pyrogens (2.2.8), using not less than 50 mg per kg of the rabbit’s weight, dissolved in 1 ml of water for injection.

Sterility (2.2.11). Complies with the test for sterility.
Storage. Store protected from light and moisture at a temperature not exceeding 30°. The constituted solution should be used within 24 hours when stored at a temperature not exceeding 20° or within 4 days when stored in a refrigerator (2° to 8°).

Labelling. The label states (1) the weight of Cephaloridine contained in the sealed container; (2) whether the contents are Cephaloridine (α-form) or Cephaloridine (δ-form).

Cetirizine Hydrochloride

\[
\begin{align*}
\text{Cl} & \quad \text{N} \\
& \quad \text{N} \\
& \quad \text{O} \\
& \quad \text{COOH} \\
& \quad 2\text{HCl}
\end{align*}
\]

C₂₁H₂₅ClN₂O₃·2HCl  Mol. Wt. 461.8


Cetirizine Hydrochloride contains not less than 99.0 per cent and not more than 100.5 per cent of C₂₁H₂₅ClN₂O₃·2HCl, calculated on the dried basis.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cetirizine hydrochloride RS or with the reference spectrum of cetirizine hydrochloride.

B. Dissolve 20.0 mg in 50 ml of a 1.03 per cent w/v solution of hydrochloric acid and dilute to 100.0 ml with the same acid. Dilute 10.0 ml of this solution to 100.0 ml with the acid.

When examined in the range 210 nm to 350 nm (2.4.7), the resulting solution shows an absorption maximum at about 231 nm. The specific absorbance at 231 nm = 3.59 to 3.81.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF 254.

Mobile phase. A mixture of 1 volume of ammonia, 10 volumes of methanol and 90 volumes of dichloromethane.

Test solution. Dissolve 10 mg of the substance under examination in water and dilute to 5 ml with the same solvent.

Reference solution (a). Dissolve 10 mg of cetirizine hydrochloride RS in water and dilute to 5 ml with the same solvent.

Reference solution (b). Dissolve 10 mg of chlorphenamine maleate RS in water and dilute to 5 ml with the same solvent. To 1 ml of the solution add 1 ml of reference solution (a). Apply to the plate 5 µl of each solution. After development, dry in a current of cold air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

D. It gives reaction A of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in carbon dioxide-free water (solution A) is clear (2.4.1) and not more intensely coloured than reference solution BY7 (2.4.1).

pH (2.4.24). 1.2 to 1.8, determined in solution A.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in the mobile phase and dilute to 100 ml with the mobile phase.

Reference solution (a). A solution containing 0.02 per cent w/v each of cetirizine dihydrochloride RS and (RS)-1-[(4-chlorophenyl)phenylmethyl]piperazine RS (cetirizine impurity A) in the mobile phase. Dilute 1 ml of the solution to 100 ml with the mobile phase.

Reference solution (b). Dilute 2 ml of the test solution to 50 ml with the mobile phase. Dilute 5 ml of the solution to 100 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecysilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 0.4 volume of dilute sulphuric acid, 6.6 volumes of water and 93 volumes of acetonitril,
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- a 20 µl loop injector.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to cetirizine and impurity A is not less than 3 and the tailing factors are not more than 2.0.

Inject the test solution and reference solution (b). Run the chromatogram for 3 times the retention time of cetirizine. In the chromatogram obtained with the test solution, the area of any impurity peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of the areas of all such peaks is not more than 1.5 times the area of the principal peak.
in the chromatogram obtained with reference solution (b) (0.3 per cent). Ignore any peak with an area 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 100º to 105º.

**Assay.** Weigh accurately about 0.1 g, dissolve in 70 ml of a mixture of 30 volumes of water and 70 volumes of acetone. Titrate with 0.1 M sodium hydroxide to the second point of inflexion. Determine the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.01539 g of C_{21}H_{27}Cl_{3}N_{2}O_{3}.

**Storage.** Store protected from light.

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**Cetirizine Tablets**

Cetirizine Hydrochloride Tablets

Cetirizine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cetirizine hydrochloride, C_{21}H_{25}ClN_{2}O_{3}.2HCl.

**Identification**

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

**Tests**

**Dissolution** (2.5.2).

Apparatus No. 1

Medium. 900 ml of 0.1 M hydrochloric acid.

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted with the dissolution medium if necessary, at the maximum at about 230 nm (2.4.7). Calculate the content of C_{21}H_{25}ClN_{2}O_{3}.2HCl in the medium from the absorbance obtained from a solution of known concentration of cetirizine hydrochloride RS in the same medium.

D. Not less than 75 per cent of the stated amount of C_{21}H_{25}ClN_{2}O_{3}.2HCl.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 20 mg of Cetirizine Hydrochloride, add 50 ml of the mobile phase, mix and dilute to 100 ml with the mobile phase.

**Reference solution (a).** A solution containing 0.02 per cent w/v each of cetirizine hydrochloride RS and (RS)-1-[(4-chlorophenyl)phenylmethyl]piperazine RS (cetirizine impurity A) in the mobile phase. Dilute 1 ml of the solution to 100 ml with the mobile phase.

**Reference solution (b).** Dilute 1 ml of the test solution to 100 ml with the mobile phase.

**Chromatographic system**

- a stainless steel column 25 cm x 4.6 mm, packed with octadecysilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 0.4 volume of dilute sulphuric acid, 6.6 volumes of water and 93 volumes of acetonitrile,
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- a 20 µl loop injector.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to cetirizine and impurity A is not less than 3 and the tailing factors are not more than 2.0. Inject the test solution and reference solution (b). Run the chromatogram 3 times the retention time of cetirizine. In the chromatogram obtained with the test solution the area of any impurity peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). The sum of the areas of all such peaks is not more than 2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Uniformity of content.** Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.17), as described under Assay, using the following solution as the test solution.

**Test solution.** Disperse 1 tablet in the mobile phase, mix and dilute to 100.0 ml with the mobile phase, filter. Dilute 5.0 ml of the solution to 10.0 ml with mobile phase.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 25 mg of Cetirizine Hydrochloride, add the mobile phase, mix and dilute to 50.0 ml with the mobile phase, filter. Dilute 1.0 ml of the solution to 10.0 ml with mobile phase.

**Reference solution.** A 0.05 per cent w/v solution of cetirizine hydrochloride RS in the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.
Cetostearyl Alcohol

Cetostearyl Alcohol is a mixture of solid aliphatic alcohols consisting chiefly of stearyl and cetyl alcohols.

Description. A white or pale yellow, wax like mass, plates, flakes or granules.

Tests

Melting range (2.4.21). 47° to 56°, determined by Method II. Introduce the substance under examination into the capillary tubes and allow to stand at 2° to 8° for 12 hours before carrying out the determination.

Appearance of solution. Dissolve the substance under examination into the light petroleum (40° to 60°) and allow to stand for 12 hours before carrying out the determination.

Acid value (2.3.23). Not more than 1.0.

Hydroxyl value (2.3.27). 208 to 228.

Saponification value (2.3.37). Not more than 2.0.

Iodine value (2.3.28). Not more than 3.0, determined by Method B in a 8.0 per cent w/v solution in chloroform.

Hydrocarbons. Dissolve 2.0 g in 100 ml of light petroleum (40° to 60°), warming slightly if necessary, and transfer the solution to a column (25 cm x 10 mm) of anhydrous alumina which has been slurried with light petroleum (40° to 60°). Elute with two portions, each of 50 ml, of light petroleum (40° to 60°) into a flask, remove the light petroleum and dry at 80°; the residue weighs not more than 30 mg.

Cetrimide

Cetrimide consists chiefly of tetradecyltrimethylammonium bromide together with smaller amounts of dodecyl- and hexadecyltrimethylammonium bromides.

Cetrimide contains not less than 96.0 per cent and not more than 101.0 per cent of alkyltrimethylammonium bromides, calculated as C_{17}H_{38}BrN (336.4) on the dried basis.

Description. A white or creamy-white, voluminous, free-flowing powder; odour, faint and characteristic.

Identification

A. To 10 ml of a 1 per cent w/v solution add 2 ml of potassium ferricyanide solution; a yellow precipitate is produced.

B. To 10 ml of a 1 per cent w/v solution add 2 ml of a 10 per cent w/v solution of sodium silicate; a white flocculent precipitate is produced.

C. To 10 ml of a 1 per cent w/v solution add 2 ml of dilute nitric acid; a yellow precipitate is produced. Filter and to the filtrate add 2 ml of dilute nitric acid and 1 ml of silver nitrate solution; a yellow precipitate is produced.

D. Dissolve 0.25 g in sufficient ethanol (95 per cent) to produce 25 ml. Absorbance of the resulting solution between 260 nm and 280 nm is not more than 0.05 (2.4.7).

Tests

Appearance of solution. A 2.0 per cent w/v solution is clear (2.4.1) and colourless (2.4.1).

Acidity or alkalinity. Dissolve 1.0 g in 50 ml of water and add 2 drops of bromocresol purple solution. Not more than 0.1 ml of either 0.1 M hydrochloric acid or 0.1 M sodium hydroxide is required to change the colour of the solution.

Amine salts. Carry out the Assay described below using a further 25.0 ml of the original solution and 10 ml of 0.1 M hydrochloric acid instead of the 0.1 M sodium hydroxide. The difference between the volume of 0.05 M potassium iodate required in the titration and that required in the Assay is not more than 1.0 ml for each g of the substance used.

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

Assay. Weigh accurately about 2.0 g and dissolve in sufficient water to produce 100.0 ml. Transfer 25.0 ml of the solution to a separator, add 25 ml of chloroform, 10 ml of 0.1 M sodium hydroxide and 10.0 ml of a freshly prepared 5.0 per cent w/v solution of potassium iodide. Shake well, allow to separate, and discard the chloroform layer. Shake the aqueous solution with three quantities, each of 10 ml, of chloroform and discard.
the chloroform solution. Add 40 ml of hydrochloric acid, allow to cool and titrate with 0.05 M potassium iodate until the deep brown colour is almost discharged. Add 2 ml of chloroform and continue the titration, with shaking, until the chloroform layer no longer changes colour. Carry out a blank titration on a mixture of 20 ml of water, 10.0 ml of the freshly prepared potassium iodide solution and 40 ml of hydrochloric acid. The difference between the titrations represents the amount of potassium iodate required.

1 ml of 0.05 M potassium iodate is equivalent to 0.03364 g of C\textsubscript{7}H\textsubscript{3}BrN.

**Cetyl Alcohol**

Palmityl Alcohol; \(n\)-Hexadecyl Alcohol; 1-Hexadecanol

Cetyl Alcohol is a mixture of solid alcohols consisting mainly of 1-hexadecanol, C\textsubscript{16}H\textsubscript{33}O.

**Description**. A white, unctuous mass, powder, flakes or granules; odour, slight.

**Tests**

**Melting range** (2.4.21). 46° to 52°, determined by Method II. Introduce the substance under examination into the capillary tubes and allow to stand at 2° to 8° for 12 hours before carrying out the determination.

**Appearance of solution**. Dissolve 0.5 g in boiling ethanol (95 per cent), cool and dilute to 20 ml with the same solvent. The resulting solution is clear (2.4.1) and not more intensely coloured than reference solution BS6 (2.4.1).

**Acid value** (2.3.23). Not more than 1.0.

**Hydroxyl value** (2.3.27). 218 to 238.

**Saponification value** (2.3.37). Not more than 2.0.

**Iodine value** (2.3.28). Not more than 2.0, determined by Method B in a 8.0 per cent w/v solution in chloroform.

**Activated Charcoal**

Decolorising Charcoal

Activated Charcoal is obtained from vegetable matter by suitable carbonisation processes intended to confer a high adsorbing power.

**Description**. A light, black powder, free from grittiness; odourless.

**Identification**

A. When heated to redness, burns slowly without flame.

B. Complies with the test for Adsorbing power.

**Tests**

**Acidity or alkalinity**. Boil 2.0 g with 40 ml of water for 5 minutes. Cool, restore to the original volume with carbon dioxide-free water and filter, discarding the first 20 ml of the filtrate. To 10 ml of the filtrate add 0.25 ml of bromothymol blue solution and 0.25 ml of 0.02 M sodium hydroxide. The solution is blue and not more than 0.75 ml of 0.02 M hydrochloric acid is required to change the colour to yellow.

**Acid-soluble substances**. Boil 1.0 g with a mixture of 20 ml of water and 5 ml of hydrochloric acid for 5 minutes, filter whilst hot and collect the filtrate in a previously weighed porcelain crucible, wash the residue with 10 ml of hot water, adding the washing to the filtrate. To the combined filtrate and washing add 1 ml of hydrochloric acid, evaporate to dryness and ignite gently to constant weight; the residue weighs not more than 30 mg.

**Ethanol-soluble substances**. Boil 2.0 g with 50 ml of ethanol (95 per cent) under a reflux condenser for 10 minutes. Filter immediately, cool and adjust the volume to 50 ml with ethanol (95 per cent). The filtrate is not more intensely coloured than reference solution BS6 or YS6 (2.4.1). Evaporate 40 ml of the filtrate to dryness; the residue, after drying to constant weight at 105°, weighs not more than 8 mg.

**Alkali-soluble coloured matter**. Boil 0.25 g with 10 ml of 2 M sodium hydroxide for 1 minute, cool and filter; the filtrate, when diluted to 10 ml with water, is not more intensely coloured than reference solution GYS4 (2.4.1).

**Chlorides** (2.3.12). Boil 3.0 g with 75 ml of water for 5 minutes, cool. Dilute to 100.0 ml with water and filter; 6.0 ml of the filtrate complies with the limit test for chlorides. (0.14 per cent).

**Sulphates** (2.3.17). 10.0 ml of the filtrate obtained in the test for Chloride complies with the limit test for sulphates (450 ppm).

**Sulphide**. Heat 1.0 g with a mixture of 20 ml of water and 5 ml of 7 M hydrochloric acid to boiling; the fumes evolved do not turn lead acetate paper brown.

**Uncarbonised constituents**. Boil 0.25 g with 10 ml of 1 M sodium hydroxide for few seconds and filter; the filtrate is colourless.

**Copper**. Determine by atomic absorption spectrophotometry (2.4.2), measuring at 325.0 nm using an air-acetylene flame and a solution prepared in the following manner. Boil 2.0 g with 50 ml of 2 M hydrochloric acid under a reflux condenser for 1 hour. Filter, wash the filter with 2 M hydrochloric acid and evaporate the combined filtrate to dryness on a water-bath. Dissolve the residue in sufficient 0.1 M hydrochloric acid to produce 50.0 ml. Use copper solution AAS, suitably diluted with 0.1 M hydrochloric acid, for preparing the standard solutions (25 ppm).
Reserve the solution for the tests for Lead and Zinc.

**Lead.** Determine by atomic absorption spectrophotometry (2.4.2), measuring at 283.3 nm or 217.0 nm using an air-acetylene flame. Use the solution prepared in the test for Copper as the test solution and lead solution AAS, suitably diluted with 0.1 M hydrochloric acid, for preparing the standard solutions (10 ppm).

**Zinc.** Determine by atomic absorption spectrophotometry (2.4.2), measuring at 214.0 nm using an air-acetylene flame. Use the solution prepared in the test for Copper as the test solution and zinc solution AAS, suitably diluted with 0.1 M hydrochloric acid, for preparing the standard solutions (25 ppm).

**Sulphated ash** (2.3.18). Not more than 5.0 per cent.

**Loss on drying** (2.4.19). Not more than 15.0 per cent, determined on 1.0 g by drying in an oven at 120° for 4 hours.

**Adsorbing power.** Not less than 40 per cent of its own weight of phenazone, calculated on the dried basis, determined by the following method. To 0.3 g add 25 ml of a freshly prepared 1 per cent w/v solution of potassium bromide and 20 ml of 2 M hydrochloric acid and titrate with 0.0167 M potassium bromate, using 0.1 ml of methyl red solution as indicator, until the colour changes from reddish pink to yellowish pink and titrate slowly towards the end of the titration (a ml). Repeat the titration using 10 ml of the phenazone solution beginning at the words “add 1 g... titration” (b ml). Calculate the percentage of phenazone adsorbed with reference to the dried substance using the expression 2.353 \( \frac{(a-b)}{w} \) where w is the weight, in g, of the substance under examination.

**Storage.** Store protected from moisture.

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**Chlorambucil**

\[
\text{Cl} \quad \text{N} \quad \text{COOH}
\]

C\(_{14}\)H\(_{19}\)Cl\(_2\)NO\(_2\)  Mol. Wt. 304.2

Chlorambucil is 4-[4-bis(2-chloroethyl)amino]phenylbutyric acid.

Chlorambucil contains not less than 98.0 per cent and not more than 101.0 per cent of C\(_{14}\)H\(_{19}\)Cl\(_2\)NO\(_2\), calculated on the anhydrous basis.

**Description.** A white, crystalline powder.

**CAUTION —** Chlorambucil must be handled with care; contact with the skin and inhalation of airborne particles must be avoided.

**Identification**

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with chlorambucil RS.

B. Shake 0.4 g with 10 ml of 2 M hydrochloric acid and allow to stand for 30 minutes, shaking occasionally. Filter, wash the residue with two quantities, each of 10 ml, of water and add 0.5 ml of potassium mercuri-iodide solution to 10 ml of the mixed filtrate and washings; a buff precipitate is produced. To a further 10 ml add 0.5 ml of potassium permanganate solution; the purple colour is immediately discharged.

C. Dissolve 50 mg in 5 ml of acetone and dilute to 10 ml with water. Add 0.05 ml of 2 M nitric acid and 0.2 ml of dilute silver nitrate solution; no opalescence is produced immediately. Heat on a water-bath; an opalescence is produced.

**Tests**

**Related substances.** Carry out all operations as rapidly as possible, protected from light, and prepare the solutions immediately before use.

Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 40 volumes of toluene, 25 volumes of methanol, 20 volumes of 2-butanone and 20 volumes of n-heptane.

**Test solution.** A 2 per cent w/v solution in acetone.

**Reference solution (a).** Dilute 1 ml of the test solution to 50 ml with acetone.

**Reference solution (b).** Dilute 5 ml of reference solution (a) to 20 ml with acetone.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 0.5 per cent, determined on 1.0 g

**Assay.** Weigh accurately about 0.2 g, dissolve in 10 ml of acetone, add 10 ml of water and titrate with 0.1 M sodium hydroxide using dilute phenolphthalein solution as indicator.
1 ml of 0.1 M sodium hydroxide is equivalent to 0.03042 g of C₁₄H₁₉Cl₂NO₂.

**Storage.** Store protected from light.

### Chlorambucil Tablets

Chlorambucil Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of chlorambucil, C₁₄H₁₉Cl₂NO₂. The tablets are coated.

**Identification**

Shake 0.4 g of the powdered tablets with 10 ml of 2 M hydrochloric acid and allow to stand for 30 minutes, shaking occasionally. Filter, wash the residue with two quantities, each of 10 ml, of water and add 0.5 ml of potassium mercuri-iodide solution to 10 ml of the mixed filtrate and washings; a buff precipitate is produced. To a further 10 ml add 0.5 ml of potassium permanganate solution; the purple colour is immediately discharged.

**Tests**

**Uniformity of content.** Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve one tablet as completely as possible in 10 ml of 0.1 M hydrochloric acid, add 40 ml of acetonitrile and mix in an ultrasonic bath for 5 minutes. Add sufficient acetonitrile to produce a solution containing 0.002 per cent w/v of Chlorambucil. Filter the solution, preferably through a glass microfibre filter paper (such as Whatman GF/C), discarding the first 20 ml of the filtrate.

**Reference solution.** A 0.002 per cent w/v solution of chlorambucil RS in a mixture of 90 volumes of acetonitrile and 10 volumes of 0.1 M hydrochloric acid.

Carry out the chromatographic procedure described under Uniformity of content.

Calculate the content of C₁₄H₁₉Cl₂NO₂ in the tablets.

### Chloramphenicol

C₁₁H₁₂Cl₂N₂O₅ Mol. Wt. 323.1

Chloramphenicol is 2,2-dichloro-N-[(1R,2R)-2-hydroxy-1-hydroxymethyl-2-(4-nitrophenyl)ethyl]acetamide. It is produced by the growth of certain strains of *Streptomyces venezuelae* in a suitable medium, but is normally prepared by synthesis.

Chloramphenicol contains not less than 98.0 per cent and not more than 102.0 per cent of C₁₁H₁₂Cl₂N₂O₅, calculated on the dried basis.

**Description.** A white to greyish-white or yellowish-white, fine crystalline powder or fine-crystals, needles or elongated plates; odourless.

**Identification**

**Test A** may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with chloramphenicol RS or with the reference spectrum of chloramphenicol.

B. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

C. Dissolve 10 mg in 1 ml of ethanol (50 per cent), add 3 ml of a 1 per cent w/v solution of calcium chloride and 50 mg of
zinc powder and heat on a water-bath for 10 minutes. Decant the clear supernatant liquid into a test-tube, add 0.1 g of anhydrous sodium acetate and 0.1 ml of benzoyl chloride, shake for 1 minute and add 0.5 ml of a 10.5 per cent w/v solution of ferric chloride hexahydrate and, if necessary, add sufficient dilute hydrochloric acid to produce a clear solution; a red-violet to purple colour is produced. Repeat the test omitting the zinc powder; no red colour is produced.

D. Heat 50 mg with 2 ml of ethanolic potassium hydroxide solution in a covered test-tube on a water-bath for 15 minutes; the resulting solution gives the reactions of chlorides (2.3.1).

**Tests**

**pH** (2.4.24). 4.5 to 7.5, determined in a suspension prepared by shaking 50 mg with 10 ml of carbon dioxide-free water.

**Specific optical rotation** (2.4.22). +17.0° to +20.0°, determined in a 5.0 per cent w/v solution in ethanol.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 90 volumes of chloroform, 10 volumes of methanol and 1 volume of water.

**Test solution.** Dissolve 1 g of the substance under examination in 100 ml of acetone.

**Reference solution (a).** A 1 per cent w/v solution of chloramphenicol RS in acetone.

**Reference solution (b).** Dilute 0.5 ml of reference solution (a) to 100 ml with acetone.

Apply to the plate 1 µl and 20 µl of the test solution, 1 µl of reference solution (a) and 20 µl of reference solution (b). After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with 20 µl of the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

**Chlorides** (2.3.12). To 2.0 g add 20 ml of water and 10 ml of nitric acid and shake for 5 minutes. Filter through a filter paper previously washed by filtering 5-ml quantities of water until 5 ml of the filtrate is no longer opalescent on addition of 0.1 ml of nitric acid and 0.1 ml of a 4.25 per cent w/v solution of silver nitrate. The resulting filtrate complies with the limit test for chlorides (125 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.125 g and dissolve in sufficient water to produce 250.0 ml. Dilute 10.0 ml with sufficient water to produce 250.0 ml. Measure the absorbance of the resulting solution at the maximum at about 278 nm (2.4.7).

Calculate the content of $C_{11}H_{12}Cl_{2}N_{2}O_{5}$ taking 297 as the specific absorbance at 278 nm.

**Chloramphenicol intended for use in the manufacture of parenteral preparations without a further process for the removal of bacterial endotoxins complies with the following additional requirement.**

**Bacterial endotoxins** (2.2.3). Not more than 0.2 Endotoxin Unit per mg of chloramphenicol.

**Chloramphenicol intended for use in the manufacture of parenteral or ophthalmic preparations without a further sterilisation procedure complies with the following additional requirement.**

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage.** Store protected from light and moisture. If the material is intended for use in the manufacture of parenteral or ophthalmic preparations without further appropriate procedure of sterilisation, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

**Labelling.** The label states whether or not the contents are intended for use in the manufacture of parenteral or ophthalmic preparations.

**Chloramphenicol Capsules**

Chloramphenicol Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of chloramphenicol, $C_{11}H_{12}Cl_{2}N_{2}O_{5}$.

**Identification**

Suspend a quantity of the contents of the capsules containing about 1.25 g of Chloramphenicol in 60 ml of water and extract with two quantities, each of 20 ml, of light petroleum (60° to 80°) or light petroleum (100° to 120°). Wash the combined extracts with two quantities, each of 15 ml, of water; add the washings to the aqueous layer, extract with four quantities, each of 50 ml, of ether and remove the ether from the combined extracts. The residue, after drying to constant weight at 105°, complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with chloramphenicol RS or with the reference spectrum of chloramphenicol.

B. Heat 50 mg with 2 ml of ethanolic potassium hydroxide solution in a covered test-tube on a water-bath for 15 minutes; the resulting solution gives the reactions of chlorides (2.3.1).

**Tests**

**Specific optical rotation** (2.4.22). +17.0° to +20.0°, determined in a 5.0 per cent w/v solution in ethanol of the residue obtained in the test for Identification.
Apparatus. No. 1
Medium. 900 ml of 0.1 M hydrochloric acid.
Speed and time. 100 rpm and 30 minutes.
Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate.
Dilute 5.0 ml of the filtrate to 100.0 ml with the same solvent.
Measure the absorbance of the resulting solution at the maximum at about 278 nm (2.4.7). Calculate the content of C\textsubscript{11}H\textsubscript{12}Cl\textsubscript{2}N\textsubscript{2}O\textsubscript{5} taking 297 as the specific absorbance at 278 nm.
D. Not less than 85 per cent of the stated amount of C\textsubscript{11}H\textsubscript{12}Cl\textsubscript{2}N\textsubscript{2}O\textsubscript{5}.

Other tests. Comply with the tests stated under Capsules.

Assay. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 0.2 g of Chloramphenicol, dissolve in 800 ml of water, warming if necessary to effect solution and add sufficient water to produce 1000.0 ml. Dilute 10.0 ml of this solution to 100.0 ml with water and measure the absorbance of the resulting solution at the maximum at about 278 nm (2.4.7). Calculate the content of C\textsubscript{11}H\textsubscript{12}Cl\textsubscript{2}N\textsubscript{2}O\textsubscript{5}, taking 297 as the specific absorbance at 278 nm.

Storage. Store protected from moisture.

Chloramphenicol Eye Drops
Chloramphenicol Eye Drops are a sterile solution of Chloramphenicol in Purified water.

Chloramphenicol Eye Drops contain not less than 90.0 per cent and not more than 130.0 per cent of the stated amount of chloramphenicol, C\textsubscript{11}H\textsubscript{12}Cl\textsubscript{2}N\textsubscript{2}O\textsubscript{5}.

Identification
To a volume containing 50 mg of Chloramphenicol add 15 ml of water and extract with four quantities, each of 25 ml, of ether. Combine the extracts and evaporate to dryness. The residue complies with the following tests.

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G F254.

Mobile phase. A mixture of 90 volumes of chloroform, 10 volumes of methanol and 1 volume of water.

Test solution. Dissolve 0.1 g of the residue in sufficient ethanol (95 per cent) to produce 10 ml.

Reference solution. Dissolve 0.1 g of chloramphenicol RS in sufficient ethanol (95 per cent) to produce 10 ml.

Storage. Store in light resistant containers at a temperature not exceeding 30°.

Chloramphenicol Eye Ointment
Chloramphenicol Eye Ointment contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of chloramphenicol, C\textsubscript{11}H\textsubscript{12}Cl\textsubscript{2}N\textsubscript{2}O\textsubscript{5}.

Identification
Mix a quantity of the ointment containing 30 mg of Chloramphenicol with 10 ml of light petroleum (40° to 60°).
centrifuge and discard the supernatant liquid. Repeat this procedure using three quantities, each of 10 ml, of the same solvent. Combine the extracts and evaporate to dryness. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with chloramphenicol RS or with the reference spectrum of chloramphenicol.

B. Heat 50 mg with 2 ml of ethanolic potassium hydroxide solution in a covered test-tube on a water-bath for 15 minutes; the resulting solution gives the reactions of chlorides (2.3.1).

**Tests**

**Other tests.** Complies with the tests stated under Eye Ointments.

**Assay.** Determine by liquid chromatography (2.4.14).

*Test solution.* Transfer an accurately weighed quantity of the ointment, containing about 25 mg of Chloramphenicol, to a suitable conical flask, add 20 ml of cyclohexane, mix with the aid of ultrasound for about 2 minutes add 60 ml of methanol, and mix. Filter this mixture, collecting the filtrate in a 100-ml volumetric flask. Wash the filter with methanol, collecting the washings in the volumetric flask. Dilute with methanol to volume, and mix. Transfer 50.0 ml of the resulting solution to a suitable round-bottom flask, and evaporate to dryness by rotating the flask under vacuum in a water-bath at 35°. Dissolve the residue in 50.0 ml of methanol. Transfer 10.0 ml of the resulting solution to a 25 ml-volumetric flask, dilute with the mobile phase to volume, and mix. Filter a portion of this solution through a 0.5 µm or finer porosity filter, and use the clear filtrate.

*Reference solution.* A 0.01 per cent w/v solution of chloramphenicol RS in the mobile phase. Filter this solution through a 0.5 µm or finer porosity filter and use the clear filtrate.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 55 volumes of water, 45 volumes of methanol and 0.1 volume of glacial acetic acid,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- a 20 µl loop injector.

Inject alternately the test solution and the reference solution. Calculate the content of C_{11}H_{12}Cl_{2}N_{2}O_{5} in the ointment.

**Storage.** Store at a temperature not exceeding 30°.
C. Dissolve 10 mg in 4 ml of ethanol (95 per cent) and add 1 ml of 1 M sulphuric acid and 50 mg of zinc powder and allow to stand for 10 minutes. Filter, cool the filtrate in ice and add 0.5 ml of sodium nitrite solution and, after 2 minutes, 1 g of urea followed by 1 ml of 2-naphthol solution and 2 ml of 10 M sodium hydroxide; a red colour develops. Repeat the test omitting the zinc powder; no red colour is produced.

D. Heat 50 mg with 2 ml of ethanolic potassium hydroxide solution in a covered test-tube on a water-bath for 15 minutes; the resulting solution gives the reactions of chlorides (2.3.1).

Tests

Free acid. Dissolve 1.0 g by warming to 35° in 5 ml of a mixture of an equal volumes of ethanol (95 per cent) and ether and add 0.2 ml of phenolphthalein solution; not more than 0.4 ml of 0.1 M sodium hydroxide is required to produce a pink colour persisting for 30 seconds.

Specific optical rotation (2.4.22). +21.0° to +25.0°, determined in a 5.0 per cent w/v solution in ethanol.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 50 volumes of cyclohexane, 40 volumes of chloroform and 10 volumes of methanol.

Test solution. Dissolve 1 g of the substance under examination in 100 ml of acetone.

Reference solution. Dilute 2 ml of the test solution to 100 ml with acetone.

Apply to the plate 10 μl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Free chloramphenicol. Not more than 450 ppm, determined by the following method. Dissolve, with the aid of gentle heat, 1.0 g in 80 ml of xylene, cool and extract with three successive quantities, each of 15 ml, of water; discard the xylene and dilute the combined aqueous extracts to 50 ml with water. Extract the solution with 10 ml of carbon tetrachloride, allow to separate, discard the carbon tetrachloride and centrifugate a portion of the aqueous solution. Measure the absorbance of the clear aqueous solution at the maximum at about 278 nm, using as the blank a solution obtained by repeating the procedure without the substance under examination; the absorbance of this blank solution must not be greater than 0.05 (2.4.7). Calculate the content of free chloramphenicol, in ppm, from the expression (A x 10⁴)/5.96, where A is the absorbance of the clear aqueous solution of the substance under examination.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven over phosphorus pentoxide at 80° at a pressure not exceeding 0.1 kPa for 3 hours.

Assay. Weigh accurately about 60 mg and dissolve in sufficient ethanol (95 per cent) to produce 100.0 ml. Dilute 10.0 ml of this solution to 200.0 ml with ethanol (95 per cent) and measure the absorbance of the resulting solution at the maximum at about 271 nm (2.4.7). Calculate the content of C₁₁H₁₂Cl₂N₂O₅ taking 178 as the specific absorbance at 271 nm.

Storage. Store protected from light and moisture.

Chloramphenicol Oral Suspension

Chloramphenicol Palmitate Oral Suspension; Chloramphenicol Palmitate Mixture

Chloramphenicol Oral Suspension is a suspension of Chloramphenicol Palmitate in a suitable flavoured vehicle.

Chloramphenicol Oral Suspension contains not less than 95.0 per cent and not more than 115.0 per cent of the stated amount of chloramphenicol, C₁₁H₁₂Cl₂N₂O₅.

Identification

Extract a quantity of the suspension containing about 7.5 mg of chloramphenicol with 10 ml of chloroform and carefully evaporate the clear chloroform solution on a water-bath to dryness. Dissolve the residue in 250 ml of ethanol (95 per cent). When examined in the range 230 nm to 360 nm (2.4.7) the resulting solution shows an absorption maximum only at about 271 nm.

Tests

pH (2.4.24). 4.5 to 7.0.

Polymorph A. To a volume of the suspension containing 125 mg of chloramphenicol add 35 ml of water, mix, centrifuge for 40 minutes at not less than 18,000 rpm and discard the supernatant liquid. Wash the residue by adding 2 ml of water, triturating to form a paste, adding 18 ml of water; mixing thoroughly centrifuging and discarding the supernatant liquid. Wash the residue twice more in a similar manner, dry at 20° for 16 hours at a pressure not exceeding 0.7 kPa and grind to a fine powder. Prepare a mull of the residue by triturating a small quantity with about twice its weight of liquid paraffin until a smooth creamy paste is obtained. Determine by infrared absorption spectrophotometry (2.4.6) over the range 770 cm⁻¹ to 910 cm⁻¹ using conditions such that between 20 per cent and 30 per cent transmittance occurs at 810 cm⁻¹ to 910 cm⁻¹. Repeat the operation using a mull prepared with a standard mixture obtained by mixing together thoroughly 1 part by weight of chloramphenicol palmitate (polymorph A) RS and 9 parts by weight of chloramphenicol palmitate RS. On each
of the spectra, draw a straight base line between the minima occurring at about 880 cm⁻¹ and 790 cm⁻¹ and using these base lines measure the heights of the peaks occurring at the maxima at about 858 cm⁻¹ and 840 cm⁻¹. In the spectrum obtained with preparation under examination, the ratio of the peak height at about 858 cm⁻¹ to that at the maximum at about 840 cm⁻¹ is greater than the corresponding ratio in the spectrum obtained with the standard mixture.

Other tests. Complies with the tests stated under Oral Liquids.

Assay. Weigh accurately a quantity of the suspension containing about 125 mg of chloramphenicol, add 10 ml of water and shake with four quantities, each of 20 ml, of chloroform, filtering each extract through cotton wool, previously washed with chloroform, into a 100-ml volumetric flask. Dilute to volume with chloroform and mix well. Dilute 2.0 ml of this solution to 100.0 with ethanol (95 per cent) and measure the absorbance of the resulting solution at the maximum at about 271 nm using 1 ml of chloroform diluted to 50 ml with ethanol (95 per cent) as the blank (2.4.7). Calculate the content of chloramphenicol palmitate, C₂₇H₄₂Cl₂N₂O₆, taking 178 as the specific absorbance at 271 nm.

Determine the weight per ml of the suspension (2.4.29) and calculate the content of chloramphenicol, C₁₅H₁₅Cl₂N₂NaO₈, weight in volume using a factor of 0.575 for the conversion of the content of chloramphenicol palmitate to chloramphenicol.

Storage. Store protected from light.

Labelling. The label states (1) the strength in terms of the equivalent amount of chloramphenicol; (2) that if the preparation is diluted, it must be used immediately after dilution.

Chloramphenicol Sodium Succinate

![Chemical structure of Chloramphenicol Sodium Succinate](image)

\[ \text{C}_{15}\text{H}_{15}\text{Cl}_{2}\text{N}_{2}\text{NaO}_{8} \]

\[ \text{Mol. Wt. 445.2} \]

Chloramphenicol Sodium Succinate is a mixture of variable proportions of sodium (2R,3R)-2-(2,2-dichloacetamido)-3-hydroxy-3-(4-nitrophenyl)propyl succinate (3-isomer) and of sodium (1R,2R)-2-(2,2-dichloacetamido)-3-hydroxy-1-(4-nitrophenyl)propyl succinate (1-isomer).

Chloramphenicol Sodium Succinate contains not less than 98.0 per cent and not more than 102.0 per cent of C₁₅H₁₅Cl₂N₂NaO₈, calculated on the anhydrous basis.

Description. A white or yellowish-white powder; hygroscopic.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 85 volumes of chloroform, 14 volumes of methanol and 1 volume of 2 M acetic acid.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of acetone.

Reference solution (a). A 1 per cent w/v solution of chloramphenicol sodium succinate RS in acetone.

Reference solution (b). A 1 per cent w/v solution of chloramphenicol RS in acetone.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The two principal spots in the chromatogram obtained with the test solution are similar in position and size to those in the chromatogram obtained with reference solution (a) and their positions are different from that of the principal spot in the chromatogram obtained with reference solution (b).

B. Dissolve 10 mg in 2 ml of ethanol (95 per cent) add 4.5 ml of 1 M sulphuric acid and 50 mg of zinc powder, allow to stand for 10 minutes and decant the supernatant liquid or filter, if necessary. Cool the resulting solution in ice and add 0.5 ml of sodium nitrite solution and, after 2 minutes, 1 g of urea followed by 1 ml of 2-naphthol solution and 2 ml of 10 M sodium hydroxide; a red colour develops. Repeat the test omitting the zinc powder; no red colour is produced.

C. To 5 ml of a 0.1 per cent w/v solution add a few drops of silver nitrate solution; no precipitate is produced. Heat 50 mg with 2 ml of ethanolic potassium hydroxide solution on a water-bath for 15 minutes, add 50 mg of decolorising charcoal, shake and filter. The filtrate when treated with silver nitrate solution, yields a curdy precipitate which is insoluble in nitric acid but soluble, after being well washed with water; in dilute ammonia solution from which it is precipitated on addition of nitric acid.

D. A 5 per cent w/v solution gives the reactions of sodium salts (2.3.1).

Tests

pH (2.4.24). 6.4 to 7.0, determined in a 25.0 per cent w/v solution.

Specific optical rotation (2.4.22). +5.0° to +8.0°, determined in a 5.0 per cent w/v solution.

Free chloramphenicol. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 90 volumes of chloroform, 10 volumes of methanol and 1 volume of water.
Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of acetone.

Reference solution. A 0.02 per cent w/v solution of chloramphenicol RS in acetone.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any spot corresponding to chloramphenicol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Water (2.3.43). Not more than 2.0 per cent, determined on 0.3 g.

Assay. Weigh accurately about 0.2 g and dissolve in sufficient water to produce 500.0 ml; dilute 5.0 ml of this solution to 100.0 ml with water and measure the absorbance of the resulting solution at the maximum at about 276 nm (2.4.7). Calculate the content of \( \text{C}_{11} \text{H}_{12} \text{Cl}_{2} \text{N}_{2} \text{O}_{5} \) taking 220 as the specific absorbance at 276 nm.

Chloramphenicol Sodium Succinate intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.2 Endotoxin Unit per mg of chloramphenicol.

Chloramphenicol Sodium Succinate intended for use in the manufacture of parenteral preparations without a further sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light and moisture. If the material is intended for use in the manufacture of parenteral preparations, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

Labelling. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

Chloramphenicol Sodium Succinate Injection

Chloramphenicol Sodium Succinate Injection is a sterile material consisting of Chloramphenicol Sodium Succinate with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Chloramphenicol Sodium Succinate Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of chloramphenicol, \( \text{C}_{11} \text{H}_{12} \text{Cl}_{2} \text{N}_{2} \text{O}_{5} \).

Description. A white or yellowish-white powder; hygroscopic.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 85 volumes of chloroform, 14 volumes of methanol and 1 volume of 2 M acetic acid.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of acetone.

Reference Solution (a). A 1 per cent w/v solution of chloramphenicol sodium succinate RS in acetone.

Reference solution (b). A 1 per cent w/v solution of chloramphenicol RS in acetone.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The two principal spots in the chromatogram obtained with the test solution are similar in position and size to those in the chromatogram obtained with reference solution (a) and their positions are different from that of the principal spot in the chromatogram obtained with reference solution (b).

B. Dissolve 10 mg in 2 ml of ethanol (95 per cent) add 4.5 ml of 1 M sulphuric acid and 50 mg of zinc powder, allow to stand for 10 minutes and decant the supernatant liquid or filter, if necessary. Cool the resulting solution in ice and add 0.5 ml of sodium nitrite solution and, after 2 minutes, 1 g of urea followed by 1 ml of 2-naphthol solution and 2 ml of 10 M sodium hydroxide; a red colour develops. Repeat the test omitting the zinc powder; no red colour is produced.

C. To 5 ml of a 0.1 per cent w/v solution add a few drops of silver nitrate solution; no precipitate is produced. Heat 50 mg with 2 ml of ethanolic potassium hydroxide solution on a water-bath for 15 minutes, add 50 mg of decolorising charcoal, shake and filter. The filtrate when treated with silver nitrate solution, yields a curdy precipitate which is insoluble in nitric acid but soluble, after being well washed with water; in dilute ammonia solution from which it is reprecipitated on addition of nitric acid.

D. A 5 per cent w/v solution gives the reactions of sodium salts (2.3.1).
Tests

**pH** (2.4.24). 6.4 to 7.0, determined in a 25.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +5.0° to +8.0°, determined in a 5.0 per cent w/v solution.

**Free chloramphenicol.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel* GF254.

**Mobile phase.** A mixture of 90 volumes of *chloroform*, 10 volumes of *methanol* and 1 volume of *water*.

**Test solution.** Dissolve 0.1 g of the substance under examination and dissolve in 10 ml of *acetone*.

**Reference solution.** A 0.02 per cent w/v solution of chloramphenicol RS in *acetone*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any spot corresponding to chloramphenicol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Water** (2.3.43). Not more than 2.0 per cent, determined on 0.3 g.

**Bacterial endotoxins** (2.2.3). Not more than 0.2 Endotoxin Unit per mg of chloramphenicol.

**Sterility** (2.2.11). Complies with the test for sterility.

**Assay.** Determine the weight of the contents of 10 containers. Weigh accurately about 0.2 g of the mixed contents of the 10 containers and dissolve in sufficient *water* to produce 500.0 ml; dilute 5.0 ml of this solution to 100.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 276 nm (2.4.7). Calculate the content of C_{15}H_{15}Cl_{2}N_{2}NaO_{8} taking 220 as the specific absorbance at 276 nm.

**Storage.** Store protected from light and moisture.

**Labelling.** The label states the quantity of Chloramphenicol Sodium Succinate in the sealed container in terms of the equivalent amount of chloramphenicol.

**Chlorbutol**

![Chlorbutol chemical structure](image)

C_{6}H_{12}Cl_{3}O, ½H_{2}O  
Mol.Wt. 186.5

Chlorbutol is 1,1,1-trichloro-2-methylpropan-2-ol hemihydrate.

Chlorbutol contains not less than 98.0 per cent and not more than 101.0 per cent of C_{6}H_{12}Cl_{3}O, calculated on the anhydrous basis.

**Description.** Colourless crystals or a white, crystalline powder; odour, characteristic and somewhat camphoraceous; sublimes readily.

**Identification**

A. To 5 ml of a freshly prepared 0.5 per cent w/v solution add 1 ml of 1 M sodium hydroxide and then, slowly, 2 ml of *iodine solution*; a yellow precipitate of iodoform is produced.

B. Heat about 20 mg with 2 ml of 10 M sodium hydroxide and 1 ml of *pyridine* on a water-bath and shake; the separated pyridine layer becomes red.

C. Warm gently about 20 mg with 5 ml of *ammoniacal silver nitrate solution*; a black precipitate is produced.

**Tests**

**Appearance of solution.** A 50.0 per cent w/v solution in *ethanol (95 per cent)* is not more opalescent than opalescence standard OS2 (2.4.1), and not more intensely coloured than reference solution BYS5 (2.4.1).

**Acidity.** Dissolve 2.0 g in 20 ml of *ethanol (95 per cent)*, add 0.1 ml of bromothymol blue solution and titrate with 0.1 M sodium hydroxide; not more than 0.1 ml of 0.1 M sodium hydroxide is required to change the colour of the solution.

**Chlorides** (2.3.12). 0.5 g dissolved in 10 ml of *ethanol (95 per cent)* complies with the limit test for chlorides (500 ppm). Use 5 ml of *ethanol (95 per cent)* in place of 5 ml of *water* to prepare the standard.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). 4.5 per cent to 6.0 per cent, determined on 0.3 g.

**Assay.** Weigh accurately about 0.2 g and dissolve in 5 ml of *ethanol (95 per cent)*. Add 5 ml of sodium hydroxide solution and boil under a reflux condenser for 15 minutes. Cool, dilute with 20 ml of *water*; add 5 ml of *nitric acid*, 1 ml of *nitrobenzene* and 50.0 ml of 0.1 M *silver nitrate* and shake vigorously for 1 minute. Add 4 ml of ferric ammonium sulphate solution and titrate the excess of silver nitrate with 0.1 M ammonium thiocyanate.

1 ml of 0.1 M silver nitrate is equivalent to 0.005917 g of C_{6}H_{12}Cl_{3}O.

**Storage.** Store protected from moisture at a temperature not exceeding 30°.
Chlorcyclizine Hydrochloride

**Formula**: C₁₈H₂₁ClN₂.HCl

**Molar Weight**: 337.3

Chlorcyclizine Hydrochloride is 1-(4-chlorobenzhydryl)-4-methylpiperazine hydrochloride.

Chlorcyclizine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of the stated amount of C₁₈H₂₁ClN₂.HCl, calculated on the dried basis.

**Description**. A white crystalline powder.

**Identification**

*Test A* may be omitted if *tests B, C and D* are carried out. *Tests B and C* may be omitted if *tests A and D* are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with chlorcyclizine hydrochloride RS or with the reference spectrum of chlorcyclizine hydrochloride.

B. Weigh accurately about 10 mg, dissolve in 100 ml of 0.5 per cent w/v of sulphuric acid. Dilute 10 ml of the solution to 100 ml with 0.5 per cent w/v sulphuric acid. When examined in the range 215 to 300 nm (2.4.7), exhibits maximum only at about 231 nm; absorbance at about 231 nm, about 0.475 to 0.525.

C. In the test for Related substances, the principle spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

D. Gives the reactions of chlorides (2.3.1).

**Tests**

**Appearance of solution**. A 5.0 per cent w/v solution is clear (2.4.1), and colourless (2.4.1).

**pH** (2.4.24). 5.0 to 6.0, determined in a 1.0 per cent w/v solution.

**Related substance**. Determine by thin layer chromatography (2.4.17). coating the plate with silica gel.

*Mobile phase*. A mixture of 85 volumes of dichloromethane, 13 volumes of methanol and 2 volumes of strong ammonia solution.

*Test solution (a)*. A 2.0 per cent w/v solution of the substance under examination in methanol.

*Test solution (b)*. A 0.1 per cent w/v solution of the substance under examination in methanol.

*Reference solution (a)*. A 0.10 per cent w/v solution of chlorcyclizine hydrochloride RS in methanol.

*Reference solution (b)*. A 0.01 per cent w/v solution of methylpiperazine RS in methanol.

*Reference solution (c)*. A 0.004 per cent w/v solution of the substance under examination in methanol.

*Reference solution (d)*. 0.10 per cent w/v each of hydroxyzine hydrochloride RS and chlorcyclizine hydrochloride RS in methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and expose to iodine vapours for 10 minutes. Any secondary spot in the chromatogram obtained with the test solution (a) is not more intense than the corresponding spot in the chromatogram obtained with the test solution (b). Any spot other than the principle spot but corresponding to the spot obtained with reference solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.50 per cent). The test is not valid unless the chromatogram obtained with reference solution (d) shows to clearly separated spot.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 130°.

**Assay**. Weigh accurately about 0.2 g, dissolve in 1 ml of 0.1 M hydrochloric acid and add 50 ml of methanol. Titrate with 0.1 M sodium hydroxide, determining the end-point potentiometrically (2.4.25). Carry out a blank titration. 1 ml of 0.1 M sodium hydroxide is equivalent to 0.03373 g of C₁₈H₂₁ClN₂.HCl.

**Storage**. Store protected from light and moisture.

Chlordiazepoxide

**Formula**: C₁₆H₁₄ClN₃O

**Molar Weight**: 299.8

Chlordiazepoxide is 7-chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepine 4-oxide.
Chlordiazepoxide contains not less than 99.0 per cent and not more than 101.0 per cent of C\textsubscript{16}H\textsubscript{14}ClN\textsubscript{3}O, calculated on the dried basis.

**Description.** An almost white to light yellow, crystalline powder; practically odourless.

**Identification**

*Test A* may be omitted if *tests B and C* are carried out. *Tests B and C* may be omitted if *test A* is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with chlordiazepoxide RS.

B. When examined in the range 230 nm to 360 nm (2.4.7) a 0.0005 per cent w/v solution prepared immediately before use in subdued light in 0.1 M hydrochloric acid shows absorption maxima at about 246 nm and 308 nm. Absorbance at the maximum at about 246 nm, 0.56 to 0.60 and at the maximum at about 308 nm, 0.16 to 0.17.

C. Dissolve 0.2 g in 4 ml of hot dilute hydrochloric acid, heat at 100° for 10 minutes, cool and filter. 2 ml of the filtrate gives the reactions of primary aromatic amines (2.3.1).

**Tests**

**Related substances.** Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with silica gel GF\textsubscript{254}.

*Mobile phase.* A mixture of 70 volumes of toluene, 15 volumes of ethyl acetate, 10 volumes of ethanol (95 per cent), 4 volumes of diethylamine and 1 volume of water.

*Prepare the following solutions immediately before use.*

*Test solution.* Dissolve 0.2 g in 10 ml of a mixture of 12 volumes of methanol and 8 volumes of toluene.

*Reference solution (a).* Dilute 1 ml of the test solution to 10 ml with the same solvent mixture.

*Reference solution (b).* Dilute 5 ml of reference solution (a) to 100 ml with the same solvent mixture.

*Reference solution (c).* A 0.005 per cent w/v solution of 2-amino-5-chlorobenzophenone in the same solvent mixture.

*Reference solution (d).* A 0.2 per cent w/v solution of chlordiazepoxide RS in the same solvent mixture.

Apply to the plate 25 µl of the test solution as five quantities, each of 5 µl, at one point, allowing the solvent to evaporate between applications, and 5 µl of each of reference solutions (a), (b), (c) and (d). After development dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b). Spray the plate with a freshly prepared 1 per cent w/v solution of sodium nitrite in 1 M hydrochloric acid, dry it in a current of air and spray with 0.4 per cent w/v solution of N-(1-naphthyl)ethylenediamine dihydrochloride in ethanol (95 per cent). Any violet spot in the chromatogram obtained with the test solution corresponding to 2-amino-5-chlorobenzophenone is not more intense than the spot in the chromatogram obtained with reference solution (c).

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.25 g and dissolve by heating, if necessary, in 80 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration. 1 ml of 0.1 M perchloric acid is equivalent to 0.02998 g of C\textsubscript{16}H\textsubscript{14}ClN\textsubscript{3}O.

**Storage.** Store protected from light and moisture.

### Chlordiazepoxide Tablets

Chlordiazepoxide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of chlordiazepoxide, C\textsubscript{16}H\textsubscript{14}ClN\textsubscript{3}O. The tablets may be coated.

**Identification**

A. Dilute 1 ml of the final solution obtained in the Assay to 2 ml with 0.1 M hydrochloric acid. When examined in the range 230 nm to 360 nm (2.4.7) the resulting solution shows absorption maxima at about 246 nm and 308 nm.

B. To a quantity of the powdered tablets containing 0.2 g of Chlordiazepoxide add 4 ml of hot 2 M hydrochloric acid, heat at 100° for 10 minutes, cool and filter; 2 ml of the filtrate gives the reactions of primary aromatic amines (2.3.1).

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF\textsubscript{254}.

*Mobile phase.* A mixture of 85 volumes of chloroform, 14 volumes of methanol and 1 volume of strong ammonia solution.

*Test solution.* Shake a quantity of the powdered tablets containing 0.1 g of Chlordiazepoxide with 10 ml of a mixture of
Chlorhexidine Gluconate Solution

Chlorhexidine Gluconate Solution is an aqueous solution of 1,1’-hexamethylenbis [5-(4-chlorophenyl)biguanide] digluconate.

Chlorhexidine Gluconate Solution contains not less than 19.0 per cent w/v and not more than 21.0 per cent w/v of C_{22}H_{30}Cl_{2}N_{10.2}C_{6}H_{12}O_{7}.

Description. An almost colourless or pale yellowish, clear or slightly opalescent liquid; almost odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.

A. To 2 ml add 80 ml of water, cool in ice, add 5 M sodium hydroxide dropwise with stirring until the solution is slightly alkaline to titan yellow paper and add 2 ml in excess. Filter, wash the precipitate with water until the washings are free from alkali, dissolve it in about 25 ml of ethanol on a boiling water-bath and heat until the volume is reduced to about 5 ml. Cool in ice, induce crystallisation, if necessary, by scratching the side of the vessel with a glass rod, filter and dry the crystals at 105°. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with chlorhexidine RS or with the reference spectrum of chlorhexidine. Examine the substance as a dispersion in potassium bromide IR without excessive grinding.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 50 volumes of ethanol (95 per cent), 30 volumes of water, 10 volumes of strong ammonia solution and 10 volumes of ethyl acetate.

Test solution. Dilute 10 ml of the substance under examination to 50 ml with water.

Reference solution. A 2.5 per cent w/v solution of calcium gluconate RS in water.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate at 100° for 20 minutes, allow to cool, spray with a 5 per cent w/v solution of potassium dichromate in a 40 per cent w/w solution of sulphuric acid and allow to stand for 5 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. To 0.5 ml add 10 ml of water and 0.5 ml of cupric sulphate solution; a white precipitate is produced which on boiling flocculates and changes to a pale purple colour.
D. To 0.05 ml add 5 ml of a 1 per cent w/v solution of cetrimide, 1 ml of 10 M sodium hydroxide and 1 ml of bromine water; a deep red colour is produced.

**Tests**

**pH** (2.4.24). 5.5 to 7.0, determined in a solution obtained by diluting 5 ml to 100 ml.

**Weight per ml** (2.4.29). 1.06 g to 1.07 g, determined at 20°.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating a 0.5-mm thick plate with a slurry consisting of 8 g of silica gel GF254 and 16 ml of water containing 1 g of sodium formate.

**Mobile phase.** A mixture of 50 volumes of chloroform, 50 volumes of ethanol (95 per cent) and 7 volumes of formic acid.

**Test solution.** Dilute 1 ml of the substance under examination to 20 ml with 1.5 M acetic acid.

Apply to the plate, in the form of a band 4 cm wide, 20 µl of the test solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Mark the area around each group of bands above and below the principal band, transfer quantitatively the enclosed areas of silica gel to a glass-stoppered tube, add 5.0 ml of methanol, shake for 15 minutes, centrifuge and measure the absorbance of the clear, supernatant liquid at the maximum at about 256 nm (2.4.7), using as the blank a solution prepared by heating in a similar manner equivalent-sized areas of silica gel removed from the coating adjacent to the areas previously removed. The absorbance is not more than that obtained with a solution prepared by diluting 2 ml of the substance under examination with sufficient 1.5 M acetic acid to produce 10 ml and diluting 0.2 ml of this solution to 50 ml with methanol.

**4-Chloroaniline.** Not more than 0.25 per cent, calculated with reference to chlorhexidine solution at a nominal concentration of 20 per cent w/v, determined by the following method. Dilute 2.0 ml to 100.0 with water. To 10.0 ml of this solution add 2.5 ml of 2 M hydrochloric acid and dilute to 20 ml with water. Add rapidly, with continuous mixing after each addition, 0.35 ml of sodium nitrite solution, 2 ml of a 5 per cent w/v solution of ammonium sulphamate and 5 ml of a 0.01 per cent w/v solution of N-(1-naphthyl) ethylenediamine dihydrochloride. Add 1 ml of ethanol (95 per cent) and sufficient water to produce 50 ml, mix and set aside for 30 minutes. Any reddish blue colour produced is not more intense than that produced by treating at the same time in the same manner a mixture of 10.0 ml of 0.001 per cent w/v solution of 4-chloroaniline in 2 M hydrochloric acid and 10 ml of water in place of the dilution of the substance under examination.

**Assay.** Weigh accurately about 1.0 g and evaporate to a low bulk. Dissolve in 50 ml of anhydrous glacial acetic acid.

Titr ate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02244 g of C₂₂H₃₀Cl₂N₁₀₂C₂H₄O₇.

Determine the weight per ml (2.4.29) and calculate the percentage content of C₂₂H₃₀Cl₂N₁₀₂C₆H₁₂O₇, weight in volume.

**Storage.** Store protected from light.

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**Chlorhexidine Acetate**

![Chemical Structure](image)

C₂₂H₃₀Cl₂N₁₀₂C₂H₄O₇  Mol. Wt. 625.6

Chlorhexidine Acetate is 1,1’-(hexane-1,6-diyl)bis[5-(4-chlorophenyl)biguanide] diacetate.

Chlorhexidine Acetate contains not less than 98.0 per cent and not more than 101.0 per cent of chlorhexidine diacetate, C₂₂H₃₀Cl₂N₁₀₂C₆H₁₂O₇, calculated on the dried basis.

**Description.** A white or almost white, microcrystalline powder.

**Identification**

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with chlorhexidine acetate RS.

B. Dissolve about 5 mg in 5 ml of a warm 1.0 per cent w/v solution of cetrimide and add 1 ml of strong sodium hydroxide solution and 1 ml of bromine water. A deep red colour is produced.

C. Dissolve 0.3 g in 10 ml of a mixture of equal volumes of hydrochloric acid and water. Add 40 ml of water, filter if necessary and cool in ice water. Make alkaline to titan yellow paper by adding dropwise and with stirring strong sodium hydroxide solution and add 1 ml in excess. Filter, wash the precipitate with water until the washings are free from alkali and recrystallise from alcohol (70 per cent w/v). Dry at 100° to 105°. Melting point (2.4.21). 132° to 136°.
D. It gives reaction (a) of acetates (2.3.1).

**Tests**

**Chloroaniline.** Dissolve 0.2 g of the substance under examination in 25 ml of water with shaking if necessary. Add 1 ml of hydrochloric acid and dilute to 30 ml with water. Add rapidly and with thorough mixing after each addition, 2.5 ml of dilute hydrochloric acid, 0.35 ml of sodium nitrite solution, 2 ml of a 5.0 per cent w/v solution of ammonium sulphamate and 1 ml of alcohol, dilute to 50.0 ml with water and allow to stand for 30 minutes. Any reddish-blue colour in the solution is not more intense than that in a standard prepared at the same time in the same manner using a mixture of 10.0 ml of 0.001 per cent w/v solution of chloroaniline in dilute hydrochloric acid and 20 ml of dilute hydrochloric acid instead of the solution of the substance under examination (500 ppm).

**Related substances.** Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.2 g of the substance under examination in 100 ml of the mobile phase.

Reference solution (a). A 0.15 per cent w/v solution of chlorhexidine acetate RS in the mobile phase.

Reference solution (b). Dilute 2.5 ml of the test solution to 100 ml with the mobile phase.

Reference solution (c) Dilute 2 ml of reference solution (b) to 10 ml with the mobile phase. Further dilute 1 ml of this solution to 10 ml with the mobile phase.

Chromatographic system
- a stainless steel column 20 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 2.0 g of sodium octanesulphonate in a mixture of 120 ml of glacial acetic acid, 270 ml of water and 730 ml of methanol,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 10 µl loop injector.

Equilibrate the column with the mobile phase for at least 1 hour. Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is at least 50 per cent of the full scale of the recorder.

Inject the test solution and reference solutions (a), (b) and (c). Record the chromatograms of reference solutions (b) and (c) until the peak due to chlorhexidine has been eluted and record the chromatogram of the test solution for six times the retention time of the peak due to chlorhexidine. In the chromatogram obtained with the test solution, the sum of the areas of all the peaks, other than the principal peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent). Ignore any peak with a relative retention time of 0.25 or less with respect to the principal peak and any peak whose area is less than that of the principal peak in the chromatogram obtained with reference solution (c).

**Loss on drying** (2.4.19). Not more than 3.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Sulphated ash** (2.3.18). Not more than 0.15 per cent.

**Assay.** Dissolve 0.14 g in 100 ml of anhydrous acetic acid and titrate with 0.1 M perchloric acid determining the end-point potentiometrically (2.4.25).

1 ml of 0.1 M perchloric acid is equivalent to 0.01564 g of C_{22}H_{30}Cl_{2}N_{10}O_{4}.

**Chlorhexidine Hydrochloride**

![Chlorhexidine Hydrochloride molecule](image)

\[ \text{C}_{22}\text{H}_{30}\text{Cl}_{2}\text{N}_{10}\text{2HCl} \quad \text{Mol. Wt. 578.4} \]

Chlorhexidine Hydrochloride is 1,1'-(hexane-1,6-diyl)bis[5-(4-chlorophenyl)biguanide] dihydrochloride.

Chlorhexidine Hydrochloride contains not less than 98.0 per cent and not more than 101.0 per cent of chlorhexidine dihydrochloride, C_{22}H_{30}Cl_{2}N_{10}·2HCl calculated on the dried basis.

**Description.** A white or almost white, crystalline powder.

**Identification**

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with chlorhexidine hydrochloride RS.

B. Dissolve about 5 mg in 5 ml of a warm 1.0 per cent w/v solution of cetrimide and add 1 ml of strong sodium hydroxide solution and 1 ml of bromine water. A deep red colour is produced.
C. Dissolve 0.3 g in 10 ml of a mixture of equal volumes of hydrochloric acid and water. Add 40 ml of water, filter if necessary and cool in ice water. Make alkaline to titan yellow paper by adding dropwise and with stirring strong sodium hydroxide solution and add 1 ml in excess. Filter, wash the precipitate with water until the washings are free from alkali and recrystallise from alcohol (70 per cent v/v). Dry at 100° to 105°. Melting point (2.4.21). 132° to 136°.

D. It gives reaction (a) of chlorides (2.3.1).

Tests

Chloroaniline. To 0.2 g of the substance under examination, add 1 ml of hydrochloric acid, dilute to 30 ml with water and shake until a clear solution is obtained. Add rapidly and with thorough mixing after each addition, 2.5 ml of dilute hydrochloric acid, 0.35 ml of sodium nitrite solution, 2 ml of a 5.0 per cent w/v solution of ammonium sulphamate, 5 ml of a 0.1 per cent w/v solution of naphthylenediamine dihydrochloride and 1 ml of alcohol, dilute to 50.0 ml with water and allow to stand for 30 minutes. Any reddish-blue colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using a mixture of 10.0 ml of a 0.001 per cent solution of chloroaniline in dilute hydrochloric acid and 20 ml of dilute hydrochloric acid instead of the solution of the substance under examination (500 ppm).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.2 g of the substance under examination in 100 ml of the mobile phase.

Reference solution (a). A 0.15 per cent w/v solution of chlorhexidine hydrochloride RS in the mobile phase.

Reference solution (b). Dilute 2.5 ml of the test solution to 100 ml with the mobile phase.

Reference solution (c). Dilute 2 ml of reference solution (b) to 10 ml with the mobile phase. Dilute 1 ml of the solution to 10 ml with the mobile phase.

Chromatographic system

- a stainless steel column 20 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 2.0 g of sodium octanesulphonate in a mixture of 120 ml of glacial acetic acid, 270 ml of water and 730 ml of methanol,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 10 µl loop injector.

Equilibrate the column with the mobile phase for at least 1 hour. Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is at least 50 per cent of the full scale of the recorder.

Inject the test solution and reference solutions (a), (b) and (c). Record the chromatograms until the peak due to chlorhexidine has been eluted and record the chromatogram of the test solution for six times the retention time of the peak due to chlorhexidine. In the chromatogram obtained with the test solution, the sum of the areas of all the peaks, other than the principal peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent). Ignore any peak with a relative retention time of 0.25 or less with respect to the principal peak and any peak whose area is less than that of the principal peak in the chromatogram obtained with reference solution (c).

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Dissolve 0.1 g in 5 ml of anhydrous formic acid and add 70 ml of acetic anhydride. Titrate with 0.1 M perchloric acid determining the end-point potentiometrically (2.4.25).

1 ml of 0.1 M perchloric acid is equivalent to 0.01446 g of C₂₂H₃₂Cl₄N₁₀.

Chlorobutanol

\[\text{H}_3\text{C} - \overset{\text{OH}}{\text{C}} - \overset{\text{CCl}_3}{\text{C}}\text{H}_3, \frac{1}{2}\text{H}_2\text{O}\]

\[\text{C}_9\text{H}_7\text{Cl}_3\text{O}, \frac{1}{2}\text{H}_2\text{O}\]  Mol. Wt. 186.5

Chlorobutanol is 1,1,1-trichloro-2-methylpropan-2-ol.

Chlorobutanol contains not less than 98.0 per cent and not more than 101.0 per cent of chlorobutanol, C₉H₇Cl₃O, calculated on the anhydrous basis.

Description. A white crystalline powder or colourless crystals.

Identification

A. Dissolve 20 mg in a mixture of 1 ml of pyridine and 2 ml of strong sodium hydroxide solution. Heat in a water-bath and shake. Allow to stand. The pyridine layer becomes red.

B. Dissolve 20 mg in 5 ml of ammoniacal silver nitrate solution and warm slightly. A black precipitate is formed.

C. Dissolve 20 mg in 3 ml of J M sodium hydroxide. Add 5 ml of water and then, slowly, 2 ml of iodinated potassium iodide solution. A yellowish precipitate is formed.
Tests

Appearance of solution. A 50 per cent w/v solution in ethanol (95 per cent) (Solution A), is not more opalescent than reference suspension II (2.4.1) and not more intensely coloured than reference solution BY5 (2.4.1).

Acidity. To 4 ml of solution A add 15 ml of ethanol (95 per cent) and 0.1 ml of bromothymol blue solution. Not more than 1.0 ml of 0.01 M sodium hydroxide is required to change the colour of the indicator to blue.

Chlorides (2.3.12). To 1 ml of solution A add 4 ml of ethanol (95 per cent) and dilute to 15 ml with water. The solution complies with the limit test for chlorides (100 ppm).

Water (2.3.43). 4.5 per cent to 5.5 per cent, determined on 0.3 g.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Dissolve 0.1 g in 20 ml of ethanol (95 per cent), add 10 ml of dilute sodium hydroxide solution, heat in a water-bath for 5 minutes and cool. Add 20 ml of dilute nitric acid, 25.0 ml of 0.1 M silver nitrate and 2 ml of dibutyl phthalate and shake vigorously. Add 2 ml of ferric ammonium sulphate solution and titrate with 0.1 M ammonium thiocyanate until an orange colour is obtained.

1 ml of 0.1 M silver nitrate is equivalent to 0.00592 g of C₇H₇ClO.

Storage. Store protected from moisture.

Chlorocresol

\[
\begin{align*}
\text{C}_7\text{H}_7\text{ClO} & \quad \text{Mol. Wt. 142.6} \\
\end{align*}
\]

Chlorocresol is 4-chloro-3-methylphenol.

Chlorocresol contains not less than 98.0 per cent and not more than 101.0 per cent of C₇H₇ClO.

Description. Colourless or almost colourless crystals or a white, crystalline powder; odour, characteristic and not tarry; volatile in steam.

Identification

A. To a saturated solution in water add one drop of ferric chloride test solution; a bluish colour is produced.

B. To 0.1 g add 0.2 ml of benzoil chloride and 0.5 ml of 2 M sodium hydroxide. Shake vigorously until a white precipitate is produced, add 5 ml of water and filter. The melting range of the residue, after crystallisation from methanol and drying at 70°, is 85° to 88° (2.4.21).

Tests

Appearance of solution. A 5.0 per cent w/v solution in ethanol (95 per cent) is clear (2.4.1) and not more intensely coloured than reference solution BYS6 (2.4.1).

Acidity or alkalinity. To 10 ml of a 5.0 per cent w/v solution add 0.1 ml of methyl red solution. The solution is orange or red and not more than 0.2 ml of 0.01 M sodium hydroxide is required to change the colour of the solution to yellow.

Related substances. Determine by gas chromatography (2.4.13).

Test solution. A 1 per cent w/v solution of the substance under examination in acetone.

Chromatographic system

- a glass column 1.8m x 3.5 mm, packed with silanised diatomaceous support (80 to 120 mesh) impregnated with 3 to 5 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) (such as OV-17),
- temperature: column.125°, inlet port. 210°, detector. 230°,
- flow rate. 30 ml per minute of the carrier gas (nitrogen).

Allow the chromatography to proceed for three times the retention time of chlorocresol (about 8 minutes).

The sum of the areas of any secondary peaks in the chromatogram is not greater than 1.0 per cent of the total area of the peaks.

Non-volatile matter. Not more than 0.1 per cent, determined on 2.0 g by volatilising on a water-bath and drying at 105°.

Assay. Weigh accurately about 70 mg, dissolve in 30 ml of glacial acetic acid, add 25.0 ml of 0.0167 M potassium bromate, 20.0 ml of a 15 per cent w/v solution of potassium bromide and 10 ml of hydrochloric acid. Stopper the flask and allow to stand in the dark for 15 minutes, shaking occasionally. Add 1 g of potassium iodide and 100 ml of water. Titrate with 0.1 M sodium thiosulphate, shaking vigorously and using starch solution, added towards the end of the titration, as indicator. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of potassium bromate required.

1 ml of 0.0167 M potassium bromate is equivalent to 0.003565 g of C₇H₇ClO.

Storage. Store protected from light and moisture.
Chloroform

CHCl₃  Mol. Wt. 119.4

Chloroform is trichloromethane to which either 1.0 per cent to 2.0 per cent v/v of ethanol or 50 mg per litre of amylene has been added.

Description. A colourless, volatile liquid; odour, characteristic.

NOTE - Care should be taken not to vaporise chloroform in the presence of a flame because of the production of harmful gases.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Shake with an equal volume of water and dry with anhydrous sodium sulphate. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with the reference spectrum of chloroform.

B. Non-flammable. The vapour introduced into a Bunsen flame produces a green colour and gives rise to noxious vapours having a characteristic odour.

C. Warm 0.5 ml with 0.05 ml of aniline and 1 ml of 5 M sodium hydroxide. The characteristic odour of phenyl isocyanide is produced.

Tests

Weight per ml (2.4.29). 1.474 g to 1.478 g.

Boiling range (2.4.8). Not more than 5.0 per cent v/v distils below 60° and the remainder distils between 60° and 62°.

Acidity or alkalinity. Shake 10 ml with 20 ml of freshly boiled and cooled water for 3 minutes and allow to separate. To 5 ml of the aqueous layer (solution A) add 0.1 ml of litmus solution; the colour produced is similar to that produced on adding 0.1 ml of litmus solution to 5 ml of freshly boiled and cooled water.

Chlorides. To 5 ml of solution A add 5 ml of water and 0.2 ml of silver nitrate solution; the solution is clear.

Free chlorine. To 10 ml of solution A add 1 ml of cadmium iodide solution and 2 drops of starch solution; no blue colour is produced.

Aldehyde. Shake 5 ml with 5 ml of water and 0.2 ml of alkaline potassium mercuri-iodide solution in a stoppered bottle and set aside in the dark for 15 minutes; not more than a pale yellow colour is produced.

Foreign chlorine compounds. Shake 20 ml with 10 ml of sulphuric acid in a stoppered flask for 5 minutes, allow to stand in the dark for 30 minutes and discard the acid layer. Shake 15 ml of the chloroform layer with 30 ml of water in a stoppered flask for 3 minutes and allow to separate. To the aqueous layer add 0.2 ml of silver nitrate solution and set aside in the dark for 5 minutes; no opalescence is produced.

Related substances. Determine by gas chromatography (2.4.13).

Test solution (a). A solution containing 0.2 per cent v/v of carbon tetrachloride, 0.2 per cent v/v of 1,1,1-trichloroethane (internal standard), 0.2 per cent v/v of dichloromethane, 0.2 per cent v/v of ethanol, 0.5 per cent v/v of bromochloromethane and 0.2 per cent v/v of the substance under examination in 1-propanol.

Test solution (b). The substance under examination.

Reference solution (a). A solution containing 0.2 per cent v/v of the internal standard in the substance under examination.

Reference solution (b). 1-propanol.

Chromatographic system

– a glass column 4 m x 3 mm, packed with acid-washed kieselguhr (60 to 100 mesh) coated with 15 per cent w/w of di-2-cyanoethyl ether,

– temperature:
  column, 40°,
  inlet port and detector, 100°,

– flow rate. 30 ml per minute of the carrier gas.

– Inject 0.1 µl of each solution.

The test is not valid unless the column efficiency, determined using the chloroform peak in the chromatogram obtained with test solution (a), is greater than 700 plates per metre and the total number of plates is greater than 2,500.

In the chromatogram obtained with test solution (a) the peaks, in the order of emergence, are due to carbon tetrachloride, 1,1,1-trichloroethane, dichloromethane, chloroform, ethanol, bromochloromethane and 1-propanol (solvent).

Using the chromatogram obtained with reference solution (b) make any corrections due to the contribution of secondary peaks from the solvent to the peaks in the chromatogram obtained with test solution (a).

In the chromatogram obtained with reference solution (a), the ratio of the areas of any peaks due to carbon tetrachloride, dichloromethane and bromochloromethane to the area of the peak due to the internal standard is not greater than the corresponding ratios in the chromatogram obtained with test solution (a) and the ratio of the area of any other secondary peak that elutes prior to the solvent peak, except for the peak corresponding to ethanol, to the area of the peak due to the internal standard is not greater than the ratio of the area of the peak due to chloroform to the area of the peak due to the internal standard in the chromatogram obtained with test solution (a).
Calculate the percentage content of each of the specified impurities and also calculate the percentage content of each of any other impurities assuming the same response per unit volume as with chloroform. The total content of all impurities is not more than 1.0 per cent v/v.

**Ethanol (if present).** Determine by gas chromatography (2.4.13).

**Test solution (a).** The substance under examination.

**Test solution (b).** A solution containing 1.0 per cent v/v of 1-propanol (internal standard) in the substance under examination.

**Reference solution.** A solution containing 1.0 per cent v/v of ethanol and 1.0 per cent v/v of the internal standard in water.

Inject 0.1 µl of each solution. Follow the chromatographic procedure described under Related substances.

The test is not valid unless the height of the trough separating the ethanol peak from the chloroform peak in the chromatogram obtained with test solution (a) is less than 15 per cent of the height of the ethanol peak.

Calculate the percentage content of ethanol from the areas of the peaks due to ethanol and the internal standard in the chromatograms obtained with test solution (a) and test solution (b).

**Non-volatile matter.** Not more than 0.004 per cent w/v, determined on 25 ml by evaporation to dryness and drying at 105°.

**Storage.** Store protected from light in tightly-closed, glass-stoppered containers.

**Labelling.** The label states whether it contains ethanol or amyline.

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**Chloroquine Phosphate**

Chloroquine Phosphate contains not less than 98.5 per cent and not more than 101.0 per cent of \( \text{C}_{18}\text{H}_{26}\text{ClN}_3\cdot2\text{H}_3\text{PO}_4 \), calculated on the anhydrous basis.

**Description.** A white or almost white, crystalline powder; odourless. It slowly gets discoloured on exposure to light. It may exist in two polymorphic forms differing in their behaviour, one of which melts at about 195° and the other at about 218°.

**Identification**

**Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.**

A. Dissolve 0.1 g in 10 ml of water, add 2 ml of 2 M sodium hydroxide and extract with two quantities, each of 20 ml, of chloroform. Wash the combined chloroform extracts with water, dry over anhydrous sodium sulphate, evaporate to dryness and dissolve the residue in 2 ml of chloroform. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with 80 mg of chloroquine phosphate RS treated in the same manner.

B. When examined in the range 210 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution shows absorption maxima at about 220 nm, 235 nm, 256 nm, 329 nm and 342 nm; absorbance at about 220 nm, 0.60 to 0.66, at about 235 nm, 0.35 to 0.39, at about 256 nm, 0.30 to 0.33, at about 329 nm, 0.325 to 0.355 and at about 342 nm, 0.36 to 0.39.

C. Dissolve 25 mg in 20 ml of water and add 8 ml of picric acid solution; the precipitate, after washing successively with water, ethanol (95 per cent) and ether, melts at 205° to 210° (2.4.21).

D. Neutralise with dilute nitric acid the aqueous layer obtained in test A. Add an equal volume of ammonium molybdate solution and warm; a yellow precipitate is produced.

**Tests**

**Appearance of solution.** A 10.0 per cent w/v solution in carbon dioxide-free water is clear (2.4.1), and not more intensely coloured than reference solution BYS5 or GYS5 (2.4.1).

**pH** (2.4.24). 3.5 to 4.5, determined in a 10.0 per cent w/v solution.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 50 volumes of chloroform, 40 volumes of cyclohexane and 10 volumes of diethylamine.

**Test solution.** A 5 per cent w/v solution of the substance under examination in water.

**Reference solution (a).** Dilute 1 ml of the test solution to 100 ml with water.
Reference solution (b). Dilute 25 ml of reference solution (a) to 50 ml with water.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

Water (2.3.43). Not more than 2.0 per cent, determined on 1.0 g.

Assay. Weigh accurately about 0.2 g and dissolve in 50 ml of anhydrous glacial acetic acid with the aid of heat (if necessary, heat under a reflux condenser). Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration. 1 ml of 0.1 M perchloric acid is equivalent to 0.02579 g of C_{18}H_{26}ClN_{3}.2H_{3}PO_{4}.

Storage. Store protected from light.

Chloroquine Phosphate Injection

Chloroquine Phosphate Injection is a sterile solution of Chloroquine Phosphate in Water for Injections.

Chloroquine Phosphate Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of chloroquine, C_{18}H_{26}ClN_{3}.

Description. A clear, colourless or almost colourless solution.

Identification

A. To a volume of the injection containing 60 mg of chloroquine add 2 ml of 2 M sodium hydroxide and extract with two quantities, each of 20 ml, of chloroform. Wash the combined chloroform extracts with water, dry over anhydrous sodium sulphate, evaporate to dryness and dissolve the residue in 2 ml of chloroform. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with 80 mg of chloroquine phosphate RS treated in the same manner.

B. Dilute a volume of the injection containing 15 mg of chloroquine to 20 ml with water and add 8 ml of picric acid solution; the precipitate, after washing successively with water, ethanol (95 per cent) and ether, melts at about 207° (2.4.21).

C. Neutralise the aqueous layer obtained in test A with dilute nitric acid, add an equal volume of ammonium molybdate solution and warm; a yellow precipitate is produced.

Tests

pH (2.4.24). 3.5 to 4.5.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. To an accurately measured volume of the injection containing 0.4 g of chloroquine add 20 ml of 1 M sodium hydroxide and extract with four quantities, each of 25 ml, of chloroform. Combine the chloroform extracts and evaporate to a volume of about 10 ml. Add 40 ml of anhydrous glacial acetic acid and mix. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration. 1 ml of 0.1 M perchloric acid is equivalent to 0.01599 g of chloroquine, C_{18}H_{26}ClN_{3}.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of chloroquine in a suitable dose-volume.

Chloroquine Phosphate Suspension

Chloroquine Phosphate Suspension is a suspension of Chloroquine Phosphate in a suitable flavoured vehicle.

Chloroquine Phosphate Suspension contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of chloroquine, C_{18}H_{26}ClN_{3}.

Identification

To a volume of the suspension containing 50 mg of chloroquine add 2 ml of 2 M sodium hydroxide and extract with two quantities, each of 20 ml, of chloroform. Wash the combined chloroform extracts with water; dry over anhydrous sodium sulphate, evaporate to dryness and dissolve the residue in 2 ml of chloroform. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with 80 mg of chloroquine phosphate RS treated in the same manner.

Tests

pH (2.4.24). 5.5 to 6.5.

Other tests. Complies with the tests stated under Oral Liquids.

Assay. Weigh accurately a quantity of the suspension containing about 100 mg of chloroquine, add 50 ml of 1 M
hydrochloric acid, shake well and dilute to 100.0 ml with 1 M hydrochloric acid. Filter and discard the first few ml of the filtrate. Dilute 10.0 ml of the filtrate to 100.0 ml with 1 M hydrochloric acid and mix. Further dilute 10.0 ml to 100.0 ml with the same solvent and mix. Measure the absorbance of the resulting solution at the maximum at about 342 nm (2.4.7). Calculate the content of C_{18}H_{26}ClN_{3}.2H_{3}PO_{4} from the absorbance obtained by repeating the operation using chloroquine phosphate RS in place of the substance under examination.

**Chloroquine Phosphate Tablets**

Chloroquine Phosphate Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of chloroquine phosphate, C_{18}H_{26}ClN_{3}.2H_{3}PO_{4}. The tablets are coated.

**Identification**

A. To a quantity of the powdered tablets containing 0.1 g of Chloroquine Phosphate add 10 ml of water and 2 ml of 2 M sodium hydroxide and extract with two quantities, each of 20 ml, of chloroform. Wash the combined chloroform extracts with water, dry over anhydrous sodium sulphate, evaporate to dryness and dissolve the residue in 2 ml of chloroform. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with 80 mg of chloroquine phosphate RS treated in the same manner.

B. Extract a quantity of the powdered tablets containing 25 mg of Chloroquine Phosphate with 20 ml of water; filter and to the filtrate add 8 ml of picric acid solution; the precipitate, after washing successively with water, ethanol (95 per cent) and ether, melts at about 207° (2.4.21).

C. Extract a quantity of the powdered tablets containing 0.5 g of Chloroquine Phosphate with 25 ml of water and filter. To the filtrate add 2.5 ml of 5 M sodium hydroxide and extract with three quantities, each of 10 ml, of ether. The aqueous layer, after neutralisation with 2 M nitric acid, gives the reactions of phosphates (2.3.1).

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 50 volumes of chloroform, 40 volumes of cyclohexane and 10 volumes of diethylamine.

**Test solution.** Shake a quantity of the powdered tablets containing 1 g of Chloroquine Phosphate with 20 ml of water for 30 minutes, centrifuge and use the clear, supernatant liquid.

**Reference solution (a).** Dilute 1 ml of the test solution to 100 ml with water.

**Reference solution (b).** Dilute 25 ml of reference solution (a) to 50 ml with water.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Dissolution (2.5.2).**

Apparatus. No 1

Medium. 900 ml of 0.1 M hydrochloric acid

Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with 0.1 M hydrochloric acid. Measure the absorbance of the resulting solution at the maximum at about 344 nm (2.4.7). Calculate the content of C_{18}H_{26}ClN_{3}.2H_{3}PO_{4} per tablet taking 371 as the specific absorbance at 344 nm.

D. Not less than 70 per cent of the stated amount of C_{18}H_{26}ClN_{3}.2H_{3}PO_{4}.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.5 g of Chloroquine Phosphate, add 20 ml of 1 M sodium hydroxide and extract with four quantities, each of 25 ml, of chloroform. Combine the chloroform extracts and evaporate to a volume of about 10 ml. Add 40 ml of anhydrous glacial acetic acid and mix. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02579 g of C_{18}H_{26}ClN_{3}.2H_{3}PO_{4}.

**Storage.** Store protected from light.

**Chloroquine Sulphate**

C_{18}H_{26}ClN_{3}.H_{2}SO_{4}.H_{2}O

Mol. Wt. 435.9

Chloroquine Sulphate is (RS)-4-(7-chloro-4-quinolylamino) pentyldiethylamine sulphate monohydrate.

Chloroquine Sulphate contains not less than 98.5 per cent and not more than 101.0 per cent of C_{18}H_{26}ClN_{3}.H_{2}SO_{4}, calculated on the anhydrous basis.
**Description.** A white or almost white, crystalline powder; odourless.

**Identification**

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Dissolve 0.1 g in 10 ml of *water*, add 2 ml of 2 *M sodium hydroxide* and extract with two quantities, each of 20 ml, of *chloroform*. Wash the combined chloroform extracts with *water*, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *chloroform*. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained by treating 0.1 g of *chloroquine sulphate RS* in the same manner.

B. When examined in the range 210 nm to 360 nm, a 0.001 per cent w/v solution shows absorption maxima at about 220 nm, 235 nm, 256 nm, 329 nm and 342 nm; absorbance at about 220 nm, 0.73 to 0.81, at about 235 nm, 0.43 to 0.47, at about 256 nm, 0.37 to 0.41, at about 329 nm, 0.40 to 0.44 and at about 342 nm, 0.43 to 0.47 (2.4.7).

C. Dissolve 25 mg in 20 ml of *water* and add 8 ml of *picric acid solution*; the precipitate, after washing successively with *water*, *ethanol (95 per cent)* and *ether*, melts at 205° to 210° (2.4.21).

D. Gives reaction A of sulphates (2.3.1).

**Tests**

**Appearance of solution.** An 8.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution BYS5 or GYS5 (2.4.1).

**pH** (2.4.24). 4.0 to 5.0, determined in an 8.0 per cent w/v solution.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase.* A mixture of 50 volumes of *chloroform*, 40 volumes of *cyclohexane* and 10 volumes of *diethylamine*.

*Test solution.* A 5 per cent w/v solution of the substance under examination in *water*.

*Reference solution (a).* Dilute 1 ml of the test solution to 100 ml with *water*.

*Reference solution (b).* Dilute 25 ml of reference solution (a) to 50 ml with *water*.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Heavy metals** (2.3.13). 1.0 g dissolved in 25 ml of *water* complies with the limit test for heavy metals, Method A (20 ppm).

**Chlorides** (2.3.12). 1.25 g complies with the limit test for chlorides (200 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). 3.0 to 5.0 per cent, determined on 0.5 g.

**Assay.** Weigh accurately about 0.5 g and dissolve in 50 ml of *anhydrous glacial acetic acid*. Titrate with 0.1 *M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 *M perchloric acid* is equivalent to 0.0418 g of C\textsubscript{18}H\textsubscript{26}ClN\textsubscript{3}.H\textsubscript{2}SO\textsubscript{4}.

**Storage.** Store protected from light.

**Chloroquine Sulphate Injection**

Chloroquine Sulphate Injection is a sterile solution of Chloroquine Sulphate in Water for Injections.

Chloroquine Sulphate Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of chloroquine, C\textsubscript{18}H\textsubscript{26}ClN\textsubscript{3}.

**Description.** A clear, colourless or almost colourless solution.

**Identification**

A. To a volume of the injection containing 70 mg of chloroquine add sufficient *water* to produce 10 ml, add 2 ml of 2 *M sodium hydroxide* and extract with two quantities, each of 20 ml, of *chloroform*. Wash the combined chloroform extracts with *water*, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *chloroform*. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained by treating 0.1 g of *chloroquine sulphate RS* in the same manner.

B. When examined in the range 210 nm to 360 nm, a 0.001 per cent w/v solution shows absorption maxima at about 220 nm, 235 nm, 256 nm, 329 nm and 342 nm; absorbance at about 220 nm, 0.73 to 0.81, at about 235 nm, 0.43 to 0.47, at about 256 nm, 0.37 to 0.41, at about 329 nm, 0.40 to 0.44 and at about 342 nm, 0.43 to 0.47 (2.4.7).

C. Gives reaction A of sulphates (2.3.1).

**Tests**

**pH** (2.4.24). 4.0 to 5.5.
Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. To an accurately measured volume of the injection containing 0.4 g of chloroquine add 20 ml of 1 M sodium hydroxide and extract with four quantities, each of 25 ml, of chloroform. Combine the chloroform extracts and evaporate to a volume of about 10 ml. Add 40 ml of anhydrous glacial acetic acid and mix. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml 0.1 M perchloric acid is equivalent to 0.01599 g of C₁₈H₂₆ClN₃.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of chloroquine in a suitable dose-volume.

Chloroquine Sulphate Tablets

Chloroquine Sulphate Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of chloroquine sulphate, C₁₈H₂₆ClN₃.H₂SO₄. The tablets are coated.

Identification

A. To a quantity of the powdered tablets equivalent to 0.1 g of Chloroquine Sulphate add 10 ml of water and 2 ml of 2 M sodium hydroxide and extract with two quantities, each of 20 ml, of chloroform. Wash the combined chloroform extracts with water, dry with anhydrous sodium sulphate, evaporate to dryness and dissolve the residue in 2 ml of chloroform. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained by treating 0.1 g of chloroquine sulphate RS in the same manner.

B. Extract a quantity of the powdered tablets containing 25 mg of Chloroquine Sulphate with 20 ml of water, filter and to the filtrate add 8 ml of picric acid solution; the precipitate, after washing successively with water, ethanol (95 per cent) and ether, melts at about 207° (2.4.21).

C. Extract a quantity of the powdered tablets containing about 0.1 g of Chloroquine Sulphate with 10 ml of water and 1 ml of dilute hydrochloric acid and filter. To the filtrate add 1 ml of barium chloride solution; a white precipitate is produced.

Tests

Dissolution (2.5.2).

Apparatus. No 1

Medium. 900 ml of 0.1 M hydrochloric acid

Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with 0.1 M hydrochloric acid. Measure the absorbance of the resulting solution at the maximum at about 344 nm (2.4.7). Calculate the content of C₁₈H₂₆ClN₃.H₂SO₄ per tablet taking 450 as the specific absorbance at 344 nm.

D. Not less than 70 per cent of the stated amount of C₁₈H₂₆ClN₃.H₂SO₄.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.5 g of Chloroquine Sulphate, add 20 ml of 1 M sodium hydroxide and extract with four quantities, each of 25 ml, of chloroform. Combine the chloroform extracts and evaporate to a volume of about 10 ml. Add 40 ml of anhydrous glacial acetic acid and mix. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.0436 g of C₁₈H₂₆ClN₃.H₂SO₄.

Storage. Store protected from light.

Chloroquine Syrup

Chloroquine Syrup is a solution of Chloroquine Phosphate or Chloroquine Sulphate in a suitable flavoured vehicle.

Chloroquine Syrup contains Chloroquine Phosphate or Chloroquine Sulphate equivalent to not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of chloroquine, C₁₈H₂₆ClN₃.

Identification

To a volume of the syrup containing 50 mg of chloroquine add 2 ml of 2 M sodium hydroxide and extract with two quantities, each of 20 ml, of chloroform. Wash the combined chloroform extracts with water, dry with anhydrous sodium sulphate, evaporate to dryness and dissolve the residue in 2 ml of chloroform. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained by treating 0.1 g of chloroquine sulphate RS in the same manner.

Tests

pH (2.4.24). 4.0 to 6.5.
**Other tests.** Complies with the tests stated under Oral Liquids.

**Assay.** To an accurately measured volume of the syrup containing about 0.4 g of chloroquine add 20 ml of 1 M sodium hydroxide and extract with four quantities, each of 25 ml, of chloroform. Combine the chloroform extracts and evaporate to a volume of about 10 ml. Add 40 ml of anhydrous glacial acetic acid and mix. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.01599 g of C_{18}H_{26}ClN_{3}.

**Storage.** Store protected from light.

**Labelling.** The label states (1) whether the syrup contains Chloroquine Phosphate or Chloroquine Sulphate; (2) the strength in terms of equivalent amount of chloroquine in each 5 ml.

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### Chloroxylenol

\[
\begin{align*}
\text{C}_9\text{H}_8\text{ClO} & \quad \text{Mol. Wt. 156.6} \\
& \text{Chloroxylenol is 4-chloro-3,5-dimethylphenol.} \\
& \text{Chloroxylenol contains not less than 98.0 per cent and not more than 103.0 per cent of C}_9\text{H}_8\text{ClO}. \\
\text{Description.} & \text{ A white or creamy-white crystals or crystalline powder; odour characteristic. It is volatile in steam.} \\
\text{Identification} & \text{ A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with chloroxylenol RS or with the reference spectrum of chloroxylenol.} \\
& \text{B. Dissolve 0.1 g in 5 ml of chloroform and add 0.5 ml of a filtered 1 per cent w/v solution of ferric chloride in chloroform and 0.1 ml of pyridine; a blue colour is produced.} \\
& \text{C. To 5 ml of a saturated solution in water add 0.5 ml of ferric chloride test solution; no blue colour is produced.} \\
& \text{D. Mix 50 mg with 0.5 g of anhydrous sodium carbonate and ignite strongly, cool, boil the residue with 5 ml of water, acidify with nitric acid, filter and add 2 ml of silver nitrate solution; a white precipitate is produced.} \\
\end{align*}
\]

**Tests**

**Related substances.** Determine by gas chromatography (2.4.13).

**Test solution.** A 2 per cent w/v solution of the substance under examination in chloroform.

**Reference solution.** A solution containing 2 per cent w/v of the substance under examination and 0.04 per cent w/v of 4-chloro-o-cresol (internal standard) in chloroform.

**Chromatographic system**
- a glass column 1.5m × 4 mm, packed with acid-washed diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of polyethylene glycol (such as Carbowax 20M),
- temperature: column, 160°, inlet port and detector, 220°,
- a flame ionisation detector,
- flow rate, 30 ml per minute of the carrier gas.

In the chromatogram obtained with the reference solution the sum of the areas of any secondary peaks is not greater than the area of the peak due to internal standard.

**Assay.** Weigh accurately about 70 mg, dissolve in 30 ml of glacial acetic acid, add 25.0 ml of 0.0167 M potassium bromate, 20 ml of a 15 per cent w/v solution of potassium bromide and 10 ml of hydrochloric acid, stopper the flask and allow to stand protected from light for 15 minutes. Add 1 g of potassium iodide and 100 ml of water and titrate with 0.1 M sodium thiosulphate, shaking vigorously and using 1 ml of starch solution as indicator. Repeat the procedure without the substance under examination. The difference between the titerations represents the amount of potassium bromate required.

1 ml of 0.0167 M potassium bromate is equivalent to 0.003915 g of C_{9}H_{8}ClO.

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**Chloroxylenol Solution**

Chloroxylenol solution is a solution of Chloroxylenol solubilised in a saponaceous base containing Ethanol (95 per cent) and essential oils. Ethanol (95 per cent) may be replaced by Industrial Methylated Spirit in making Chloroxylenol Solution.

Chloroxylenol Solution contains not less than 4.75 per cent and not more than 5.25 per cent of C_{9}H_{8}ClO.

**Tests**

**pH (2.4.24).** 7.0 to 11.0.

**Ethanol content (2.3.45).** 16 to 21 per cent v/v.
**Assay.** Determine by gas chromatography (2.4.13).

**Test solution.** Extract 4 ml of the solution under examination with 20.0 ml of chloroform after adding 4 ml of 2 M hydrochloric acid. Extract with two further quantities, each of 10.0 ml, of chloroform. Combine the chloroform extracts, shake with anhydrous sodium sulphate and filter.

**Reference solution (a).** Dissolve 0.1 g of chloroxylenol RS in 10.0 ml of a 0.8 per cent w/v solution of 4-chloro-o-cresol (internal standard) in chloroform (solution A) and dilute to 20.0 ml with chloroform.

**Reference solution (b).** Prepare in the same manner as the test solution but use 20.0 ml of solution A instead of 20 ml of chloroform.

**Chromatographic system**
- a glass column 1.5m × 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of polyethylene glycol (such as Carbowax 20M),
- temperature: column, 160°; inlet port and detector, 220°,
- a flame ionisation detector,
- flow rate, 30 ml per minute of the carrier gas.

Calculate the content of C₈H₉ClO in the solution as a percentage w/v.

**Labelling.** The label states that the preparation is meant for external use only.

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**Chlorpheniramine Maleate**

\[
\text{C}_{16}\text{H}_{19}\text{ClN}_{2}\text{C}_{4}\text{H}_{4}\text{O}_{4} \quad \text{Mol. Wt. 390.9}
\]

Chlorpheniramine Maleate is (RS)-3-(4-chlorophenyl)-3-(pyrid-2-yl)propyldimethylamine hydrogen maleate.

Chlorpheniramine Maleate contains not less than 98.0 per cent and not more than 101.0 per cent of C₆H₁₅ClN₂C₄H₅O₄, calculated on the dried basis.

**Description.** A white, crystalline powder; odourless.

**Identification**

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*
**Assay.** Weigh accurately about 0.2 g and dissolve in 20 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.01954 g of C16H19ClN2,C4H4O4.

**Storage.** Store protected from light and moisture.

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**Chlorpheniramine Injection**

Chlorpheniramine Injection is a sterile solution of Chlorpheniramine Maleate in Water for Injections free from dissolved air and containing suitable buffering and stabilising agents.

Chlorpheniramine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of chlorpheniramine maleate, C16H19ClN2,C4H4O4.

**Description.** A colourless solution.

**Identification**

Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254. Heat the plate at 105° for 30 minutes before use.

**Mobile phase.** A mixture of 50 volumes of ethyl acetate, 30 volumes of methanol and 20 volumes of 1 M acetic acid.

**Test solution.** Evaporate an appropriate volume of the injection to dryness in a current of nitrogen using the minimum amount of heat, dissolve the residue as completely as possible in sufficient chloroform to produce a solution containing 0.5 per cent w/v of Chlorpheniramine Maleate and centrifuge. For the reference solution, dilute 1 volume of the test solution to 500 volumes with chloroform. After development, dry the plate in air and spray with dilute potassium iodobismuthate solution. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** Dilute an accurately measured volume of the injection containing 10 mg of Chlorpheniramine Maleate to 500.0 ml with 0.25 M sulphuric acid. Measure the absorbance of the resulting solution at the maximum at about 265 nm (2.4.7). Calculate the content of C16H19ClN2,C4H4O4 taking 212 as the specific absorbance at 265 nm.

**Storage.** Store protected from light.

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**Chlorpheniramine Tablets**

Chlorpheniramine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of chlorpheniramine maleate, C16H19ClN2,C4H4O4.

**Identification**

Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254. Heat the plate at 105° for 30 minutes before use.

**Mobile phase.** A mixture of 50 volumes of ethyl acetate, 30 volumes of methanol and 20 volumes of 1 M acetic acid.

**Test solution.** Extract a quantity of the powdered tablets containing 5 mg of Chlorpheniramine Maleate with chloroform, filter, evaporate the filtrate to dryness and dissolve the residue in 1 ml of chloroform.

**Reference solution.** A 0.5 per cent w/v solution of chlorpheniramine maleate RS in chloroform.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The two principal spots in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution. Spray with dilute potassium iodobismuthate solution. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

**Tests**

**pH** (2.4.24). 4.0 to 5.2.
the plate with dilute potassium iodobismuthate solution. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 50 volumes of cyclohexane, 40 volumes of chloroform and 10 volumes of diethylamine.

**Test solution.** Extract a quantity of the powdered tablets containing 100 mg of Chlorpheniramine Maleate with chloroform, filter, evaporate to dryness and dissolve the residue in 2 ml of chloroform.

**Reference solution.** Dilute 1 ml of the test solution to 50 ml with chloroform and dilute 1.0 ml of the resulting solution to 10.0 ml with the same solvent.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution. Ignore any spot remaining on the line of application.

**Uniformity of content.** Comply with test stated under Tablets.

Powder one tablet and carry out the Assay beginning at the words "shake with 20 ml of 0.05 M sulphuric acid...". Calculate the content of C_{16}H_{19}ClN_{2}C_{4}H_{4}O_{4} in the tablet.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 4 mg of Chlorpheniramine Maleate, shake with 20 ml of 0.05 M sulphuric acid for 5 minutes, add 20 ml of ether; shake carefully and filter the acid layer into a second separator. Extract the ether layer with two quantities, each of 10 ml, of 0.05 M sulphuric acid, filter each acid layer into the second separator and wash the filter with 0.05 M sulphuric acid. Make the combined acid extracts and washing just alkaline to litmus paper with 1 M sodium hydroxide, add 2 ml in excess, and extract with two quantities, each of 50 ml, of ether. Wash each ether extract with the same 20 ml of water and extract in succession with 20, 20 and 5 ml of 0.25 M sulphuric acid, dilute the combined acid extracts to 50.0 ml with 0.25 M sulphuric acid; dilute 10.0 ml to 50.0 ml with 0.25 M sulphuric acid and measure the absorbance of the resulting solution at the maximum at about 265 nm (2.4.7). Calculate the content of C_{16}H_{19}ClN_{2}C_{4}H_{4}O_{4} taking 212 as the specific absorbance at 265 nm.

**Storage.** Store protected from light and moisture.

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**Chlorpromazine Hydrochloride**

![Chemical Structure](image)

C_{17}H_{19}ClN_{2}S.HCl  
Mol. Wt. 355.3

Chlorpromazine Hydrochloride is 2-chloro-10-(3-dimethylaminopropyl)phenothiazine hydrochloride.

Chlorpromazine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of C_{17}H_{19}ClN_{2}S.HCl, calculated on the dried basis.

**Description.** A white or creamy-white, crystalline powder; odourless. It decomposes on exposure to air and light becoming yellow, pink and finally violet.

**Identification**

*Test A may be omitted if tests B, C and D are carried out. Test B may be omitted if tests A, C and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with chlorpromazine hydrochloride RS or with the reference spectrum of chlorpromazine hydrochloride.

B. When examined in the range 230 nm to 360 nm, a 0.0005 per cent w/v solution in 0.1 M hydrochloric acid shows absorption maxima at about 254 nm and 306 nm; absorbance at about 254 nm, 0.45 to 0.48 (2.4.7).

C. Complies with the test for identification of phenothiazines (2.3.3)

D. A 5 per cent w/v solution gives reaction B of chlorides (2.3.1).

**Tests**

**pH** (2.4.24). 3.5 to 4.5, determined in a 10.0 per cent solution.

**Related substances** (2.3.5). Use mobile phase (a).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.6 g, dissolve in 200 ml of acetone and add 15 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid, using a saturated solution of
methyl orange in acetone as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.03553 g of C_{17}H_{19}ClN_{2}S.HCl.

Storage. Store protected from light and moisture.

**Chlorpromazine Injection**

Chlorpromazine Hydrochloride Injection

Chlorpromazine Injection is a sterile solution of Chlorpromazine hydrochloride in Water for Injections free from air and containing buffering and stabilizing agents.

Chlorpromazine Hydrochloride contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of chlorpromazine hydrochloride, C_{17}H_{19}ClN_{2}S.HCl.

NOTE — Protect the solutions from light throughout the tests.

Description. A colourless or almost colourless solution.

Identification

A. To a volume containing 0.1 g of Chlorpromazine Hydrochloride add 20 ml of water and 2 ml of 10 M sodium hydroxide. Extract with 25 ml of ether; wash the ether extract with two quantities, each of 5 ml, of water; dry the ether extract with anhydrous sodium sulphate; evaporate the ether and dissolve the residue in 1 ml of chloroform. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with chlorpromazine hydrochloride RS treated in the same manner or with the reference spectrum of chlorpromazine hydrochloride.

B. Dilute a volume of the injection with sufficient 0.1 M hydrochloric acid to produce a solution containing 0.0005 per cent w/v of Chlorpromazine Hydrochloride. The resulting solution, when examined in the range 230 nm to 360 nm shows absorption maxima at about 254 nm and 306 nm; absorbance at about 254 nm, 0.45 to 0.48 (2.4.7).

C. Gives reaction B of chlorides (2.3.1).

Tests

Related substances (2.3.5). Use mobile phase (a).

Test solution. Dilute a volume of the injection with sufficient of a mixture of 95 volumes of methanol and 5 volumes of diethylamine to produce a solution containing 2.0 per cent of Chlorpromazine Hydrochloride.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Dilute an accurately measured volume of the injection with sufficient 0.1 M hydrochloric acid to produce a solution containing 0.0005 per cent w/v of Chlorpromazine Hydrochloride and measure the absorbance of the resulting solution at the maximum at about 254 nm (2.4.7). Calculate the content of C_{17}H_{19}ClN_{2}S.HCl, taking 915 as the specific absorbance at 254 nm.

Storage. Store protected from light.

**Chlorpromazine Tablets**

Chlorpromazine Hydrochloride Tablets

Chlorpromazine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of chlorpromazine hydrochloride, C_{17}H_{19}ClN_{2}S.HCl. The tablets are coated.

NOTE — Protect the solutions from light throughout the tests.

Identification

A. To a quantity of the powdered tablets containing 40 mg of Chlorpromazine Hydrochloride add 10 ml of water and 2 ml of 10 M sodium hydroxide. Extract with 15 ml of ether and wash the ether extract with two quantities, each of 5 ml, of water; dry the ether extract with anhydrous sodium sulphate; evaporate the ether and dissolve the residue in 0.4 ml of chloroform. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with chlorpromazine hydrochloride RS treated in the same manner or with the reference spectrum of chlorpromazine hydrochloride.

B. Digest a quantity of the powdered tablets containing 25 mg of Chlorpromazine Hydrochloride with 25 ml of water and filter. Reserve a portion of the filtrate for Identification C. Dilute a volume of the filtrate with sufficient 0.1 M hydrochloric acid to produce a solution containing 0.0005 per cent w/v of Chlorpromazine Hydrochloride. The resulting solution, when examined in the range 230 nm to 360 nm shows absorption maxima at about 254 nm and 306 nm; absorbance at about 254 nm, 0.45 to 0.48 (2.4.7).

C. The filtrate reserved in test B gives reaction B of chlorides (2.3.1).

Tests

Related substances. (2.3.5). Use mobile phase (a).

Test solution. Extract a quantity of the powdered tablets containing 0.2 g of Chlorpromazine Hydrochloride with 10 ml of a mixture of 95 volumes of methanol and 5 volumes of diethylamine and filter.
Reference solution. Dilute 1 volume of the test solution to 200 volumes with the same solvent mixture.

Uniformity of content. Comply with the test stated under Tablets.

Powder one tablet, shake with 1 ml of dilute hydrochloric acid and 40 ml of water for 15 minutes, add sufficient water to produce 100.0 ml and mix. Centrifuge about 15 ml and to 10.0 ml of the clear, supernatant liquid add 2 ml of 1 M hydrochloric acid and sufficient water to produce a solution containing about 0.0005 per cent w/v of Chlorpromazine Hydrochloride. Measure the absorbance of the resulting solution at the maximum at about 254 nm (2.4.7). Calculate the content of C₁₀H₁₃ClN₂O₃S, HCl in the tablet taking 915 as the specific absorbance at 254 nm.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Chlorpromazine Hydrochloride, add 5 ml of dilute hydrochloric acid and 200 ml of water. Shake for 15 minutes and add sufficient water to produce 500.0 ml. Centrifuge about 15 ml and to 5.0 ml of the clear, supernatant liquid add 10 ml of dilute hydrochloric acid and sufficient water to produce 200.0 ml. Measure the absorbance of the resulting solution at the maximum at about 254 nm (2.4.7). Calculate the content of C₁₀H₁₃ClN₂O₃S.HCl, taking 915 as the specific absorbance at 254 nm.

Storage. Store protected from light.

Chlorpropamide

CH₃
H
O
N
S

C₁₀H₁₃ClN₂O₃S

Mol. Wt. 276.7

Chlorpropamide is 1-(4-chlorobenzenesulphonyl)-3-propylurea.

Chlorpropamide contains not less than 99.0 per cent and not more than 101.0 per cent of C₁₀H₁₃ClN₂O₃S, calculated on the dried basis.

Description. A white, crystalline powder; odourless or almost odourless.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B, C, D and E may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with chlorpropamide RS or with the reference spectrum of chlorpropamide.

B. Dissolve 0.16 g in 50 ml of methanol, dilute 5 ml to 100 ml with 0.01 M hydrochloric acid and dilute 5 ml of this solution to 100 ml with 0.01 M hydrochloric acid. When examined in the range 220 nm to 360 nm, the resulting solution shows an absorption maximum only at about 232 nm; absorbance at about 232 nm, about 0.48 (2.4.7).

C. Boil 0.1 g with 8 ml of a 50 per cent w/w solution of sulphuric acid under a reflux condenser for 30 minutes, cool and filter, reserving the filtrate for test D. The precipitate, after recrystallisation from water and drying, melts at about 143° (2.4.21).

D. Make the filtrate reserved in test C alkaline with sodium hydroxide solution and heat; an ammoniacal odour is produced.

E. Heat 0.1 g with 1 g of anhydrous sodium carbonate at a dull red heat for 10 minutes. Cool, extract the residue with water and filter. Acidify the filtrate with dilute nitric acid and add silver nitrate solution; a white precipitate is produced.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 100 volumes of chloroform, 50 volumes of methanol, 30 volumes of cyclohexane and 11.5 volumes of strong ammonia solution.

Test solution. Dissolve 0.6 g of the substance under examination in 10 ml of acetone.

Reference solution (a). A 0.02 per cent w/v solution of 4-chlorobenzencesulphonamide in acetone.

Reference solution (b). A 0.02 per cent w/v solution of 1,3-dipropylurea RS in acetone.

Reference solution (c). A 0.02 per cent w/v solution of the substance under examination in acetone.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of cold air, heat at 110° for 10 minutes, place the plate, while hot, in a tank of chlorine gas prepared by adding hydrochloric acid to a 5 per cent w/v solution of potassium permanganate contained in a beaker placed in the tank and allow to stand for 2 minutes. Dry it in a current of cold air until an area of the plate below the line of application gives at most a very faint blue colour with a 0.5 per cent w/v solution of potassium iodide in starch solution; avoid prolonged exposure to cold air. Any spots corresponding to 4-chlorobenzenesulphonamide and 1,3-dipropylurea in the chromatogram obtained with the test solution are not more
intense than the spots in the chromatogram obtained with reference solutions (a) and (b) respectively. Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (c).

**Heavy metals** (2.3.13). 0.66 g complies with the limit test for heavy metals, Method B (30 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent, determined on 2.0 g.

**Loss on drying** (2.4.19). Not more than 1.5 per cent, determined on 1.0 g by drying in an oven at 105°C.

**Assay.** Weigh accurately about 0.5 g and dissolve in 50 ml of ethanol (95 per cent) previously neutralised to phenolphthalein solution. Add 25 ml of water and titrate with 0.1 M sodium hydroxide using phenolphthalein solution as indicator.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.02767 g of C_{10}H_{13}ClN_{2}O_{3}S.

**Chlorpropamide Tablets**

Chlorpropamide Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of chlorpropamide, C_{10}H_{13}ClN_{2}O_{3}S.

**Identification**

Extract a quantity of the powdered tablets containing 1 g of Chlorpropamide with five quantities, each of 4 ml, of acetone, filter and carefully evaporate the filtrate to dryness on a water-bath. The residue complies with the following tests.

A. Boil 0.1 g with 8 ml of a 50 per cent w/w solution of sulphuric acid under a reflux condenser for 30 minutes, cool and filter, reserving the filtrate for test B. The precipitate, after recrystallisation from water and drying, melts at about 143°C (2.4.21).

B. Make the filtrate reserved in test A alkaline with sodium hydroxide solution and heat; an ammonical odour is produced.

C. Heat 0.1 g with 1 g of anhydrous sodium carbonate at a dull red heat for 10 minutes. Cool, extract the residue with water and filter. Acidify the filtrate with dilute nitric acid and add silver nitrate solution; a white precipitate is produced.

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 100 volumes of chloroform, 50 volumes of methanol, 30 volumes of cyclohexane and 11.5 volumes of strong ammonia solution.

**Test solution.** Shake a quantity of the powdered tablets containing 0.6 g of Chlorpropamide with 10 ml of acetone and filter.

**Reference solution (a).** A 0.02 per cent w/v solution of 4-chlorobenzenesulphonamide in acetone.

**Reference solution (b).** A 0.02 per cent w/v solution of 1,3-dipropylurea RS in acetone.

**Reference solution (c).** A 0.02 per cent w/v solution of the substance under examination in acetone.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of cold air, heat at 110°C for 10 minutes, place the plate, while hot, in a tank of chlorine gas prepared by adding hydrochloric acid to a 5 per cent w/v solution of potassium permanganate contained in a beaker placed in the tank and allow to stand for 2 minutes. Dry it in a current of cold air until an area of the plate below the line of application gives at most a very faint blue colour with a 0.5 per cent w/v solution of potassium iodide in starch solution; avoid prolonged exposure to cold air. Any spots corresponding to 4-chlorobenzenesulphonamide and 1,3-dipropylurea in the chromatogram obtained with the test solution are not more intense than the spots in the chromatogram obtained with reference solutions (a) and (b) respectively. Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (c).

**Dissolution** (2.5.2).

**Apparatus.** No 1 Medium. 900 ml of a 0.68 per cent w/v solution of potassium dihydrogen phosphate adjusted to pH 7.4 by the addition of 1 M sodium hydroxide

**Speed and time.** 100 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with 0.1 M hydrochloric acid to obtain a solution containing about 10 µg of chlorpropamide per ml. Measure the absorbance of the resulting solution at the maximum at about 232 nm (2.4.7). Calculate the content of C_{10}H_{13}ClN_{2}O_{3}S taking 598 as the specific absorbance at 232 nm.

D. Not less than 75 per cent of the stated amount of C_{10}H_{13}ClN_{2}O_{3}S.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.25 g of Chlorpropamide and shake with 40 ml of methanol for 20 minutes, add sufficient methanol to produce 50.0 ml, mix, filter and dilute 5.0 ml of the filtrate to 100.0 ml with 0.1 M
hydrochloric acid. Mix, dilute 10.0 ml of this solution to 250.0 ml with 0.1 M hydrochloric acid and measure the absorbance of the resulting solution at the maximum at about 232 nm (2.4.7). Calculate the content of C_{10}H_{13}ClN_{2}O_{3}S taking 598 as the specific absorbance at 232 nm.

Tests

Appearance of solution. Dissolve 1.0 g in sufficient 2 M sodium hydroxide to produce 10 ml. The solution is clear (2.4.1), and not more intensely coloured than degree 6 of the appropriate range of reference solutions (2.4.1).

Acidity. Dissolve 1 g in a mixture of 25 ml of acetone and 25 ml of carbon dioxide-free water with the aid of heat, cool and titrate with 0.01 M sodium hydroxide using methyl red solution as indicator. Repeat the operation without the substance under examination. The difference between the titrations is not more than 0.75 ml.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Chlorides (2.3.12). Triturate 0.5 g with 30 ml of water, shake for 5 minutes and filter. 15 ml of the filtrate complies with the limit test for chlorides. Use 5.0 ml of chloride standard solution (25 ppm Cl) to prepare the standard (500 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°C.

Assay. Weigh accurately about 0.2 g and dissolve in 50 ml of acetone. Titrate with 0.1 M tetrabutylammonium hydroxide in an atmosphere of nitrogen, determining the end-point potentiometrically (2.4.25). Carry out a blank titration. 1 ml of 0.1 M tetrabutylammonium hydroxide is equivalent to 0.03388 g of C_{10}H_{13}ClN_{2}O_{3}S.
Chlorthalidone Tablets

Chlorthalidone Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of chlorthalidone, C₁₄H₁₁ClN₂O₄S.

Identification

Heat a quantity of the powdered tablets containing 0.2 g of Chlorthalidone with 20 ml of acetone on a water-bath for 10 minutes, cool and filter. Add 40 ml of water to the filtrate and heat on a water-bath for 20 minutes using a gentle current of air to remove the solvent. Cool to room temperature and allow to stand, filter and dry the crystals at 105° for 4 hours. The crystals comply with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with chlorthalidone RS or with the reference spectrum of chlorthalidone.

B. When examined in the range 230 nm to 360 nm, a 0.01 per cent w/v solution in ethanol (95 per cent) shows absorption maxima at about 275 nm and at about 284 nm; absorbance at about 275 nm, about 0.6 and at about 284 nm, about 0.45 (2.4.7).

C. Wash with water a quantity of the crystals obtained in test A and dissolve 50 mg in 3 ml of sulphuric acid; an intense yellow colour is produced.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF₂₅₄.

Test solution. Shake a quantity of the powdered tablets containing 50 mg of Chlorthalidone with 5 ml of acetone, centrifuge and use the supernatant liquid.

Reference solution. A solution containing 0.01 per cent w/v of 2-(4-chloro-3-sulphamoylbenzoyl)benzoic acid RS in acetone.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Chlorthalidone, boil with 30 ml of methanol under a reflux condenser for 5 minutes, shake vigorously for 15 minutes, cool and filter; wash the residue with methanol and filter. Dilute the combined filtrate and washings to 100.0 ml with methanol. To 5.0 ml add 2 ml of 1 M hydrochloric acid and sufficient methanol to produce 50.0 ml. Measure the absorbance of the resulting solution at the maximum at about 275 nm (2.4.7). Calculate the content of C₁₄H₁₁ClN₂O₄S taking 57.4 as the specific absorbance at 275 nm.

Cholecalciferol

Vitamin D₃

C₂₇H₄₄O    Mol Wt. 384.6
Cholecalciferol is (5Z,7E)-(3S)-9,10-secocholesta-5,7,10(19)-trien-3-ol.

Cholecalciferol contains not less than 97.0 per cent and not more than 103.0 per cent of C₂₇H₄₄O.

Description. White or almost white crystals; odourless or almost odourless. It is sensitive to air, heat and light. A reversible isomerisation to precholecalciferol may occur in solution, depending on temperature and time.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cholecalciferol RS.

B. Dissolve 1 mg in 1 ml of 1,2-dichloroethane and 4 ml of antimony trichloride solution; a yellowish-orange colour is produced.

C. In the test for 7-Dehydrocholesterol, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (b).

D. To a solution of about 0.5 mg in 5 ml of chloroform add 0.3 ml of acetic anhydride and 0.1 ml sulphuric acid and shake vigorously; a bright red colour is produced which rapidly changes through violet and blue to green.

Tests

Specific optical rotation (2.4.22). +105° to +112°, determined, within 30 minutes of preparation, in a solution prepared by dissolving 0.2 g rapidly and without heating in sufficient aldehyde-free ethanol (95 per cent) to produce 25.0 ml.
**Light absorption.** Dissolve 10 mg, rapidly and without heating, in sufficient *aldehyde-free ethanol* (95 per cent) to produce 100.0 ml. Dilute 5.0 ml of this solution to 50.0 ml with *aldehyde-free ethanol* (95 per cent). Absorbance of the resulting solution at the maximum at about 265 nm, measured within 30 minutes of preparation, 0.46 to 0.50 (2.4.7).

**7-Dehydrocholesterol.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase.* A 0.01 per cent w/v solution of *butylated hydroxytoluene* in a mixture of equal volumes of *cyclohexane* and *peroxide-free ether*.

Prepare the following solutions immediately before use.

*Test solution.* Dissolve 0.25 g of the substance under examination in sufficient of *1,2-dichloroethane* containing 1 per cent w/v of *squalane* and 0.01 per cent w/v of *butylated hydroxytoluene* (solvent A) to produce 5 ml.

*Reference solution (a).* A solution containing 0.005 per cent w/v of 7-dehydrocholesterol *RS* in solvent A.

*Reference solution (b).* A solution containing 2.5 per cent w/v of *cholecalciferol* *RS* in solvent A.

*Reference solution (c).* Mix equal volumes of reference solutions (a) and (b).

Apply to the plate 10 µl of each solution. Develop the chromatograms immediately, protected from light. After development, dry the plate in air and spray three times with *antimony trichloride reagent*. Examine the chromatograms for not more than 4 minutes after spraying. The principal spot in the chromatogram obtained with the test solution is initially orange-yellow but becomes brown later. In the chromatogram obtained with the test solution any violet spot with an Rf value slightly lower than that of the principal spot (due to 7-dehydrocholesterol and appearing slowly) is not more intense than the spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

**Assay.** Carry out the following procedure as rapidly as possible in subdued light and protected from air.

Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh accurately about 50.0 mg of the substance under examination, dissolve in 10 ml of *toluene* without heating and dilute to 100.0 ml with the mobile phase; dilute 5.0 ml of this solution to 50.0 ml with the mobile phase; further dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

*Reference solution (a).* Dissolve 50.0 mg of *cholecalciferol* *RS* in 10 ml of toluene without heating and dilute to 100.0 ml with the mobile phase; dilute 5.0 ml of this solution to 50.0 ml with the mobile phase (Solution A); further dilute 5.0 ml of solution A to 50.0 ml with the mobile phase.

*Reference solution (b).* Reflux 5.0 ml of solution A, under nitrogen, on a water-bath for 60 minutes to obtain a solution of cholecalciferol, precholecalciferol and *trans*-cholecalciferol.

**Chromatographic system**
- a stainless steel column 25 cm × 4.6 mm, packed with porous silica particles (5 μm) (such as Nucleosil 50-S 5 μm),
- mobile phase: a mixture of 997 volumes of *hexane* and 3 volumes of 1-pentanol,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 μl loop injector.

Inject reference solution (b) and record the chromatogram adjusting the sensitivity so that the height of the peak due to cholecalciferol is more than 50 per cent of full-scale deflection. The approximate relative retention times calculated with reference to cholecalciferol are 0.4 for precholecalciferol and 0.5 for *trans*-cholecalciferol. The resolution between precholecalciferol and *trans*-cholecalciferol should be not less than 1.0; if necessary adjust the proportions of the constituents and flow rate of the mobile phase to obtain the required resolution.

Inject reference solution (a) and record the chromatogram adjusting the sensitivity so that the height of the peak due to cholecalciferol is more than 50 per cent of full-scale deflection. Inject the test solution. Measure the areas for the major peaks. Calculate the content of C_{27}H_{44}O.

**Storage.** Store protected from light in hermetically sealed containers under nitrogen in a refrigerator. The contents of an opened container should be used immediately.

**Chorionic Gonadotrophin**

**Human Chorionic Gonadotrophin**

Chorionic Gonadotrophin is a dry, sterile preparation of placental glycoproteins that has luteinising activity. It is extracted from the urine of pregnant women. The material is sterilised by filtration and dried under reduced pressure or freeze-dried.

Chorionic Gonadotrophin contains not less than 2500 Units

**Description.** A white or almost white, amorphous powder.

**Identification**

It causes an increase in the weight of the seminal vesicles or of the prostate glands of immature male rats when administered as directed in the Assay.
Tests

**Appearance of solution.** A 1.0 per cent w/v solution is clear (2.4.1), and colourless (2.4.1).

**Water.** Not more than 5 per cent, determined by the following method.

Determine by gas chromatography (2.4.13).

*Use throughout dry glassware that may be siliconised.*

**Internal standard.** Dilute 15 µl of anhydrous methanol with sufficient anhydrous 2-propanol to produce 100 ml.

**Test solution (a).** Dissolve 4 mg of the substance under examination in 0.5 ml of anhydrous 2-propanol.

**Test solution (b).** Dissolve 4 mg of the substance under examination in 0.5 ml of test solution (a).

**Reference solution.** Add 10 µl of water to 50 ml of test solution (a).

Chromatographic system

- a stainless steel column 1m × 2 mm, packed with porous polymer beads (60 to 80 mesh) (such as Chromosorb 102),
- temperature:
  - column: 120°,
  - inlet port and detector: 150°,
- thermal conductivity detector,
- flow rate: 30 ml per minute of the carrier gas (helium).

From the chromatograms obtained, and taking into account any water detectable in test solution (a), calculate the percentage of water taking 0.9960 g as the weight per ml at 25°.

**Assay.** Carry out the biological assay of chorionic gonadotrophin described below.

**Standard preparation.** The 3rd International Standard for Chorionic Gonadotrophin, human, established in 1986, consisting of a freeze-dried extract of human chorionic gonadotrophin with human albumin (supplied in ampoules containing 650 Units), or another suitable preparation the potency of which has been determined in relation to the International Standard.

Dissolve a sufficient quantity corresponding to the daily doses to be used in sufficient albumin-phosphate buffer pH 7.2 so that the daily dose is about 0.2 ml. Add a suitable antimicrobial preservative such as 0.4 per cent w/v of phenol or 0.002 per cent w/v of thiomersal. Store the solution at a temperature of 2° to 8°.

Use immature male rats of the same strain, approximately 21 days old and of approximately equal weight within the range 25 to 35 g. Assign the rats at random to four equal groups of at least eight animals. If sets of four littersmates are available, allot one littersmate from each set at random to each group and mark according to the litter.

Choose two doses of the standard preparation and two of the test solution such that the smaller dose is sufficient to produce a positive response in some of the rats and the larger dose does not produce a maximum response in all of the rats. As an initial approximation, doses of 7.5 and 15 Units may be tried although the dose will depend on the sensitivity of the animals used, which may vary widely.

Inject subcutaneously into each rat the daily dose allocated to its group on 4 consecutive days at the same time each day. On the fifth day, about 24 hours after the last injection, kill the rats and remove the seminal vesicles or the prostate glands from each animal. Remove any extraneous fluid and tissue from the vesicles or glands and weigh them immediately. Calculate the result of the assay by standard statistical methods using the weight of the vesicles or prostate glands as the response.

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The fiducial limits of error are not less than 64 per cent and not more than 156 per cent of the stated potency.

**Chorionic Gonadotrophin intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.**

**Bacterial endotoxins** (2.2.3). Not more than 15 Endotoxin Units per ml of a solution prepared in the following manner. Dissolve a quantity in water BET to obtain a solution containing 500 units of chorionic gonadotrophin per ml. Carry out the test using Maximum Valid dilution of this solution calculated from the declared sensitivity of the lysate used in the test.

**Chorionic Gonadotrophin intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirements.**

**Sterility** (2.2.11). Complies with the test for sterility.

**Abnormal toxicity** (2.2.1). Complies with the test for abnormal toxicity (2.2.1) using a quantity equivalent to 1000 Units dissolved in 0.5 ml of sodium chloride injection and observing the animals for 48 hours.
Storage. Store protected from light in a tamper-evident container, which is sealed so as to exclude micro-organisms, in a refrigerator (2° to 8°).

Labelling. The label states (1) the number of Units contained in the container; (2) the number of Units per mg; (3) whether or not it is intended for use in the manufacture of parenteral preparations.

Chorionic Gonadotrophin Injection

Chorionic Gonadotrophin Injection is a sterile material consisting of Chorionic Gonadotrophin with or without excipients such as buffers, diluents or other inert substances such as Lactose or Sodium Chloride. It may also contain an antimicrobial agent. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Chorionic Gonadotrophin Injection contains not less than 80.0 per cent and not more than 125.0 per cent of the stated potency.

Description. A white or almost white powder.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Identification

It causes an increase in the weight of the seminal vesicles or of the prostate glands of immature male rats when administered as directed in the Assay.

Tests

pH (2.4.24). 6.0 to 8.0, determined in a 1.0 per cent w/v solution.

Water. Not more than 5.0 per cent, determined by the following method.

Determine by gas chromatography (2.4.13).

Use throughout dry glassware that may be siliconised.

Internal standard. Dilute 15 µl of anhydrous methanol with sufficient anhydrous 2-propanol to produce 100 ml.

Test solution (a). Dissolve 4 mg of the substance under examination in 0.5 ml of anhydrous 2-propanol.

Test solution (b). Dissolve 4 mg of the substance under examination in 0.5 ml of test solution (a).

Reference solution. Add 10 µl of water to 50 ml of test solution (a).

Chromatographic system

− a stainless steel column 1m x 2 mm, packed with porous polymer beads (60 to 80 mesh) (such as Chromosorb 102),
− temperature: column.120°, inlet port and detector. 150°,
− thermal conductivity detector,
− flow rate. 30 ml per minute of the carrier gas (helium).

From the chromatograms obtained, and taking into account any water detectable in test solution (a), calculate the percentage of water taking 0.9960 g as the weight per ml at 25°.

Assay. Carry out the biological assay of chorionic gonadotrophin described below.

Standard preparation. The 3rd International Standard for Chorionic Gonadotrophin, human, established in 1986, consisting of a freeze-dried extract of human chorionic gonadotrophin with human albumin (supplied in ampoules containing 650 Units), or another suitable preparation the potency of which has been determined in relation to the International Standard.

Dissolve a sufficient quantity corresponding to the daily doses to be used in sufficient albumin-phosphate buffer pH 7.2 so that the daily dose is about 0.2 ml. Add a suitable antimicrobial preservative such as 0.4 per cent w/v of phenol or 0.002 per cent w/v of thiomersal. Store the solution at a temperature of 2° to 8°.

Test preparation. Dissolve a sufficient quantity of the injection under examination corresponding to the daily doses to be used in sufficient albumin-phosphate buffer pH 7.2 so that the daily dose is about 0.2 ml. Add a suitable antimicrobial preservative such as 0.4 per cent w/v of phenol or 0.002 per cent w/v of thiomersal. Store the solution at a temperature of 2° to 8°.

Use immature male rats of the same strain, approximately 21 days old and of approximately equal weight within the range 25 to 35 g. Assign the rats at random to four equal groups of at least eight animals. If sets of four littersmates are available, allot one littermate from each set at random to each group and mark according to the litter.

Choose two doses of the standard preparation and two of the test solution such that the smaller dose is sufficient to produce a positive response in some of the rats and the larger dose does not produce a maximum response in all of the rats. As an
initial approximation, doses of 7.5 and 15 Units may be tried although the dose will depend on the sensitivity of the animals used that may vary widely.

Inject subcutaneously into each rat the daily dose allocated to its group on 4 consecutive days at the same time each day. On the fifth day, about 24 hours after the last injection, kill the rats and remove the seminal vesicles or the prostate glands from each animal. Remove any extraneous fluid and tissue from the vesicles or glands and weigh them immediately. Calculate the result of the assay by standard statistical methods using the weight of the vesicles or prostate glands as the response.

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The fiducial limits of error are not less than 64 per cent and not more than 156 per cent of the stated potency.

**Bacterial endotoxins (2.2.3).** Not more than 15 Endotoxin Units per ml of a solution prepared in the following manner. Dissolve the contents of a sealed container in water BET to obtain a solution containing 500 units of chorionic gonadotrophin per ml. Carry out the test using Maximum Valid Dilution of this solution calculated from the declared sensitivity of the lysate used in the test.

**Abnormal toxicity (2.2.1).** Use a quantity equivalent to 1000 Units dissolved in 0.5 ml of sodium chloride injection and observing the animals for 48 hours.

**Storage.** Store protected from light in containers, which are sealed so as to exclude micro-organisms, at a temperature not exceeding 20°.

**Labelling.** The label states (1) the number of Units contained in the sealed container; (2) the name(s) of any added substance(s).

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**Ciclesonide**

Ciclesonide contains not less than 98.0 per cent and not more than 102.0 per cent of ciclesonide, C_{32}H_{44}O_{7}, calculated on the anhydrous basis.

**Description.** A white to-off white powder.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ciclesonide RS or with the reference spectrum of ciclesonide.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

**Tests**

**Specific optical rotation** (2.4.22). +90.0° to +98.0°, determined in a 0.5 per cent w/v solution in methanol.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 50 mg of the substance under examination in 50 ml of methanol.

**Reference solution.** A 0.001 per cent w/v solution of ciclesonide RS in methanol.

**Chromatographic system**

- a stainless steel column 25 cm × 4.6 mm, packed with octylsilyl silica gel (5 µm),
- mobile phase: A. dilute 1 volume of orthophosphoric acid to 1000 ml with water, B. acetonitrile
- flow rate. 1.5 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 240 nm,
- a 20 µl loop injector.

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>65</td>
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<tr>
<td>55</td>
<td>65</td>
<td>35</td>
</tr>
</tbody>
</table>

Inject reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 30000 theoretical plates.

Inject the test solution. Any individual impurity is not more than 0.5 per cent and the sum of all the impurities found is not more than 1.0 per cent.
Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent, determined on 1.0 g.

Water (2.3.43). Not more than 0.5 per cent, determined on 1 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 50.0 ml of methanol. Dilute 5 ml of the resulting solution to 50.0 ml with methanol.

Reference solution. A 0.01 per cent w/v solution of ciclesonide RS in methanol.

Chromatographic system
– a stainless steel column 25 cm x 4.6 mm, packed with octylsilyl silica gel (5 µm),
– mobile phase: a mixture of 30 volumes of 0.1 per cent orthophosphoric acid and 70 volumes of acetonitrile,
– flow rate. 1.5 ml per minute,
– spectrophotometer set at 245 nm,
– a 20 µl loop injector.

Inject the reference solution. The test is not valid unless relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of C$_{32}$H$_{44}$O$_{7}$.

Storage. Store protected from light, at a temperature not exceeding 30º.

Ciclesonide Inhalation

Ciclesonide Inhalation is a suspension of microfine Ciclesonide in a suitable liquid filled in a suitable pressurized container. It may contain suitable pharmaceutical aids such as surfactants, stabilizing agents.

Ciclesonide Inhalation delivers not less than 80.0 per cent and not more than 120.0 per cent of the stated amount of ciclesonide, C$_{32}$H$_{44}$O$_{7}$, per inhalation by actuation of the valve.

Identification

In the Assay the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with reference solution (b).

Tests

Other tests. Complies with the tests stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

Follow the procedure described under Assay with suitable dilution of the reference solution wherever the amount of active substance is to be determined in any test.

Assay. Carry out the test for Content of active ingredient delivered per actuation stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of equal volumes of water and acetonitrile.

Test solution. Prepare using the solvent mixture as described under the test for Content of active ingredient delivered per actuation stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

Reference solution (a). A 0.04 per cent w/v solution of ciclesonide RS in acetonitrile.

Reference solution (b). Dilute reference solution (a) with the solvent mixture to obtain a solution containing 32 µg of Ciclesonide per ml.

Chromatographic system
– a stainless steel column 15 cm x 4.6 mm, packed with octylsilyl silica gel (5 µm),
– mobile phase: a mixture of 30 volumes of a buffer solution prepared by diluting 1 ml of orthophosphoric acid to 1000 ml with water, and 70 volumes of acetonitrile,
– flow rate. 3 ml per minute,
– spectrophotometer set at 245 nm,
– inject 200 µl.

Inject reference solution (b). The test is not valid unless the column efficiency is not less than 3500 theoretical plates and the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution (b).

Calculate the content of C$_{32}$H$_{44}$O$_{7}$ in the solution and the amount of C$_{32}$H$_{44}$O$_{7}$ delivered per actuation of the valve.

Determine the content of active ingredient a second and third time by repeating the procedure on the middle ten and on the last ten successive combined actuations of the valve. For each of the three determinations the average content of C$_{32}$H$_{44}$O$_{7}$ delivered per actuation of the valve meets the requirements.

Storage. Store protected from moisture at a temperature not exceeding 30º.

Labelling. The label states the amount of active ingredient delivered per inhalation.
Cimetidine

\[
\begin{align*}
\text{HN} & \quad \text{S} & \quad \text{NHCH}_3 \\
\text{NCN} & \quad \text{CH}_3
\end{align*}
\]

\(\text{C}_{10}\text{H}_{16}\text{N}_6\text{S}\). Mol Wt. 252.3

Cimetidine is 2-cyano-1-methyl-3-[2-(5-methylimidazol-4-ylmethylthio)ethyl]guanidine.

Cimetidine contains not less than 98.5 per cent and not more than 101.5 per cent of \(\text{C}_{10}\text{H}_{16}\text{N}_6\text{S}\), calculated on the dried basis.

**Description.** A white or almost white powder.

**Identification**

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6), using a potassium bromide dispersion obtained from the solid state without prior solvent treatment. Compare the spectrum with that obtained with cimetidine RS or with the reference spectrum of cimetidine. No shoulder or peak should be discernible at 1190 cm\(^{-1}\).

B. When examined in the range 210 nm to 360 nm, a 0.0008 per cent w/v solution in 1 M sulphuric acid shows an absorption maximum at about 218 nm and a minimum at about 260 nm (2.4.7).

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (d).

D. Dissolve about 1 mg in a mixture of 1 ml of ethanol and 5 ml of a freshly prepared 2 per cent w/v solution of citric acid in acetic anhydride. Heat in a water-bath for 10 to 15 minutes; a reddish violet colour is produced.

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase (a).** A mixture of 65 volumes of ethyl acetate, 20 volumes of methanol and 15 volumes of strong ammonia solution.

**Mobile phase (b).** A mixture of 84 volumes of ethyl acetate, 8 volumes of methanol and 8 volumes of strong ammonia solution.

**Test solution (a).** Dissolve 0.5 g of the substance under examination in sufficient methanol to produce 10 ml.

**Test solution (b).** Dilute 1 ml of test solution (a) to 10 ml with methanol.

**Reference solution (a).** Dilute 1 ml of solution (a) to 100 ml with methanol and dilute 20 ml of this solution to 100 ml with methanol.

**Reference solution (b).** Dilute 5 ml of reference solution (a) to 10 ml with methanol.

**Reference solution (c).** Dilute 5 ml of reference solution (b) to 10 ml with methanol.

**Reference solution (d).** Dissolve 10 mg of cimetidine RS in 2 ml of methanol.

Apply separately to two plates 4 µl of each solution. Allow the first plate to stand for 15 minutes in the tank saturated with vapour from mobile phase (a). Develop the second plate using mobile phase (b). After development, dry the plates in a current of air, expose to iodine vapour until maximum contrast of the spots has been obtained and examine in ultraviolet light at 254 nm. The following limits apply to both methods. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (c) shows a clearly visible spot.

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.25 g and dissolve in 75 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02523 g of \(\text{C}_{10}\text{H}_{16}\text{N}_6\text{S}\).

**Storage.** Store protected from light.

**Cimetidine Tablets**

Cimetidine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of cimetidine, \(\text{C}_{10}\text{H}_{16}\text{N}_6\text{S}\).

**Identification**

A. Shake a quantity of the powdered tablets containing 0.1 g of Cimetidine with 10 ml of methanol, filter, evaporate the
filtrate to dryness using gentle heat and dry the residue at 60° at a pressure not exceeding 0.7 kPa. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cimetidine RS or with the reference spectrum of cimetidine.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (d).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase (a). A mixture of 65 volumes of ethyl acetate, 20 volumes of methanol and 15 volumes of strong ammonia solution.

Mobile phase (b). A mixture of 84 volumes of ethyl acetate, 8 volumes of methanol and 8 volumes of strong ammonia solution.

Test solution (a). Add 20 ml of methanol to a quantity of the powdered tablets containing 1 g of Cimetidine, mix with the aid of ultrasound for 2 minutes, shake for 3 minutes and filter using a suitable 0.2 µm filter.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with methanol.

Reference solution (a). Dilute 1 ml of test solution (a) to 100 ml with methanol and dilute 20 ml of this solution to 100 ml with methanol.

Reference solution (b). Dilute 5 ml of reference solution (a) to 10 ml with methanol.

Reference solution (c). Dilute 5 ml of reference solution (b) to 10 ml with methanol.

Reference solution (d). Dissolve 10 mg of cimetidine RS in 2 ml of methanol.

Apply separately to two plates 4 µl of each solution. Allow the first plate to stand for 15 minutes in the tank saturated with vapour from mobile phase (a). Develop the second plate using mobile phase (b). After development, dry the plates in a current of air, expose to iodine vapour until maximum contrast of the spots has been obtained and examine in ultraviolet light at 254 nm. The following limits apply to both methods. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (c) shows a clearly visible spot.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.25 g of Cimetidine and stir with 20 ml of warm methanol. Filter and repeat the extraction with three quantities, each of 20 ml, of warm methanol. Evaporate the combined filtrate and washings to dryness and dissolve the residue in 75 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02523 g of C10H16N6S.

Cinnarizine

\[
\text{C}_{26}\text{H}_{28}\text{N}_{2} \quad \text{Mol. Wt. 368.5}
\]

Cinnarizine is \((E)-1-(diphenylmethyl)-4-(3-phenylprop-2-enyl)piperazine.

Cinnarizine contains not less than 99.0 per cent and not more than 101.0 per cent of \(\text{C}_{26}\text{H}_{28}\text{N}_{2}\), calculated on the dried basis.

Description. A white or almost white powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cinnarizine RS.

B. In the test for Related substances the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. Dissolve 0.2 g of anhydrous citric acid in 10 ml of acetic anhydride in a water-bath at 80° and maintain the temperature of the water-bath at 80° for 10 minutes. Add about 20 mg of the substance under examination; a purple colour is produced.
Tests

Appearance of solution. A 2.5 per cent w/v solution in dichloromethane is clear (2.4.1) and not more intensely coloured than reference solution BYS6 (2.4.1).

Acidity or Alkalinity. Suspend 0.5 g in 15 ml of water. Boil for 2 minutes, cool and filter. Dilute the filtrate to 20 ml with carbon dioxide-free water. To 10 ml add 0.1 ml of phenolphthalein solution and 0.25 ml of 0.01 M sodium hydroxide; the solution is pink. To 10 ml add 0.1 ml of methyl red solution and 0.25 ml of 0.01 M hydrochloric acid; the solution is red.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF 254.

Mobile phase. A mixture of 90 volumes of toluene and 10 volumes of methanol.

Test solution (a). Dissolve 0.5 g of the substance under examination in 10 ml of dichloromethane.

Test solution (b). Dilute 5 ml of test solution (a) to 100 ml with dichloromethane.

Reference solution (a). A 0.25 per cent w/v solution of cinnarazine RS in dichloromethane.

Reference solution (b). A 0.0125 per cent w/v solution of the substance under examination in dichloromethane.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, heat at 105° for 5 minutes and examine in ultraviolet light at 254 nm. Expose the plate to iodine vapours for 15 minutes and examine in daylight. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b).

Heavy metals (2.3.13). Dissolve 1.0 g in a mixture of 85 volumes of acetone and 15 volumes of water and add dilute hydrochloric acid until dissolution is complete. Dilute to 20 ml with the same mixture of acetone and water. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (20 ppm). Prepare the standard using 10 ml of lead standard solution (1 ppm Pb) obtained by diluting lead standard solution (100 ppm Pb) with the mixture of acetone and water.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 4 hours.

Assay. Weigh accurately about 0.15 g and dissolve in a mixture of 70 volumes of 2-butanone and 10 volumes of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, using á-naphtholbenzein solution as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.01843 of C26H28N2.

Cinnarizine Tablets

Cinnarizine tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cinnarizine, C26H28N2.

Identification

A. Extract a quantity of the powdered tablets containing 0.1 g of Cinnarizine with 20 ml of dichloromethane, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cinnarizine RS.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution (a). Shake a suitable quantity of the powdered tablets containing 25 mg of Cinnarizine with methanol, dilute to 10 ml with the same solvent and filter.

Reference solution (a). Dissolve 12.5 mg of cinnarizine RS and 15 mg of flunarizine hydrochloride RS in methanol and dilute to 100 ml with the same solvent. Dilute 1 ml of this solution to 20 ml with methanol.

Reference solution (b). Dilute 1 ml of the test solution to 100 ml with methanol. Dilute 5 ml of this solution to 20 ml with methanol.

Chromatographic system

- a stainless steel column 10 cm × 4 mm, packed with base-deactivated octadecylsilyl silica gel (3 µm),
- mobile phase: A. a 10 per cent w/v solution of ammonium acetate,
- B. a 0.2 per cent v/v solution of glacial acetic acid in acetonitrile,
- flow rate. 1.5 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 230 nm,
- a 10 µl loop injector.
Ciprofloxacin

\[
\text{C}_{17}\text{H}_{18}\text{FN}_3\text{O}_3 \quad \text{Mol. Wt. 331.4}
\]

Ciprofloxacin is 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylic acid.

Ciprofloxacin contains not less than 98.0 per cent and not more than 102.0 per cent of \(\text{C}_{17}\text{H}_{18}\text{FN}_3\text{O}_3\), calculated on the dried basis.

Description. A white to pale yellow, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained from ciprofloxacin RS or with the reference spectrum of ciprofloxacin.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 40 volumes of dichloromethane, 40 volumes of methanol, 20 volumes of strong ammonia solution and 10 volumes of acetonitrile.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of 6 M ammonia.

Reference solution. A 1 per cent w/v solution of ciprofloxacin RS in 6 M ammonia.

Apply to the plate, as 1-cm bands, 5 µl of each solution. Place the plate in an atmosphere of ammonia for about 15 minutes and transfer it to an unsaturated chamber containing the mobile phase. Allow the mobile phase to rise 12 cm. Dry the plate in air for 15 minutes and examine in ultraviolet light at 254 nm and at 365 nm. The principal band in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution (b).

Other tests. Comply with the tests stated under tablets.

Assay. Determine by liquid chromatography (2.4.14) as given under the test for Related substances using the following solutions.

Test solution. Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing about 25 mg of Cinnarazine with methanol, dilute to 50.0 ml with the same solvent and filter. Dilute 5.0 ml of this solution to 50.0 ml with methanol.

Reference solution. A 0.005 per cent w/v solution of cinnarazine RS in methanol.

Calculate the content of \(\text{C}_{26}\text{H}_{28}\text{N}_2\) in the tablets.

Storage. Store protected from light.

<table>
<thead>
<tr>
<th>Time</th>
<th>Mobile phase A</th>
<th>Mobile phase B</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>(min)</td>
<td>(per cent v/v)</td>
<td>(per cent v/v)</td>
<td></td>
</tr>
<tr>
<td>0 – 20</td>
<td>75</td>
<td>10</td>
<td>linear gradient</td>
</tr>
<tr>
<td>20 – 25</td>
<td>10</td>
<td>90</td>
<td>isocratic elution</td>
</tr>
<tr>
<td>25 – 30</td>
<td>75</td>
<td>25</td>
<td>switch to initial eluent composition</td>
</tr>
<tr>
<td>30 = 0</td>
<td>75</td>
<td>25</td>
<td>restart gradient</td>
</tr>
</tbody>
</table>

Equilibrate the column for at least 30 minutes at the initial eluent composition.

Inject reference solution (b). Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained is at least 50 per cent of the full scale of the recorder. If necessary, adjust the concentration of glacial acetic acid in mobile phase B to obtain a horizontal base-line in the chromatogram.

Inject reference solution (a). When the chromatogram is recorded in the prescribed conditions, the retention times are: cinnarizine about 11 min and flunarizine about 11.5 min. The test is not valid unless the resolution between the peaks corresponding to cinnarizine and flunarizine is at least 5.0. If necessary, adjust the time programme for the gradient elution.

Inject the blank, the test solution and reference solution (b). In the chromatogram obtained with the test solution: the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent); the sum of the areas of the peaks, other than the principal peak, is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak due to the blank and any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Appearance of solution. A 2.5 per cent w/v solution in 0.1 M hydrochloric acid is clear (2.4.1).

Related substances. Carry out the method described in the Assay and calculate the percentage of each impurity from the chromatogram obtained with the test solution. The content of ciprofloxacin ethylenediamine analog or of any other individual impurity peak found is not more than 0.2 per cent and the sum of all the impurity peaks is not more than 0.5 per cent.
**Fluoroquinolonic acid.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 40 volumes of dichloromethane, 40 volumes of methanol, 20 volumes of strong ammonia solution and 10 volumes of acetonitrile.

**Test solution.** Dissolve 0.1 g of the substance under examination in 10 ml of 0.1 M acetic acid.

**Reference solution.** Weigh 10 mg of fluoroquinolonic acid RS, add 0.1 ml of 6 M ammonia and dilute to 100.0 ml with water. Dilute 2.0 ml of this solution to 10.0 ml with water.

Apply to the plate 5 µl of each solution. Place the plate in an atmosphere of ammonia for about 15 minutes. Remove the plate and place it in a chamber containing the mobile phase. After development, dry the plate in air for 15 minutes and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution corresponding to the spot of fluoroquinolonic acid is not more intense than the spot in the chromatogram obtained with the reference solution.

**Heavy metals (2.3.13).** 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Chlorides (2.3.12).** To 2.0 g add 30 ml of water, shake for 5 minutes and filter through a chloride-free filter paper. 15 ml of the filtrate complies with the limit test for chlorides (250 ppm).

**Sulphates (2.3.17).** Dissolve 0.75 g in 5.0 ml of 2 M acetic acid and 20.0 ml of water. 10 ml of the resulting solution complies with the limit test for sulphates (400 ppm).

**Sulphated ash (2.3.18).** Not more than 0.1 per cent.

**Loss on drying (2.4.19).** Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 120° for 6 hours at a pressure not exceeding 0.7 kPa.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh accurately about 25 mg, add 0.2 ml of a solution containing 7 per cent v/v of phosphoric acid and add sufficient of the mobile phase to produce 50.0 ml.

**Reference solution (a).** Prepare in the same manner as the test solution using an accurately weighed quantity of ciprofloxacin RS in place of the substance under examination.

**Reference solution (b).** A 0.05 per cent w/v solution of ciprofloxacin ethylenediamine analog RS in reference solution (a).

Chromatographic system

- a stainless steel column 25 cm × 4 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 87 volumes of 0.025 M phosphoric acid, previously adjusted with triethylamine to a pH of 3.0 ± 0.1, and 13 volumes of acetonitrile,
- flow rate. 1.5 ml per minute,
- column temperature. 30° ± 1°,
- spectrophotometer set at 278 nm,
- a 10 µl loop injector.

Inject reference solution (b) and record the chromatogram adjusting the sensitivity and flow rate suitably so that the retention time for ciprofloxacin is between 6.4 and 10.8 minutes, the relative retention times are about 0.7 for ciprofloxacin ethylenediamine analog and 1.0 for ciprofloxacin and the resolution between ciprofloxacin ethylenediamine analog peak and ciprofloxacin peak is not less than 6. The column efficiency, determined from ciprofloxacin peak, is not less than 2500 theoretical plates, the tailing factor for the ciprofloxacin peak is not more than 4.0 and the relative standard deviation for replicate injections is not more than 1.5 per cent.

Inject alternately the test solution and reference solution (a). Calculate the content of C17H18FN3O3.

**Storage.** Store protected from light.

**Ciprofloxacin Injection**

Ciprofloxacin Injection is a sterile solution of Ciprofloxacin in 5 per cent Dextrose Injection or in Sodium Chloride Injection prepared with the aid of Lactic Acid.

Ciprofloxacin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ciprofloxacin, C17H18FN3O3.

**Identification**

Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G. Place the plate in an atmosphere of ammonia for about 15 minutes and transfer it to an unsaturated chamber.

**Mobile phase.** A mixture of 40 volumes of dichloromethane, 40 volumes of methanol, 20 volumes of strong ammonia solution and 10 volumes of acetonitrile.

**Test solution.** Dilute sufficient of the injection with water to obtain a solution containing the equivalent of 0.05 per cent w/v of Ciprofloxacin.

**Reference solution.** A 0.05 per cent w/v solution of ciprofloxacin RS in 6 M ammonia.

Apply to the plate, as 1-cm bands, 5 µl of each solution. Place the plate in an atmosphere of ammonia for about 15 minutes and transfer it to an unsaturated chamber containing the mobile phase. Allow the mobile phase to rise 12 cm. Dry the
plate in air for 15 minutes and examine in ultraviolet light at 254 nm and at 365 nm. The principal band in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

**Tests**

**pH** (2.4.24). 3.5 to 4.6.

**Ciprofloxacin ethylenediamine analog.** Not more than 0.5 per cent, determined by the method described in the Assay. Calculate the percentage of ciprofloxacin ethylenediamine analog from the chromatogram obtained with the test solution from the following expression.

Per cent of the analog = \[100 \times \frac{a}{b} \times \frac{c}{d} \times 0.7\]

where 0.7 is the response factor for ciprofloxacin ethylenediamine analog relative to that of ciprofloxacin, \(a\) and \(b\) are the responses of ciprofloxacin ethylenediamine analog peak and the ciprofloxacin peak respectively.

**Lactic acid.** 0.288 mg to 0.352 mg for each mg of Ciprofloxacin stated on the label.

Determine by liquid chromatography (2.4.14).

**Test solution.** The substance under examination.

**Reference solution.** A 0.08 per cent w/v solution of sodium lactate RS in water.

**Chromatographic system**

- a stainless steel column 30 cm × 7.8 mm, packed with a strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form (7 to 11 µm),
- mobile phase: a mixture of 85 volumes of 0.0025 M sulphuric acid and 15 volumes of acetonitrile,
- column temperature. 40° ± 1°,
- flow rate. 0.6 ml per minute,
- spectrophotometer set at 208 nm,
- a 20 µl loop injector.

Inject the reference solution and record the chromatograms adjusting the sensitivity and flow rate suitably so that the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and reference solution, record the chromatograms and measure the peak responses for the major peaks. Calculate the content of lactic acid, \(C_3H_6O_3\), in the substance under examination.

**NOTE - After each analysis, the column should be rinsed with a mixture of 85 volumes of 0.005 M sulphuric acid and 15 volumes of acetonitrile to elute the ciprofloxacin from the column. The column may be regenerated with 0.005 M sulphuric acid and may be reused or stored.**

**Dextrose (if present).** 4.75 per cent to 5.25 per cent w/v of \(C_6H_12O_6\cdotH_2O\), determined by the following method. To 50.0 ml add 0.2 ml of 6 \(M\) ammonia and dilute to 100.0 ml. Mix well and determine the optical rotation at 25° in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 2.085 represents the percentage of dextrose monohydrate, \(C_6H_12O_6\cdotH_2O\), in the preparation under examination.

**Sodium chloride (if present).** 0.855 per cent to 0.945 per cent w/v of NaCl, determined by the following method. To 10.0 ml add 150 ml of water and titrate with 0.1 \(M\) silver nitrate using potassium chromate solution as indicator.

1 ml of 0.1 \(M\) silver nitrate is equivalent to 0.005844 g of NaCl.

**Bacterial endotoxins** (2.2.3). Not more than 0.25 Endotoxin Unit per mg of ciprofloxacin.

**Sterility** (2.2.11). Complies with the test for sterility, using Method A.

**Particulate contamination** (2.5.9). Complies with the limit test for particulate contamination.

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dilute a volume of the injection containing 25 mg of Ciprofloxacin to 100.0 ml with the mobile phase and mix.

**Reference solution (a).** A 0.03 per cent w/v solution of ciprofloxacin hydrochloride RS in the mobile phase.

**Reference solution (b).** Dissolve a sufficient quantity of ciprofloxacin ethylenediamine analog RS in reference solution (a) so as to obtain a solution containing 0.025 per cent w/v of the reference substance.

**Chromatographic system**

- a stainless steel column 25 cm × 4 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 87 volumes of 0.025 M phosphoric acid, previously adjusted with triethylamine to a pH of 3.0 ± 0.1, and 13 volumes of acetonitrile,
- flow rate. 1.5 ml per minute,
- column temperature. 30° ± 1°,
- spectrophotometer set at 278 nm,
- a 10 µl loop injector.

Inject the reference solution and record the chromatograms adjusting the sensitivity and flow rate suitably so that the retention time for ciprofloxacin is between 6.4 and 10.8 minutes, the relative retention times are about 0.7 for ciprofloxacin ethylenediamine analog and 1.0 for ciprofloxacin and the resolution between ciprofloxacin ethylenediamine analog peak and ciprofloxacin peak is not less than 6. The column efficiency, determined from ciprofloxacin peak, is not less than 2500 theoretical plates, the tailing factor for the ciprofloxacin peak is not more than 4.0 and the relative standard deviation
Ciprofloxacin Hydrochloride

C₇₁H₁₈FN₃O₃.HCl.H₂O  Mol. Wt. 385.8

Ciprofloxacin Hydrochloride is 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid hydrochloride monohydrate.

Ciprofloxacin Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of C₁₇H₁₈FN₃O₃.HCl, calculated on the anhydrous basis.

Description. A pale yellow, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ciprofloxacin hydrochloride RS.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G. Place the plate in an atmosphere of ammonia for about 15 minutes and transfer it to an unsaturated chamber.

Mobile phase. A mixture of 40 volumes of dichloromethane, 40 volumes of methanol, 20 volumes of strong ammonia solution and 10 volumes of acetonitrile.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of water.

Reference solution. A 1 per cent w/v solution of ciprofloxacin hydrochloride RS in water.

Apply to the plate, as 1-cm bands, 5 μl of each solution. Place the plate in an atmosphere of ammonia for about 15 minutes and transfer it to an unsaturated chamber containing the mobile phase. Allow the mobile phase to rise 12 cm. Dry the plate in air for 15 minutes and examine in ultraviolet light at 254 nm and at 365 nm. The principal band in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Gives the reactions of chlorides (2.3.1).

Tests

pH (2.4.24). 3.0 to 4.5, determined in a 2.5 per cent w/v solution.

Related substances. Carry out the method described in the Assay and calculate the percentage of each impurity peak in the chromatogram obtained with the test solution. The content of ciprofloxacin ethylenediamine analog or of any other individual impurity peak found is not more than 0.2 per cent and the sum of all the impurity peaks is not more than 0.5 per cent.

Fluoroquinolonic acid. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 40 volumes of dichloromethane, 40 volumes of methanol, 20 volumes of strong ammonia solution and 10 volumes of acetonitrile.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of water.

Reference solution. Weigh 10 mg of fluoroquionolonic acid RS, add 0.1 ml of 6 M ammonia and dilute to 100.0 ml with water. Dilute 2.0 ml of this solution to 10.0 ml with water.

Apply to the plate 5 μl of each solution. Place the plate in an atmosphere of ammonia for about 15 minutes. Remove the plate and place it in a chamber containing the mobile phase. After development, dry the plate in air for 15 minutes and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution corresponding to the spot of fluoroquionolonic acid is not more intense than the spot in the chromatogram obtained with the reference solution.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphates (2.3.17). 0.375 g complies with the limit test for sulphates (400 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.4.19). 4.7 to 6.7 per cent, determined on 0.2 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately about 50 mg of the substance under examination and dissolve in 100.0 ml of water.

Reference solution (a). A 0.05 per cent w/v solution of ciprofloxacin hydrochloride RS in water.

Reference solution (b). A 0.05 per cent w/v solution of ciprofloxacin ethylenediamine analog RS in water.

Chromatographic system

– a stainless steel column 25 cm × 4 mm, packed with octadecylsilyl silica gel (5 μm),
– mobile phase: a mixture of 87 volumes of 0.025 M phosphoric acid, previously adjusted with triethylamine to a pH of 3.0 ± 0.1, and 13 volumes of acetonitrile,
– flow rate. 1.5 ml per minute,
– column temperature. 30° ± 1°.
– spectrophotometer set at 278 nm,
– a 10 µl loop injector.

Inject reference solution (b) and record the chromatogram adjusting the sensitivity and flow rate suitably so that the retention time for ciprofloxacin is between 6.4 and 10.8 minutes, the relative retention times are about 0.7 for ciprofloxacin ethylenediamine analog and 1.0 for ciprofloxacin and the resolution between ciprofloxacin ethylenediamine analog peak and ciprofloxacin peak is not less than 6. The column efficiency, determined from ciprofloxacin peak, is not less than 2500 theoretical plates, the tailing factor for the ciprofloxacin peak is not more than 4.0 and the relative standard deviation for replicate injections is not more than 1.5 per cent.

Inject alternately the test solution and reference solution (a). Calculate the content of C$_{17}$H$_{18}$FN$_3$O$_3$.HCl.

Storage. Store protected from light.

Ciprofloxacin Eye Drops

Ciprofloxacin Eye Drops are a sterile solution of Ciprofloxacin Hydrochloride in Purified water.

Ciprofloxacin Eye Drops contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ciprofloxacin, C$_{17}$H$_{18}$FN$_3$O$_3$.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Give reaction A of chlorides (2.3.1).

Tests

pH (2.4.24). 3.5 to 5.5

Other tests. Comply with the tests stated under Eye Drops.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Transfer an accurately measured volume of Eye drops containing 6 mg of ciprofloxacin, to a 50-ml volumetric flask, dilute with water to volume, and mix.

Reference solution (a). A 0.014 per cent w/v solution of ciprofloxacin hydrochloride RS in water.

Resolution solution (b). A 0.001 per cent w/v solution of ciprofloxacin ethylenediamine analog RS in water

Chromatographic system
– a stainless steel column 25 cm x 4.6 mm packed with octadecysilane bonded to porous silica (5 µm),
– column temperature 30°C,
– mobile phase: a mixture of 75 volumes of 0.005 M tetrabutylammonium phosphate, adjusted to pH 2.0 with orthophosphoric acid and 25 volumes of methanol,
– flow rate. 1.5 ml per minute,
– spectrophotometer set at 280 nm,
– a 20 µl loop injector.

Inject reference solution (b). The relative retention time are about 0.8 for the ciprofloxacin ethylenediamine analog and 1.0 for ciprofloxacin and the resolution between the ciprofloxacin ethylenediamine analog peak and the ciprofloxacin peak is not less than 1.5.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 500 theoretical plates, the tailing factor not more than 2.0, and the relative standard deviation for replicate injections is not more than 2 per cent.

Inject alternatively the test solution and reference solution (a). Calculate the content of C$_{17}$H$_{18}$FN$_3$O$_3$ in the eye drops.

Storage. Store protected from light.

Ciprofloxacin Tablets

Ciprofloxacin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ciprofloxacin, C$_{17}$H$_{18}$FN$_3$O$_3$. The tablets may be coated.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G. Place the plate in an atmosphere of ammonia for about 15 minutes and transfer it to an unsaturated chamber.

Mobile phase. A mixture of 40 volumes of dichloromethane, 40 volumes of methanol, 20 volumes of strong ammonia solution and 10 volumes of acetonitrile.

Test solution. Shake a quantity of the powdered tablets containing about 0.15 g of ciprofloxacin with 75 ml of water for 20 minutes, dilute to 100.0 ml with water, mix, centrifuge and use the clear supernatant liquid.

Reference solution. A 0.15 per cent w/v solution of ciprofloxacin hydrochloride RS in water.

Apply to the plate, as 1-cm bands, 5 µl of each solution. Place the plate in an atmosphere of ammonia for about 15 minutes and transfer it to an unsaturated chamber containing the mobile phase. Allow the mobile phase to rise 12 cm. Dry the
plate in air for 15 minutes and examine in ultraviolet light at 254 nm and at 365 nm. The principal band in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

**Tests**

**Dissolution (2.5.2).**

**Apparatus.** No 1  
**Medium.** 900 ml of *water*  
**Speed and time.** 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted with *water* if necessary, at the maximum at about 276 nm (2.4.7). Calculate the content of ciprofloxacin, C17H18FN3O3, in the medium from the absorbance obtained by repeating the determination using a solution of known concentration of ciprofloxacin hydrochloride RS.

D. Not less than 80 per cent of the stated amount of C17H18FN3O3.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 1.25 g of ciprofloxacin, add about 400 ml of 0.01 M hydrochloric acid, shake for 20 minutes, dilute to 500.0 ml with 0.01 M hydrochloric acid, and filter. Dilute 10.0 ml of the filtrate to 100.0 ml with 0.01 M hydrochloric acid.

**Reference solution (a).** A 0.03 per cent w/v solution of ciprofloxacin hydrochloride RS in 0.01 M hydrochloric acid.

**Reference solution (b).** A 0.05 per cent w/v solution of ciprofloxacin ethylenediamine analog RS in water.

**Chromatographic system**
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilyl silica gel (5 µm),  
- mobile phase: a mixture of 87 volumes of 0.025 M phosphoric acid, previously adjusted with triethylamine to a pH of 3.0 ± 0.1, and 13 volumes of acetonitrile,  
- flow rate. 1.5 ml per minute,  
- column temperature. 30° ± 1°,  
- spectrophotometer set at 278 nm,  
- a 10 µl loop injector.

Inject reference solution (b) and record the chromatogram adjusting the sensitivity and flow rate suitably so that the retention time for ciprofloxacin is between 6.4 and 10.8 minutes, the relative retention times are about 0.7 for ciprofloxacin ethylenediamine analog and 1.0 for ciprofloxacin and the resolution between ciprofloxacin ethylenediamine analog peak and ciprofloxacin peak is not less than 6. The column efficiency, determined from ciprofloxacin peak, is not less than 2500 theoretical plates, the tailing factor for the ciprofloxacin peak is not more than 4.0 and the relative standard deviation for replicate injections is not more than 1.5 per cent.

Inject alternately the test solution and reference solution (a). Calculate the content of C17H18FN3O3.HCl in the tablets.

**Storage.** Store protected from light.

**Labelling.** The label states the strength in terms of the equivalent amount of ciprofloxacin.

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**Cisplatin**

\[ \text{H}_2\text{Cl}_2\text{N}_2\text{Pt} \quad \text{Mol. Wt. 300.0} \]

Cisplatin is *cis*-diamminedichloroplatinum(II).

Cisplatin contains not less than 97.0 per cent and not more than 102.0 per cent of H6Cl2N2Pt.

**Description.** A yellow powder or orange yellow crystals.

*CAUTION - Cisplatin is potentially cytotoxic. Great care should be taken in handling the powder and preparing solutions.*

*NOTE - Carry out all the tests and the Assay, except Identification tests A and C and the test for Silver, protected from light.*

**Identification**

*Test A may be omitted if tests B and C are carried out. Test C may be omitted if tests A and B are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cisplatin RS.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Add 50 mg to 2 ml of 2 M sodium hydroxide, evaporate to dryness, dissolve the residue in a mixture of 0.5 ml of nitric acid and 1.5 ml of hydrochloric acid and evaporate to dryness again; the residue is orange. Dissolve the residue in 0.5 ml of *water* and add 0.5 ml of ammonium chloride solution; a yellow crystalline precipitate is produced.

**Tests**

**Appearance of solution.** Solution A is clear (2.4.1) and not more intensely coloured than reference solution GYS5 or BYS5.
(2.4.1). A 2.0 per cent w/v solution in dimethylformamide is clear (2.4.1).

**pH** (2.4.24). 4.5 to 6.0, determined in a 0.1 per cent w/v solution in normal saline prepared in carbon dioxide-free water (solution A), measured immediately after preparation.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with microcrystalline cellulose and activating the plate by heating at 150° for 1 hour.

**Mobile phase.** A mixture of 90 volumes of dimethylformamide and 10 volumes of acetone.

**Test solution (a).** A 2 per cent w/v solution of the substance under examination in dimethylformamide.

**Test solution (b).** Dilute 1 ml of test solution (a) to 10 ml with dimethylformamide.

**Reference solution (a).** Dilute 5 ml of test solution (b) to 25 ml with dimethylformamide.

**Reference solution (b).** A 0.2 per cent w/v solution of cisplatin RS in dimethylformamide.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and spray with a 5 per cent w/v solution of stannous chloride in 1 M hydrochloric acid. After 1 hour, the chromatogram obtained with the test solution shows no secondary spot with an Rf value lower than that of the principal spot and any secondary spot with an Rf value higher than that of the principal spot is not more intense than the spot in the chromatogram obtained with reference solution (b).

**Silver.** Determine by atomic absorption spectrophotometry (2.4.2), measuring at 328 nm using a silver hollow-cathode light as a radiation source, a fuel-lean air-acetylene flame, preferably a spectral width of 0.5 nm.

**Test solution.** Dissolve 0.1 g of the substance under examination in 15 ml of nitric acid by heating up to 80°. Cool and dilute to 25.0 ml with water.

**Reference solutions.** Add 50 ml of nitric acid to suitable volumes (10 to 30 ml) of silver solution AAS and dilute to 100.0 ml with water.

Carry out a blank determination (250 ppm).

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Prepare immediately before use a 0.05 per cent w/v solution of the substance under examination in normal saline.

**Reference solution.** A 0.05 per cent w/v solution of cisplatin RS in normal saline.

Chromatographic system

- mobile phase: a mixture of 90 volumes of methanol and 10 volumes of normal saline
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 220 nm,
- a 20 µl loop injector.

Calculate the content of H$_2$Cl$_2$N$_2$Pt.

**Storage.** Store protected from light.

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**Cisplatin Injection**

Cisplatin Injection is a sterile, freeze-dried mixture of Cisplatin, Mannitol and Sodium Chloride. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage.** The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cisplatin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cisplatin, H$_2$Cl$_2$N$_2$Pt.

**Description.** A yellow powder.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

**CAUTION — Cisplatin is potentially cytotoxic. Great care should be taken in handling the powder and preparing solutions.**

**Identification**

A. When examined in the range 230 nm to 360 nm, a 0.1 per cent w/v solution in 0.1 M hydrochloric acid shows an absorption maximum only at about 300 nm (2.4.7).

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

**Tests**

**pH** (2.4.24). 3.5 to 6.5, determined in a solution constituted as directed in the label, in water for injections.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with microcrystalline cellulose and activating the plate by heating at 150° for 1 hour.
**Mobile phase.** A mixture of 90 volumes of *dimethylformamide* and 10 volumes of *acetone*.

**Test solution.** Shake the contents of one vial with *dimethylformamide* to produce a solution containing 0.5 per cent w/v of Cisplatin, mix with the aid of ultrasound for 10 minutes and filter.

**Reference solution (a).** Dilute 5 ml of the test solution to 50 ml with *dimethylformamide*.

**Reference solution (b).** Dilute 1 ml of the test solution to 50 ml with *dimethylformamide*.

**Reference solution (c).** A solution containing 0.05 per cent w/v of cisplatin RS in *dimethylformamide*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and spray with a 5 per cent w/v solution of *stannous chloride* in 1 M *hydrochloric acid*. After 1 hour, the chromatogram obtained with the test solution shows no secondary spot with an Rf value lower than that of the principal spot and any secondary spot with an Rf value higher than that of the principal spot is not more intense than the spot in the chromatogram obtained with reference solution (b).

**Bacterial endotoxins** (2.2.3). Not more than 2.0 Endotoxin Units per mg of cisplatin.

**Sterility** (2.2.11). Comply with the test for sterility, Method A.

**Assay.** Determine by liquid chromatography (2.4.17).

**Test solution.** Determine the weight of the contents of 10 containers. Dissolve the mixed contents of 10 containers in *dimethylformamide* to obtain a solution containing about 0.1 per cent w/v of cisplatin.

**Reference solution.** A 0.1 per cent w/v solution of cisplatin RS in *dimethylformamide* (use within one hour).

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm, packed with strong anion-exchange silica gel (10 µm),
- mobile phase: a mixture of 90 volumes of *methanol* and 10 volumes of *normal saline*,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 220 nm,
- a 20 µl loop injector.

Calculate the content of H$_2$Cl$_2$N$_2$Pt in the injection.

**Storage.** Store protected from light.

---

**Citric Acid**

\[
\begin{align*}
\text{HO} & \text{COOH} \\
\text{HOOC} & \text{COOH}
\end{align*}
\]

C$_6$H$_8$O$_7$ Mol. Wt. 192.1

Citric Acid is 2-hydroxypropane-1,2,3-tricarboxylic acid.

Citric Acid Monohydrate contains not less than 99.0 per cent and not more than 101.0 per cent of C$_6$H$_8$O$_7$, calculated on the anhydrous basis.

**Description.** Colourless crystals or a white powder; slightly hygroscopic in moist dry air.

**Identification**

A. Gives reaction A of citrates (2.3.1).

B. A 10 per cent w/v solution is strongly acidic.

**Tests**

**Appearance of solution.** Dissolve 2.0 g in sufficient water to produce 10 ml. The solution is clear (2.4.1), and not more intensely coloured than reference solution YS7, BYS7 or GYS7 (2.4.1).

**Arsenic** (2.3.10). Dissolve 10.0 g in 50 ml of water and add 10 ml of stannated hydrochloric acid; the resulting solution complies with the limit test for arsenic (1 ppm).

**Barium.** Dissolve 5.0 g in several portions in 39 ml of 2 M *sodium hydroxide* and dilute to 50 ml with distilled water (solution A). To 5 ml of solution A add 5 ml of 1 M *sulphuric acid* and allow to stand for 1 hour. Any opalescence produced is not more intense than that of a mixture of 5 ml of solution A and 5 ml of distilled water.

**Calcium.** To 0.2 ml of ethanolic calcium standard solution (100 ppm Ca) add 1 ml of a 4 per cent w/v solution of ammonium oxalate. After 1 minute add 1 ml of 2 M *acetic acid* and 5 ml of solution A diluted to 10 ml with distilled water and shake. After 15 minutes any opalescence produced is not more intense than that of a standard prepared in the same manner using a mixture of 10 ml of calcium standard solution (10 ppm Ca) and 5 ml of water in place of solution A (200 ppm).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

**Iron** (2.3.14). 8 ml of solution A diluted to 10 ml with water complies with the limit test for iron (50 ppm).

**Chlorides** (2.3.12). Dissolve 5.0 gm in 10 ml of water; add 1 ml of 2 M nitric acid and dilute to 15 ml with *water*. The resulting solution complies with the limit test for chlorides. The resulting solution complies with the limit test for chlorides (50 ppm).

**Sulphates** (2.3.17). Dissolve 1.0 g in sufficient distilled *water* to produce 15 ml. The resulting solution complies with the limit test for sulphates (150 ppm).

**Oxalic acid.** Dissolve 0.8 g in 4 ml of water, add 2 ml of hydrochloric acid and 1 g of granulated zinc and heat in a water-bath for 1 minute. Allow to stand for 2 minutes, decant the liquid into a test-tube containing 0.25 ml of a 1 per cent
w/v solution of phenylhydrazine hydrochloride and heat to boiling. Cool rapidly, transfer to a graduated measuring cylinder, add an equal volume of hydrochloric acid and 0.25 ml of a 5 per cent solution of potassium ferricyanide, shake and allow to stand for 30 minutes. Any pink colour produced is not more intense than that produced by carrying out the test using 0.2 ml of oxalic acid dissolved in 4 ml of water.

**Readily carbonisable substances.** Heat 0.75 g in powder, with 10 ml of sulphuric acid (containing 94.5 to 95.5 per cent w/w of H₂SO₄) in a water-bath at 90° in the dark. Shake after one minute, continue the heating for a total of 1 hour and cool rapidly and immediately. Any colour produced is not more intense than that of a mixture of 1.0 ml of CCS and 9.0 ml of FCS (2.4.1).

**Sulphated ash (2.3.18).** Not more than 0.1 per cent.

**Water (2.3.43).** Not more than 1.0 per cent, determined on 2.0 g.

**Assay.** Weigh accurately about 2 g and dissolve in 100 ml of water. Titrate with 1 M sodium hydroxide using 0.5 ml of phenolphthalein solution as indicator.

1 ml of 1 M sodium hydroxide is equivalent to 0.06403 g of C₆H₈O₇.

---

**Citric Acid Monohydrate**

C₆H₈O₇.H₂O           Mol. Wt. 210.1

Citric Acid Monohydrate is 2-hydroxypropane-1,2,3-tricarboxylic acid monohydrate.

Citric Acid Monohydrate contains not less than 99.0 per cent and not more than 101.0 per cent of C₆H₈O₇, calculated on the anhydrous basis.

**Description.** Colourless crystals or a white, crystalline powder; slightly efflorescent in warm, dry air.

**Identification**

A. Gives reaction A of citrates (2.3.1).

B. A 10 per cent w/v solution is strongly acidic.

**Tests**

**Appearance of solution.** Dissolve 2.0 g in sufficient water to produce 10 ml. The solution is clear (2.4.1), and not more intensely coloured than reference solution YS7, BYS7 or GYS7 (2.4.1).

**Arsenic (2.3.10).** Dissolve 10 g in 50 ml of water and add 10 ml of stannated hydrochloric acid; the resulting solution complies with the limit test for arsenic (1 ppm).

**Barium.** Dissolve 5.0 g in several portions in 39 ml of 2 M sodium hydroxide and dilute to 50 ml with distilled water (solution A). To 5 ml of solution A add 5 ml of 1 M sulphuric acid and allow to stand for 1 hour. Any opalescence produced is not more intense than that of a mixture of 5 ml of solution A and 5 ml of distilled water.

**Calcium.** To 0.2 ml of ethanolic calcium standard solution (100 ppm Ca) add 1 ml of a 4 per cent w/v solution of ammonium oxalate. After 1 minute add 1 ml of 2 M acetic acid and 5 ml of solution A diluted to 10 ml with distilled water and shake. After 15 minutes any opalescence produced is not more intense than that of a standard prepared in the same manner using a mixture of 10 ml of calcium standard solution (10 ppm Ca) and 5 ml of water in place of solution A (200 ppm).

**Heavy metals (2.3.13).** 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

**Iron (2.3.14).** 8 ml of solution A diluted to 10 ml with water complies with the limit test for iron (50 ppm).

**Chlorides (2.3.12).** To 2 ml of solution A add 1 ml of 2 M nitric acid and dilute to 15 ml with water. The resulting solution complies with the limit test for chlorides (50 ppm).

**Sulphates (2.3.17).** Dissolve 1.0 g in sufficient distilled water to produce 15 ml. The resulting solution complies with the limit test for sulphates (150 ppm).

**Oxalic acid.** Dissolve 0.8 g in 4 ml of water, add 2 ml of hydrochloric acid and 1 g of granulated zinc and heat in a water-bath for 1 minute. Allow to stand for 2 minutes, decant the liquid into a test-tube containing 0.25 ml of a 1 per cent w/v solution of phenylhydrazine hydrochloride and heat to boiling. Cool rapidly, transfer to a graduated measuring cylinder, add an equal volume of hydrochloric acid and 0.25 ml of a 5 per cent solution of potassium ferricyanide, shake and allow to stand for 30 minutes. Any pink colour produced is not more intense than that produced by carrying out the test using 0.2 ml of oxalic acid dissolved in 4 ml of water.

**Readily carbonisable substances.** Heat 0.50 g in powder, with 5 ml of sulphuric acid (containing 94.5 to 95.5 per cent w/w of H₂SO₄) in a water-bath at 90° in the dark. Shake after one minute, continue heating for a total of 1 hour and cool rapidly and immediately. Any colour produced is not more intense than that of a mixture of 0.6 ml of CCS and 5.4 ml of FCS (2.4.1).

**Sulphated ash (2.3.18).** Not more than 0.1 per cent.

**Water (2.3.43).** 7.5 to 9.0 per cent, determined on 0.5 g.

**Assay.** Weigh accurately about 2 g and dissolve in 50 ml of water. Titrate with 1 M sodium hydroxide using 0.5 ml of phenolphthalein solution as indicator.

1 ml of 1 M sodium hydroxide is equivalent to 0.06403 g of C₆H₈O₇.
Clarithromycin

\[
\text{C}_{38}\text{H}_{69}\text{NO}_{13} \quad \text{Mol. Wt. 748.0}
\]

Clarithromycin is (3\text{R},4\text{S},5\text{S},6\text{R},7\text{R},9\text{R},11\text{R},12\text{R},13\text{S},14\text{R})-4-\{(2,6-\text{Dideoxy}-3-\text{C-methyl}-3-\text{O-methyl-\(\alpha\)}-\text{L-ribo-hexopyranosyl)oxy]-14-ethyl-12,13-dihydroxy-7-methoxy-3,5,7,9,11,13-hexamethyl-6-\{(3,4,6-trideoxy-3-(dimethylamino)-\(\alpha\)}-\text{D-xylo-hexopyranosyl)oxy]oxacyclotetradecane-2,10-dione (6-\text{O-methylerythromycin A}).

Clarithromycin contains not less than 96.0 per cent and not more than 102.0 per cent of \(\text{C}_{38}\text{H}_{69}\text{NO}_{13}\), calculated on the anhydrous basis.

**Description.** A white or almost white, crystalline powder.

**Identification**

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with **clarithromycin RS** or with the reference spectrum of clarithromycin.

**Tests**

**Specific optical rotation** (2.4.22). -94º to -102º, determined on a 1 per cent w/v solution in methylene chloride at 20º.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 75 mg of the substance under examination in 25 ml of acetonitrile and dilute to 50 ml with water.

**Reference solution (a).** Dissolve 7.5 mg of **clarithromycin RS** in 2.5 ml of acetonitrile and dilute to 5.0 ml with water.

**Reference solution (b).** Dilute 1 ml of reference solution (a) to 100 ml with mobile phase.

Chromatographic system as described under Assay.

Inject reference solution (b). Test is not valid unless the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 2.5 times the area of the peak in the chromatogram obtained with reference solution (b) (2.5 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the peak in the chromatogram obtained with the reference solution (b) (5.0 per cent).

**Heavy metals** (2.3.13). Dissolve 1 g in a mixture of 15 volumes of water and 85 volumes of dioxan and dilute to 20 ml with the same mixture of solvents. 12 ml of the solution complies with limit test for heavy metals, Method D (20 ppm). Prepare reference solution using 1 ml of lead standard solution (100 ppm) using the same mixture of solvents.

**Water** (2.3.43). Not more than 2.0 per cent w/w, determined on 0.5 g using pyridine as solvent.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 75 mg of the substance under examination in 25 ml of acetonitrile and dilute to 50.0 ml with water.

**Reference solution.** Dissolve 15 mg of the **clarithromycin RS** in 5 ml of acetonitrile and dilute to 10.0 ml with water.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm packed with octadecysilsyl silica (3.5µm),
- column temperature 40º,
- mobile phase: A. 0.476 per cent w/v solution of potassium dihydrogen phosphate adjusted to pH 4.4 with dilute orthophosphoric acid or a 4.5 per cent solution of potassium hydroxide, filter,
- B. acetonitrile,
- a linear gradient programme using the conditions given below,
- flow rate. 1.1 ml per minute,
- spectrophotometer set at 205 nm,
- a 10 µl loop injector.

<table>
<thead>
<tr>
<th>Time (in mins.)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>32</td>
<td>40</td>
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<td>25</td>
</tr>
<tr>
<td>42</td>
<td>75</td>
<td>25</td>
</tr>
</tbody>
</table>

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of \(\text{C}_{38}\text{H}_{69}\text{NO}_{13}\).

**Storage.** Store protected from moisture.
Clarithromycin Tablets

Clarithromycin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of clarithromycin, C\textsubscript{38}H\textsubscript{69}NO\textsubscript{13}.

**Identification**

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

**Tests**

**Dissolution** (2.5.2).

Apparatus. No. 1

Medium. 900 ml of 0.1 M sodium acetate buffer.

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate.

Reference solution. Weigh accurately a suitable quantity of clarithromycin RS, dissolve in methanol, dilute with dissolution medium to obtain a solution having a known concentration of about 0.125 mg per ml.

Chromatographic system as described under Assay.

Calculate the content of C\textsubscript{38}H\textsubscript{69}NO\textsubscript{13} in the tablet.

D. Not less than 75 per cent of the stated amount of C\textsubscript{38}H\textsubscript{69}NO\textsubscript{13}.

**Loss on drying** (2.4.19). Not more than 6.0 per cent, determined on 1 g by drying in an oven at 110\(^\circ\), under vacuum, for 3 hours.

**Assay.** Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powdered tablet containing 125 mg of Clarithromycin in 200.0 ml with methanol and filter. Dilute 5.0 ml of the solution to 25.0 ml with mobile phase.

Reference solution. A 0.0625 per cent w/v solution of clarithromycin RS in methanol. Dilute 5.0 ml of the solution to 25.0 ml with mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 μm),
- column temperature 50\(^\circ\),
- mobile phase: a mixture of 65 volumes of methanol and 35 volumes of 0.067 M monobasic potassium phosphate adjusted the pH to 4.0 with orthophosphoric acid,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- a 50 μl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 750 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of C\textsubscript{38}H\textsubscript{69}NO\textsubscript{13}.

**Storage.** Store protected from moisture.

Clobazam

![Clobazam structure](image)

C\textsubscript{16}H\textsubscript{13}ClN\textsubscript{2}O\textsubscript{2}  
Mol. Wt. 300.7

Clobazam is 7-chloro-1-methyl-5-phenyl-1,5-dihydro-3H-1,5-benzodiazepine-2,4-dione.

Clobazam contains not less than 97.0 per cent and not more than 103.0 per cent of C\textsubscript{16}H\textsubscript{13}ClN\textsubscript{2}O\textsubscript{2}, calculated on the dried basis.

**Description.** A white or almost white, crystalline powder.

**Identification**

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with clobazam RS or with the reference spectrum of clobazam.

**Tests**

**Related substances.** Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in the mobile phase and dilute to 50 ml with the mobile phase.

Reference solution (a). Dissolve 5.0 mg of 7-chloro-5-phenyl-1,5-dihydro-3H-1,5-benzodiazepine-2,4-dione RS (clobazam impurity A) in the mobile phase and dilute to 50 ml with the mobile phase. Dilute 1 ml of this solution to 100 ml with the mobile phase.

Reference solution (b). Dissolve 5 mg of chlordiazepoxide RS and 5 mg of clobazam RS in the mobile phase and dilute to 50 ml with the mobile phase. Dilute 1 ml of the solution to 100 ml with the mobile phase.
Reference solution (c). Dilute 1 ml of the test solution to 200 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of acetonitrile and 60 volumes of water,
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- a 20 µl loop injector.

Inject reference solution (b). The resolution between the peaks due to chlordiazepoxide and clobazam is not less than 1.3.

Inject the test solution and reference solutions (a) and (c). Continue the chromatography for 5 times the retention time of clobazam (about 15 minutes). In the chromatogram obtained with the test solution the area of the peak obtained due to impurity A is not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). The area of any other impurity peak is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent). The sum of the areas of all other impurity peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent, determined on the residue obtained in the test for Loss on drying.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 100°-105°.

Assay. Weigh accurately about 50 mg and dissolve in 100.0 ml of ethanol (95 per cent). Dilute 2.0 ml of the solution to 250.0 ml with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 232 nm (2.4.7), taking 1380 as the specific absorbance at 232 nm.

Calculate the content of C₁₆H₁₃ClN₂O₂.

Storage. Store protected from moisture.

Clobazam Capsules

Clobazam Capsules contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of clobazam, C₁₆H₁₃ClN₂O₂.

Identification

Shake a quantity of the capsules containing 20 mg of Clobazam with 10 ml of dichloromethane, filter and evaporate the filtrate to dryness. Dissolve the residue in the minimum amount of methanol, evaporate to dryness and dry the residue at 105° for 10 minutes. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with the spectrum obtained with clobazam RS treated in the same manner or with the reference spectrum of clobazam.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Extract a quantity of the contents of the capsules containing 40 mg of Clobazam with three quantities, each of 10 ml, of dichloromethane, combine the filtered extracts, evaporate to dryness and dissolve the residue in 2 ml of methanol.

Reference solution (a). Dilute 1 volume of the test solution to 200 volumes with methanol.

Reference solution (b). Dilute 1 volume of reference solution (a) to 2.5 volumes with methanol.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of acetonitrile and 60 volumes of water,
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- a 20 µl loop injector.

Inject the test solution and reference solutions (a) and (b). Continue the chromatography for 5 times the retention time of clobazam (about 15 minutes). In the chromatogram obtained with the test solution the area of any impurity peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b). The sum of the areas of all the impurity peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a).

Dissolution (2.5.2).

Apparatus. No 1
Medium. 900 ml of 0.1 M hydrochloric acid.
Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Determine by liquid chromatography (2.4.14).

Test solution. The filtrate obtained as given above.

Reference solution. Weigh accurately a suitable quantity of clobazam RS, dissolve in methanol, and dilute with the dissolution medium to obtain a solution having the same concentration as that of the test solution.
Chromatographic system
- a stainless steel column 12.5 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm)(such as Superspher 100RP-18),
- mobile phase: a mixture of 470 volumes of acetonitrile and 530 volumes of water,
- flow rate. 0.7 ml per minute,
- spectrophotometer set at 230 nm,
- a 50 µl loop injector.

Inject alternatively the test solution and the reference solution. Calculate the content of C_{16}H_{13}ClN_{2}O_{2} in the medium. D. Not less than 75 per cent of the stated amount of C_{16}H_{13}ClN_{2}O_{2}.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh 20 capsules. Open the capsules without losing any part of the shells and transfer the contents as completely as possible to a flask. Wash the shells with three quantities, each of 30 ml, of methanol, add the washings to the flask and dilute to 200.0 ml with methanol. Allow the shells to dry at room temperature and weigh. The difference between the weights represents the weight of the total contents. Mix the contents of the flask with the aid of ultrasound for 10 minutes and stir magnetically for 20 minutes. Centrifuge a portion of the suspension and dilute a volume of the resulting supernatant liquid containing 5 mg of Clobazam to 100.0 ml with methanol.

Reference solution (a). A 0.005 per cent w/v solution of clobazam RS in methanol.

Reference solution (b). A solution containing 0.006 per cent w/v of 7-chloro-1,5-dihydro-5-phenyl-1,5-benzodiazepine-2,4(3H)-dione RS (desmethylclobazam) and 0.0125 per cent w/v of clobazam RS in methanol.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 470 volumes of acetonitrile and 530 volumes of water,
- flow rate 0.7 ml per minute,
- spectrophotometer set at 230 nm,
- a 20 µl loop injector.

Inject reference solution (b). The resolution between the peaks corresponding to desmethylclobazam and clobazam is not less than 3.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternatively the test solution and reference solution (a). Calculate the content of C_{27}H_{22}Cl_{2}N_{4} in the capsules.

Clobazimine

C_{27}H_{22}Cl_{2}N_{4} Mol. Wt. 473.4
Clobazimine is 3-(4-chloroanilino)-10-(4-chlorophenyl)-2,10-dihydro-2-(isopropylimino)phenazine.
Clobazimine contains not less than 98.5 per cent and not more than 101.5 per cent of C_{27}H_{22}Cl_{2}N_{4}, calculated on the dried basis.

Description. Dark red crystals or a reddish-brown, fine powder; almost odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with clobazimine RS or with the reference spectrum of clofazimine.

B. When examined in the range 230 nm to 600 nm, a 0.0005 per cent w/v solution in 0.01 M methanolic hydrochloric acid shows absorption maxima, at about 283 nm and 487 nm; absorbance at about 283 nm, about 0.65 and at about 487 nm, about 0.32 (2.4.7).

C. Dissolve 2 mg in 3 ml of acetone and add 0.1 ml of hydrochloric acid; an intense violet colour is produced. Add 0.5 ml of 5 M sodium hydroxide; the colour changes to orange-red.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel F254 and exposing the plate to ammonia vapour immediately before use by suspending the plate for 30 minutes in a tank containing a shallow layer of 0.2 M ammonia.

Mobile phase. A mixture of 85 volumes of dichloromethane and 4 volumes of 1-propanol.

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of chloroform.
Reference solution (a). A 0.016 per cent w/v solution of the substance under examination in chloroform.

Reference solution (b). A 0.01 per cent w/v solution of the substance under examination in chloroform.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air for 5 minutes and replace it in the tank. When the mobile phase has again risen 12 cm dry the plate in air for 5 minutes and examine in daylight and then in ultraviolet light at 254 nm. Spray the plate with sulphuric acid (50 per cent) and examine again in daylight.

Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (b).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.5 g and dissolve in 20 ml of chloroform. Add 50 ml of acetone and titrate with 0.1 M perchloric acid in dioxan, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.04734 g of C27H22Cl2N4.

Clofazimine Capsules

Clofazimine Capsules contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of clofazimine, C27H22Cl2N4.

Identification

To 5 mg of the contents of a capsule add 3 ml of chloroform and 1 ml of 2 M hydrochloric acid; the colour of the chloroform layer changes to violet. Add 2 ml of 2 M sodium hydroxide; the colour changes to brownish-yellow.

Tests

Other tests. Comply with the tests stated under Capsules.

Assay. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 0.15 g of Clofazimine and dissolve in sufficient chloroform to produce 100.0 ml. Filter through a chloroform-washed plug of cotton wool, Dilute 5.0 ml of the clear filtrate to 100.0 ml with chloroform. To 5.0 ml add 5.0 ml of 0.1 M methanolic hydrochloric acid and sufficient chloroform to produce 50.0 ml. Measure the absorbance of the resulting solution at the maximum at about 491 nm (2.4.7), using as the blank a mixture of 5.0 ml of 0.1 M methanolic hydrochloric acid and sufficient chloroform to produce 50.0 ml. Calculate the content of C27H22Cl2N4, taking 650 as the specific absorbance at 491 nm.

Storage. Store protected from moisture.

Clomifene Citrate

Clomiphene Citrate

Clomifene citrate is a mixture of E- and Z-isomers of 2-[4-(2-chloro-1,2-diphenylvinyl)phenoxy]triethylamine dihydrogen citrate.

Clomifene Citrate contains not less than 98.0 per cent and not more than 101.0 per cent of C26H28ClNO,C6H8O7, calculated on the anhydrous basis.

Description. A white or pale yellow, crystalline powder.

Identification

Tests B and C may be omitted if tests A and D are carried out. Test A may be omitted if Tests B, C and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with clomifene citrate RS.

B. Dissolve about 5 mg in 5 ml of a mixture of 10 volumes of acetic anhydride and 50 volumes of pyridine and heat in a water-bath; a deep red colour is produced.

C. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution (a).

D. A 5 per cent w/v solution gives the reactions of citrates (2.3.1).

Tests

NOTE — In the following tests, the solutions should be protected from light in amber-coloured glassware. Ensure
minimum exposure of the solutions to daylight until they are required for chromatography.

**Related substances.** Determine by liquid chromatography (2.4.14)

**Test solution.** A 0.125 per cent w/v solution of the substance under examination in the mobile phase.

**Reference solution (a).** A solution containing 0.125 per cent w/v of clomifene citrate for performance test RS in the mobile phase.

**Reference solution (b).** Dilute 1 ml of the test solution to 50 ml with the mobile phase.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm, packed with butylsilyl silica gel (such as Vydac C4),
- mobile phase: mix 400 volumes of acetonitrile with 600 volumes of water and add 8 ml of diethylamine, adjust the pH of the mixture to 6.2 by the addition of about 1 to 2 ml of phosphoric acid taking care to reduce progressively the volume of each addition as the required pH is approached,
- flow rate, 1.2 ml per minute,
- spectrophotometer set at 233 nm,
- a 10 µl loop injector.

Equilibrate the column with the mobile phase at a flow rate of 1.2 ml per minute for about one hour.

Inject reference solution (a). Continue the chromatography for twice the retention time of the principal peak. Measure the height (a) above the baseline of the peak due to clomifene impurity A and the height (B) above the baseline of the lowest point of the curve separating this peak from the peak due to clomifene. The test is not valid unless A is greater than 15 times B and the chromatogram obtained resembles the reference chromatogram. If necessary, adjust the concentration of acetonitrile in the mobile phase.

Inject separately the test solution and reference solution (b). Continue the chromatography for four times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any peak due to 2-[4-(1,2-diphenylvinyl)phenoxy]triethylamine is not greater than that of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent) and the area of any other secondary peak is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent); the sum of the areas of any secondary peaks is not greater than 1.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent). Ignore any peak with a retention time relative to the clomifene peak of 0.2 or less and any peak with an area less than 0.025 times the area of the principal peak. In the chromatogram obtained with reference solution (b) (0.05 per cent).

**Z-isomer.** 30 to 50 per cent.

Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 25 mg of the substance under examination in 25 ml of 0.1 M hydrochloric acid, add 5 ml of 1 M sodium hydroxide and shake with three quantities, each of 25 ml, of ethanol-free chloroform. Wash the combined extracts with 10 ml of water, dry over anhydrous sodium sulphate and dilute to 100.0 ml with ethanol-free chloroform. To 20.0 ml of the solution add 0.1 ml of triethylamine and dilute to 100.0 ml with hexane.

**Reference solution.** Dissolve 25 mg of clomifene citrate RS in 25 ml of 0.1 M hydrochloric acid, add 5 ml of 1 M sodium hydroxide and shake with three quantities, each of 25 ml, of ethanol-free chloroform. Wash the combined extracts with 10 ml of water, dry over anhydrous sodium sulphate and dilute to 100.0 ml with ethanol-free chloroform. To 20.0 ml of the solution add 0.1 ml of triethylamine and dilute to 100.0 ml with hexane.

**Chromatographic system**
- a stainless steel column 30 cm x 4 mm, packed with porous silica particles (10 µm) (such as Parasol),
- mobile phase: a mixture of 1 volume of triethylamine, 200 volumes of ethanol-free chloroform and 800 volumes of hexane,
- flow rate, 2 ml per minute,
- spectrophotometer set at 302 nm,
- a 50 µl loop injector.

Equilibrate the column with the mobile phase for about 2 hours. Inject the reference solution. The chromatogram obtained shows a peak due to E-isomer just before a peak due to Z-isomer. The test is not valid unless the resolution between the peaks corresponding to E- and Z-isomers is at least 1.0. If necessary, adjust the relative proportions of ethanol-free chloroform and hexane in the mobile phase. Measure the area of the peak due to the Z-isomer in the chromatogram obtained with the test solution and the reference solution.

Calculate the content of Z-isomer as a percentage of the total clomifene citrate present.

**Water** (2.3.43). Not more than 1.0 per cent, determined on 1.0 g.

**Assay.** Weigh accurately about 0.5 g and dissolve in 50 ml of anhydrous acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.05981 g of C₂₆H₂₈ClNO₄C₆H₈O₇.
Clomifene Tablets

Clomifene Citrate Tablets; Clomiphene Tablets; Clomophene Tablets

Clomifene Tablets contain not less than 92.5 per cent and not more than 207.5 per cent of the stated amount of clomifene citrate, C₂₆H₂₈ClNO₆H₈O₇.

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows absorption maxima at about 235 nm and 292 nm.

B. Dissolve a quantity of the powdered tablets containing 5 mg of Clomifene Citrate in 5 ml of a mixture of 10 volumes of acetic anhydride and 50 volumes of pyridine and heat in a water-bath; a deep red colour is produced.

Tests

Z-isomer. 30 to 50 per cent of the content of clomifene citrate as determined in the Assay.

Determine by liquid chromatography (2.4.14)

Test solution. Shake a quantity of the powdered tablets containing about 50 mg of Clomifene Citrate with 50 ml of 0.1 M hydrochloric acid for 10 minutes and filter. To 25 ml of the filtrate add 5 ml of 1 M sodium hydroxide and extract with three quantities, each of 25 ml, of ethanol-free chloroform. Wash the combined extracts with 10 ml of water, dry over anhydrous sodium sulphate and add sufficient ethanol-free chloroform to produce 100.0 ml. To 20.0 ml of the resulting solution add 0.1 ml of triethylamine and sufficient hexane to produce 100 ml.

Chromatographic system

- a stainless steel column 30 cm x 4 mm, packed with porous silica particles (10 µm) (such as Parasol),
- mobile phase: a mixture of ethanol-free chloroform and hexane, each containing 0.10 per cent v/v of triethylamine, adjusted so that the baseline separation is obtained between E- and Z-isomers of clomifene (a mixture of 20 volumes of ethanol-free chloroform and 80 volumes of hexane is suitable),
- flow rate. 2 ml per minute,
- spectrophotometer set at 302 nm,
- a 50 µl loop injector.

Stabilise the system by passing about 250 ml of the mobile phase.

Inject the test solution. In the chromatogram a peak due to the E-isomer precedes that due to the Z-isomer of clomifene. The test is not valid unless baseline separation is achieved between E- and Z-clomifene and the column efficiency is greater than 10,000 theoretical plates per metre determine using the peak due to E-isomer.

Calculate the percentage of Z-isomer from the expression

\[ \frac{100A_Z}{1.08(A_E+A_Z)} \]

where \( A_E \) and \( A_Z \) are the areas of the peaks due to the Z- and E-isomers respectively.

Dissolution (2.5.2).

Apparatus. No 1

Medium. 900 ml of water

Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Dilute a suitable volume of the filtrate with 0.1 M hydrochloric acid. Measure the absorbance of the resulting solution at the maximum at about 232 nm (2.4.7). Calculate the content of C₂₆H₂₈ClNO₆H₈O₇ in the medium taking 317 as the specific absorbance at 232 nm.

D. Not less than 80 per cent of the stated amount of C₂₆H₂₈ClNO₆H₈O₇.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Clomifene Citrate, shake for 30 minutes with 70 ml of 0.1 M hydrochloric acid prepared in a 30 per cent v/v solution of 2-propanol (instead of water normally used for the purpose as solvent), dilute to 100.0 ml with the propanolic hydrochloric acid and filter. Dissolve 5.0 ml of the filtrate to 100.0 ml with 0.1 M hydrochloric acid and measure the absorbance of the resulting solution at the maximum at about 292 nm (2.4.7), using a solution prepared by diluting 5 ml of the propanolic hydrochloric acid to 100 ml with 0.1 M hydrochloric acid as the blank. Calculate the content of C₂₆H₂₈ClNO₆H₈O₇ taking 175 as the specific absorbance at 292 nm.

Clomipramine Hydrochloride

\[ \text{C}_{19}\text{H}_{23}\text{ClN}_2\text{HCl} \]

Mol. Wt. 351.3

Clomipramine is 3-(3-chloro-10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)-N,N-dimethylpropan-1-amine hydrochloride.

Clomipramine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of C₁₉H₂₃ClN₂HCl, calculated on the dried basis.
Description. A white or slightly yellow, crystalline powder, slightly hygroscopic.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with clomipramine hydrochloride RS.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 5 volumes of ammonia, 25 volumes of acetone and 75 volumes of ethyl acetate.

Test solution. Dissolve 20 mg of the substance under examination in 10 ml of methanol.

Reference solution. A 0.2 per cent w/v solution of clomipramine hydrochloride RS in methanol.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air, spray with 0.5 per cent solution of potassium dichromate in a 20 per cent solution of sulphuric acid. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve about 5 mg in 2 ml of nitric acid. An intense blue colour develops.

D. Dissolve about 50 mg in 5 ml of water and add 1 ml of dilute ammonia. Allow to stand for 5 minutes and filter. Acidify the filtrate with dilute nitric acid. The solution gives reaction A of chlorides (2.3.1).

Tests

Appearance of solution. A 10 per cent w/v solution in carbon dioxide-free water (Solution A) is clear (2.4.1) and not more intensely coloured than reference solution Y5 (2.4.1).

pH (2.4.24). 3.5 to 5.0, determined in solution A.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 25 volumes of mobile phase B and 75 volumes of mobile phase A.

Test solution. Dissolve 20 mg of the substance under examination in 10 ml of solvent mixture.

Reference solution (a). A 0.2 per cent w/v solution of clomipramine hydrochloride RS in solvent mixture.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with cyanopropylsilyl silica (5 µm),
- mobile phase: A. 1.2 g of sodium dihydrogen phosphate in water, add 1.1 ml of nonylamine, adjust to pH 3.0 with phosphoric acid and dilute to 1000 ml with water,
  B. acetonitrile,
- a linear gradient programme using the conditions given below,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

<table>
<thead>
<tr>
<th>Time (in min.)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
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<td>44</td>
<td>75</td>
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</table>

Inject reference solution (a). Test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

Heavy metals (2.3.13). 2 g complies with limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1 g by drying in an oven at 105°C.

Assay. Weigh accurately about 0.25 g, dissolve in 50.0 ml of ethanol and add 5.0 ml of 0.01 M hydrochloric acid. Titrate with 0.1 M sodium hydroxide. Determine the end-point potentiometrically (2.4.25).

1 ml of 0.1 M sodium hydroxide is equivalent to 0.03513 g of C19H24Cl2N2.

Storage. Store protected from light and moisture.

Clomipramine Capsules

Clomipramine Hydrochloride Capsules

Clomipramine Capsules contain Clomipramine Hydrochloride. Clomipramine Capsules contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of clomipramine hydrochloride, C19H23ClN2.HCl.

Identification

Triturate a quantity of the contents of the capsules containing 0.15 g of Clomipramine Hydrochloride with 10 ml of chloroform,
filter and evaporate the filtrate to dryness. The residue complies
with the following test.

Determine by infrared absorption spectrophotometry (2.4.6).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the mixed contents of
20 capsules containing 20 mg of Clomipramine Hydrochloride
with 5 ml of mobile phase A with the aid of ultrasound for
15 minutes, dilute to 10 ml with the same solvent and filter.

Reference solution (a). A 0.2 per cent w/v solution of
clomipramine hydrochloride RS in mobile phase A.

Reference solution (b). Dilute 1 ml of reference solution (a) to
100 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with
cyanopropylsilyl silica (5 µm) (such as Hypersil BDS CN),
- mobile phase: A. a mixture of 75 volumes of solution
prepared by dissolving 1.2 g of sodium dihydrogen
orthophosphate in 950 ml of water, add 1.1 ml of
nonylamine, adjust to pH 3.0 with orthophosphoric acid
and add sufficient water to produce 1000 ml (solution
A) and 25 volumes of acetonitrile.

B. a mixture of 65 volumes of solution A
and 35 volumes of acetonitrile,
- a linear gradient programme using the conditions given
below,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

<table>
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Inject reference solution (a). Test is not valid unless the column
efficiency is not less than 2000 theoretical plates and the tailing
factor is not more than 2.0.

Inject the test solution and reference solution (b). In the
chromatogram obtained with the test solution, the area of any
secondary peak is not more than 0.5 times the area of the peak in
the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks
is not more than the area of the peak in the chromatogram
obtained with the reference solution (b) (1.0 per cent).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of the mixed
contents of 20 capsules containing about 50 mg of
Clomipramine, disperse in 100.0 ml of methanol. Dilute 5.0 ml
of the solution to 50.0 ml with methanol.

Reference solution. A 0.0125 per cent w/v solution of clobazam
RS in methanol.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm packed with
octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 47 volumes of acetonitrile
and 53 volumes of water,
- flow rate. 0.7 ml per minute.
- spectrophotometer set at 230 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the
relative standard deviation for replicate injections is not more
than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of C_{16}H_{13}ClN_{2}O_{2}.

Clonazepam

\[
\begin{align*}
\text{O}_2\text{N} & \\
\text{Cl} & \\
\text{N} & \\
\text{N} & \\
\text{H} & \\
\text{O} & 
\end{align*}
\]

C_{15}H_{10}ClN_{3}O_{3} \quad \text{Mol. Wt. 315.7}

Clonazepam is 5-(2-chlorophenyl)-7-nitro-1,3-dihydro-2H-
1,4-benzodiazepin-2-one.

Clonazepam contains not less than 99.0 per cent and not more
than 101.0 per cent of C_{15}H_{10}ClN_{3}O_{3}, calculated on the dried
basis.

Description. A slightly yellowish, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6).

Test solution. Compare the spectrum with that obtained with clonazepam RS.

Tests

Related substances. Determine by liquid chromatography (2.4.14).
Solvent mixture. 10 volumes of tetrahydrofuran, 42 volumes of methanol and 48 volumes of water.

Test solution. Dissolve 0.05 g of the substance under examination in 10 ml of methanol and dilute to 100 ml with the solvent mixture.

Reference solution (a). Dissolve 25 mg of clonazepam RS in 5 ml of methanol and dilute to 50 ml with the solvent mixture.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm packed with octylsilyl silica (5 µm),
- mobile phase: a mixture of 10 volumes of tetrahydrofuran, 42 volumes of methanol and 48 volumes of a 0.66 per cent solution of ammonium phosphate previously adjusted to pH 8.0 with a 4 per cent w/v of sodium hydroxide or orthophosphoric acid,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 10 µl loop injector.

Inject reference solution (a). Test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1 g by drying in an oven at 105º for 4 hours.

Assay. Weigh accurately about 0.275 g, dissolve in 50 ml of acetic anhydride. Titrate with 0.1 M perchloric acid. Determine the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.03157g of C15H10ClN3O3.

Storage. Store protected from light.

Clonazepam Injection

Clonazepam Injection is a sterile material consisting of Clonazepam with or without excipients. It is filled in a sealed container.
Reference solution (a). A 0.0005 per cent w/v solution of 2-amino-2’-chloro-5-nitrobenzophenone RS (‘nitrobenzophenone’) in chloroform.

Reference solution (b). A 0.0002 per cent w/v solution of 2-amino-2’-chloro-5-nitrobenzophenone RS (‘nitrobenzophenone’) in chloroform.

Reference solution (c). A 0.0002 per cent w/v of 3-amino-4-(2-chlorophenyl)-6-nitroquinolin-2-one RS (‘carbostyril’) in chloroform.

Apply to the plate 50 µl of each solution. After development, dry the plate in a current of cold air. For the second development use a mixture of 10 volumes of ether and 90 volumes of nitromethane. After development, dry the plate, heat at a pressure of 2kPa at 120° for 3 hours, allow to cool and spray with a 10 per cent w/v solution of zinc chloride in 0.1 M hydrochloric acid. Dry the plate in air and examine in visual light. Any spots in the chromatogram obtained with test solution corresponding to the nitrobenzophenone and carbostyril impurities are not more intense than the spots in the chromatograms obtained with reference solutions (b) and (c) respectively (0.2 per cent). Any other secondary spot in the chromatogram obtained with test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

Assay. Protect the solutions from light throughout the assay.

To a volume of the solution containing 20 mg of Clonazepam, dilute to 100.0 ml with propan-2-ol. Dilute 10.0 ml of the solution to 100.0 ml with propan-2-ol. Measure the absorbance of the resulting solution at the maximum at 310 nm (2.4.7). Calculate the content of C_{15}H_{10}ClN_{3}O_{3} taking 364 as the specific absorbance at 310 nm.

Storage. Store protected from light.

Labelling. The label states (1) ‘Sterile Clonazepam Concentrate’; (2) that the diluted injection is to be given by intravenous injection.

Clonidine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of C_{15}H_{10}ClN_{3}HCl, calculated on the dried basis.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with clonidine hydrochloride RS or with the reference spectrum of clonidine hydrochloride.

B. When examined in the range 230 nm to 600 nm, a 0.03 per cent w/v solution in 0.01 M hydrochloric acid shows absorption maxima, at about 272 nm and 279 nm and an inflection at about 265 nm (2.4.7)

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

D. Gives reaction A of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in carbon dioxide-free water is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

pH (2.4.24). 4.0 to 5.0, determined in a 5.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. Shake together 50 volumes of water, 40 volumes of 1-butanol and 10 volumes of glacial acetic acid and allow the layers to separate. Use the filtered upper layer.

Test solution (a). Dissolve 0.1 g of the substance under examination in 10 ml of methanol.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10 ml with methanol.

Reference solution (a). Dilute 5 ml of test solution (b) to 100 ml with methanol.

Reference solution (b). A 0.1 per cent w/v solution of clonidine hydrochloride RS in methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with modified potassium iodosidomulate solution. Allow to dry in air for 1 hour, spray again with the same reagent and immediately spray with a 5 per cent w/v solution of sodium nitrite. Any secondary spot in the chromatogram obtained with test solution (a) is not
more intense than the spot in the chromatogram obtained with reference solution (a).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent w/w.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°C.

**Assay.** Weigh accurately about 0.2 g and dissolve in 70 ml of ethanol (95 per cent). Titrate with 0.1 M ethanolic sodium hydroxide, determining the end-point potentiometrically (2.4.25).

1 ml of 0.1 M ethanolic sodium hydroxide is equivalent to 0.02666 g of C₉H₉Cl₂N₃·HCl.

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**Clonidine Injection**

Clonidine Hydrochloride Injection

Clonidine Injection is a sterile solution of Clonidine Hydrochloride in Water for Injections.

Clonidine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of clonidine hydrochloride, C₉H₉Cl₂N₃·HCl.

**Identification**

A. Dilute a volume containing 300 µg of Clonidine Hydrochloride to 5 ml with 0.01 M hydrochloric acid. When examined in the range 230 nm to 360 nm, the resulting solution shows absorption maxima at about 272 nm and 279 nm and an inflection at about 265 nm (2.4.7).

B. To a volume containing 150 µg of Clonidine Hydrochloride add 1 ml of a 10 per cent w/v solution of ammonium reineckate and keep aside for 5 minutes; a pink precipitate is obtained.

**Tests**

**pH** (2.4.24). 4.0 to 7.0.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** Shake together 50 volumes of water, 40 volumes of 1-butanol and 10 volumes of glacial acetic acid and allow the layers to separate. Use the filtered upper layer.

**Test solution.** Add 10 ml of methanol to a volume containing 750 µg of Clonidine Hydrochloride, evaporate to dryness and dissolve the residue in 0.5 ml of methanol.

**Reference solution.** Dilute 1 volume of the test solution to 100 volumes with methanol.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and spray with modified potassium iodobismuthate solution. Allow to dry in air for 1 hour, spray again with the same reagent and immediately spray with a 5 per cent w/v solution of sodium nitrite. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** To an accurately measured volume of the injection containing 150 µg of Clonidine Hydrochloride add 25 ml of citrophosphate buffer pH 7.6, 5 ml of water, and 1 ml of a solution containing 0.15 per cent w/v of bromothymol blue and 0.15 per cent w/v of anhydrous sodium carbonate. Add 30 ml of chloroform, shake for 1 minute and centrifuge. To 15.0 ml of the chloroform layer add 10 ml of boric acid solution and measure the absorbance of the resulting solution at the maximum at about 420 nm (2.4.7), using as the blank a solution prepared by diluting 10 ml of boric acid solution to 25.0 ml with chloroform. Repeat the operation by adding to 5.0 ml of a 0.003 per cent w/v solution of clonidine hydrochloride RS, previously dried to constant weight at 105°C, 20 ml of citrophosphate buffer pH 7.6 and completing the procedure described above beginning at the words “5 ml of water”. Calculate the content of C₉H₉Cl₂N₃·HCl from the absorbance obtained using clonidine hydrochloride RS in place of the substance under examination.

**Storage.** Store in single dose containers.

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**Clonidine Tablets**

Clonidine Hydrochloride Tablets

Clonidine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of clonidine hydrochloride, C₉H₉Cl₂N₃·HCl.

**Identification**

To a quantity of the powdered tablets containing 500 µg of Clonidine Hydrochloride add 30 ml of water and 5 ml of 1 M sodium hydroxide. Swirl gently and extract with 20 ml of chloroform. Remove the chloroform layer, dry with anhydrous sodium sulphate, filter and evaporate the filtrate to dryness. Dissolve the residue in 8 ml of 0.01 M hydrochloric acid. The resulting solution complies with the following tests.

A. When examined in the range 230 nm to 360 nm, it shows absorption maxima at about 272 nm and 279 nm and an inflection at about 265 nm (2.4.7).

B. To 2 ml add 1 ml of a 10 per cent w/v solution of ammonium reineckate and allow to stand for 5 minutes; a pink precipitate is produced.
Tests

**Uniformity of content.** Comply with the test stated under Tablets.

*For tablets containing 300 µg or more of Clonidine Hydrochloride* – To one tablet add 200 ml of citrophosphate buffer pH 7.6, shake until disintegrated and dilute with citrophosphate buffer pH 7.6, if necessary, to give a solution containing about 0.0015 per cent w/v of Clonidine Hydrochloride. To 5 ml of the supernatant liquid add 1 ml of a solution containing 0.15 per cent w/v of bromothymol blue and 0.15 per cent w/v of anhydrous sodium carbonate. Add 10 ml of chloroform, shake for 1 minute and centrifuge. To 5 ml of the supernatant liquid add 5 ml of boric acid solution and measure the absorbance of a 2-cm layer of the resulting solution at the maximum at about 420 nm (2.4.7), using as the blank a mixture of 5 ml of boric acid solution and 5 ml of chloroform. Repeat the operation by using a solution prepared by diluting 5 ml of a 0.03 per cent w/v solution of clonidine hydrochloride RS to 100 ml with citrophosphate buffer pH 7.6, transferring 5 ml to a separator and completing the procedure described above beginning at the words “add 1 ml of a solution...”.

Calculate the content of C_{9}H_{9}Cl_{2}N_{3}HCl in the tablet from the absorbance obtained using clonidine hydrochloride RS in place of the tablet.

*For tablets containing less than 300 µg of Clonidine Hydrochloride* - Use the same procedure but with a concentration of 0.001 per cent w/v or 0.0005 per cent w/v of Clonidine Hydrochloride as appropriate and with corresponding smaller concentrations of clonidine hydrochloride RS.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. To an accurately weighed quantity of the powder containing about 100 µg of Clonidine Hydrochloride add 25 ml of citrophosphate buffer pH 7.6 and shake for 15 minutes. Add 5 ml of water and 1 ml of a solution containing 0.15 per cent w/v of bromothymol blue and 0.15 per cent w/v of anhydrous sodium carbonate and shake to disperse. Add 30 ml of chloroform, shake for 1 minute and centrifuge. To 15.0 ml of the chloroform layer add 10 ml of boric acid solution and measure the absorbance of the resulting solution at the maximum at about 420 nm (2.4.7), using as the blank a solution prepared by diluting 10 ml of boric acid solution to 25.0 ml with chloroform. Repeat the operation by adding to 5.0 ml of a 0.003 per cent w/v solution of clonidine hydrochloride RS, previously dried to constant weight at 105°C, 20 ml of citrophosphate buffer pH 7.6 and completing the procedure described above beginning at the words “5 ml of water”. Calculate the content of C_{9}H_{9}Cl_{2}N_{3}HCl from the absorbance obtained using clonidine hydrochloride RS in place of the substance under examination.

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**Clotrimazole**

![Clotrimazole structure](image)

\[ C_{22}H_{17}CIN_{2} \quad \text{Mol. Wt} 344.8 \]

Clotrimazole is 1-[(2-chlorophenyl)diphenylmethyl]-1H-imidazole.

Clotrimazole contains not less than 98.0 per cent and not more than 102.0 per cent of C_{22}H_{17}CIN_{2}, calculated on the dried basis.

**Description.** A white or pale yellow, crystalline powder.

**Identification**

- **A.** Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with clotrimazole RS or with the reference spectrum of clotrimazole.

- **B.** When examined in the range 230 nm to 360 nm, a 0.04 per cent w/v solution in a mixture of 1 volume of 0.1 M hydrochloric acid and 9 volumes of methanol shows absorption maxima at about 262 and 265 nm; absorbance at about 262 nm, about 0.9 and at about 265 nm, about 0.92 (2.4.7).

**Tests**

- **Appearance of solution.** A 5.0 per cent w/v solution in ethanol (95 per cent) is clear (2.4.1), and not more intensely coloured than reference solution BYS6 (2.4.1).

- **2-Chlorotritanol.** Determine by liquid chromatography (2.4.14).

  **Solvent mixture.** A mixture of 70 volumes of methanol and 30 volumes of 0.02 M phosphoric acid.

  **Test solution.** Dissolve 0.1 g of the substance under examination in 100 ml with solvent mixture.

  **Reference solution (a).** A 0.0002 per cent w/v solution of 2-chlorotritanol RS in the same solvent mixture.

  **Reference solution (b).** Dilute 1 volume of the test solution to 50 volumes with the same solvent mixture.

  **Chromatographic system**

  - a stainless steel column 25 cm x 4.6 mm, packed with octadecysilyl silica gel (5 µm),
  - mobile phase: a mixture of 70 volumes of methanol and 30 volumes of 0.02 M phosphoric acid adjusted to pH 7.5 with a 10 per cent w/v solution of triethylamine in methanol,
flow rate. 1.5 ml per minute,

- spectrophotometer set at 215 nm,
- a 20 µl loop injector.

Inject the test solution and reference solution (a). Allow the chromatography to proceed for 1.5 times the retention time of the principal peak in the chromatogram obtained with the test solution. The area of any peak corresponding to 2-chlorotritanol in the chromatogram obtained with the test solution is not greater then the area of the peak in the chromatogram obtained with reference solution (a). The test is not valid unless the column efficiency, determined using the principal peak in the chromatogram obtained with reference solution (b) is not less than 9000 theoretical plates.

**Imidazole.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 180 volumes of toluene, 20 volumes of 1-propanol and 1 volume of strong ammonia solution.

**Test solution.** Dissolve 0.5 g of the substance under examination in 10 ml of chloroform.

**Reference solution.** A 0.01 per cent w/v solution of imidazole in chloroform.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, place in a tank of chlorine prepared by the addition of hydrochloric acid to potassium permanganate and allow to stand for 2 minutes. Remove any excess chlorine from the plate with a current of air and spray with potassium iodide and starch solution. Any spot corresponding to imidazole in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Sulphated ash (2.3.18).** Not more than 0.1 per cent.

**Loss on drying (2.4.19).** Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.3 g and dissolve in 50 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, using 1-naphtholbenzein solution as indicator to a green end-point. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.03448 g of C$_2$H$_7$ClN$_2$.

**Storage.** Store protected from light.

**Clotrimazole Cream**

Clotrimazole Cream contains Clotrimazole in a suitable base. Clotrimazole Cream contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of clotrimazole, C$_{22}$H$_{17}$ClN$_2$.

**Identification**

A. Mix a quantity of the cream containing 40 mg of Clotrimazole with 20 ml of a mixture of 1 volume of 1 M sulphuric acid and 4 volumes of methanol and shake with two quantities, each of 50 ml, of carbon tetrachloride, discarding the organic layers. Make the aqueous phase alkaline with dilute ammonia solution, add a further 5 ml of dilute ammonia solution and extract with two quantities, each of 40 ml, of chloroform. Combine the chloroform extracts, shake with 5 g of anhydrous sodium sulphate, filter and add sufficient chloroform to the filtrate to produce 100 ml. Evaporate 50 ml to dryness and dissolve the residue in 50 ml of a mixture of 1 volume of 0.1 M hydrochloric acid and 9 volumes of methanol. When examined in the range 230 nm to 360 nm, the resulting solution shows absorption maxima at about 262 nm and 265 nm (2.4.7).

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** Di-isopropyl ether in a chromatography tank, containing 25 ml of strong ammonia solution in a beaker.

**Test solution.** Shake a quantity of the cream containing 20 mg of Clotrimazole with 4 ml of dichloromethane for 30 minutes, centrifuge and use the supernatant liquid.

**Reference solution.** A 0.5 per cent w/v solution of clotrimazole RS in dichloromethane.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of air and spray with dilute potassium iodosmium solution. The spot in the chromatogram obtained with the test solution is reddish brown and corresponds to the spot in the chromatogram obtained with the reference solution.

**Tests**

**2-Chlorotritanol.** Determine by liquid chromatography (2.4.14).

**Test solution.** Extract a quantity of the cream containing 20 mg of Clotrimazole by warming with 20 ml of methanol in a water-bath at 50° for 5 minutes, shaking occasionally. Remove from the water-bath, shake the mixture vigorously while cooling to room temperature, cool in ice for 15 minutes, centrifuge for 5 minutes and decant the supernatant liquid. Repeat the extraction with further quantities, each of 20 ml, of methanol. To the combined methanol extracts add 10 ml of methanol and dilute to 100.0 ml with 0.02 M phosphoric acid. Cool in ice and filter through a membrane filter.

**Reference solution (a).** A 0.0002 per cent w/v solution of 2-chlorotritanol RS in a mixture of 70 volumes of methanol and 30 volumes of 0.02 M phosphoric acid.

**Reference solution (b).** Dilute 1 volume of the test solution to 50 volumes with the same solvent mixture.
Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 μm),
- mobile phase: a mixture of 70 volumes of methanol and 30 volumes of 0.02 M phosphoric acid adjusted to pH 7.5 with a 10 per cent w/v solution of triethylamine in methanol,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- a 20 μl loop injector.

Inject reference solution (b). The column efficiency, determined using the principal peak in the chromatogram obtained is not less than 9000 theoretical plates.

Inject the test solution and reference solution (a). Allow the chromatography to proceed for 1.5 times the retention time of the principal peak in the chromatogram obtained with the test solution. The area of any peak corresponding to 2-chlorotritanol in the chromatogram obtained with the test solution is not greater then the area of the peak in the chromatogram obtained with reference solution (a).

Other tests. Complies with the tests stated under Creams.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Treat a quantity of the cream containing about 20 mg of Clotrimazole as described in the test for 2-Chlorotritanol and dilute 1.0 ml of the filtrate to 5.0 ml with a mixture of 70 volumes of methanol and 30 volumes of 0.02 M phosphoric acid.

Reference solution. Dissolve 20 mg of clotrimazole RS in 70 ml of methanol, add sufficient 0.02 M phosphoric acid to produce 100.0 ml and dilute 1.0 ml of the resulting solution to 5.0 ml with a mixture of 70 volumes of methanol and 30 volumes of 0.02 M phosphoric acid.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 μm),
- mobile phase: a mixture of 70 volumes of methanol and 30 volumes of 0.02 M phosphoric acid adjusted to pH 7.5 with a 10 per cent w/v solution of triethylamine in methanol,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- a 20 μl loop injector.

Inject the reference solution. The column efficiency, determined using the peak in the chromatogram obtained, should be not less than 9000 theoretical plates.

Inject alternately the test solution and the reference solution. Allow the chromatography to proceed for 1.5 times the retention time of the principal peak in the chromatogram obtained with the test solution.

Calculate the content of C₂₂H₁₇ClN₂ in the cream.

Storage. Store at a temperature not exceeding 30°.

Clotrimazole Pessaries

Clotrimazole Vaginal Tablets

Clotrimazole Pessaries contain Clotrimazole in a suitable base.

Clotrimazole Pessaries contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of clotrimazole, C₂₂H₁₇ClN₂.

Identification

A. Mix a quantity of the powdered pessaries containing 40 mg of Clotrimazole with 20 ml of a mixture of 1 volume of 1 M sulphuric acid and 4 volumes of methanol and shake with two quantities, each of 50 ml, of carbon tetrachloride, discarding the organic layers. Make the aqueous phase alkaline with dilute ammonia solution, add a further 5 ml of dilute ammonia solution and extract with two quantities, each of 40 ml, of chloroform. Combine the chloroform extracts, shake with 5 g of anhydrous sodium sulphate, filter and add sufficient chloroform to the filtrate to produce 100 ml. Evaporate 50 ml to dryness and dissolve the residue in 50 ml of a mixture of 1 volume of 0.1 M hydrochloric acid and 9 volumes of methanol. When examined in the range 230 nm to 360 nm, the resulting solution shows absorption maxima at about 262 nm and 265 nm (2.4.7).

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. Di-isopropyl ether in a chromatography tank, containing 25 ml of strong ammonia solution in a beaker.

Test solution. Shake a quantity of the powdered pessaries containing 20 mg of Clotrimazole with 4 ml of dichloromethane for 30 minutes, centrifuge and use the supernatant liquid.

Reference solution. A 0.5 per cent w/v solution of clotrimazole RS in dichloromethane.

Apply to the plate 10 μl of each solution. After development, dry the plate in a current of air and spray with dilute potassium iodobismuthate solution. The spot in the chromatogram obtained with the test solution is reddish brown and corresponds to the spot in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Add 50 ml of methanol to a quantity of the powdered pessaries containing 0.1 g of Clotrimazole and shake
for 20 minutes. Dilute to 100 ml with methanol and filter. To 20 ml of the filtrate add 50 ml methanol and sufficient 0.02 M phosphoric acid to produce 100 ml.

Reference solution (a). A 0.0002 per cent w/v solution of 2-chlorotritanol RS in a mixture of 70 volumes of methanol and 30 volumes of 0.02 M phosphoric acid.

Reference solution (b). Dilute 1 volume of the test solution to 50 volumes with the same solvent mixture.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecysilyl silica gel (5 µm),
- mobile phase: a mixture of 70 volumes of methanol and 30 volumes of 0.02 M phosphoric acid adjusted to pH 7.5 with a 10 per cent w/v solution of triethylamine in methanol,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- a 20 µl loop injector.

Inject the reference solution. The column efficiency, determined using the peak in the chromatogram obtained should be not less than 9000 theoretical plates.

Inject alternately the test solution and the reference solution. Allow the chromatography to proceed for 1.5 times the retention time of the principal peak in the chromatogram obtained with the test solution.

Calculate the content of C_{19}H_{17}ClN_{3} in the pessaries.

Storage. Store protected from moisture and crushing.

Cloxacillin Sodium

\[
\text{C}_{10}\text{H}_{17}\text{ClN}_{3}\text{NaO}_{5}\text{S}, \text{H}_{2}\text{O} \quad \text{Mol. Wt. 475.9}
\]

Cloxacillin Sodium is sodium (6R)-6-[3-(2-chlorophenyl)-5-methylisoxazole-4-carboxamido]penicillanate monohydrate.

Cloxacillin Sodium contains not less than 95.0 per cent and not more than 101.0 per cent of C_{19}H_{17}ClN_{3}NaO_{5}S, calculated on the anhydrous basis.

Description. A white or almost white, crystalline powder; hygroscopic.

Identification
A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cloxacillin sodium RS or with the reference spectrum of cloxacillin sodium.
B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.
C. Gives reaction A of sodium salts (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution in carbon dioxide-free water is clear (2.4.1); absorbance of the solution at about 430 nm, not more than 0.04 (2.4.7).
pH (2.4.24). 5.0 to 7.0, determined in a 10.0 per cent w/v solution.
Specific optical rotation (2.4.22). +160° to +169°, determined at 20° in a 1.0 per cent w/v solution.

N,N-Dimethylaniline (2.3.21). Not more than 20 ppm, determined by Method A.

Water (2.3.43). Not more than 4.5 per cent, determined on 0.3 g.

Assay. Determine by liquid chromatography (2.4.14).

Buffer solution. Prepare a 0.02 M monobasic potassium phosphate solution and adjust the pH to 6.6 with 2 M sodium hydroxide.

Test solution. Weigh accurately about 55 mg of the substance under examination and dilute to 100.0 ml with the buffer solution.

Reference solution. Weigh accurately a suitable quantity of cloxacillin sodium RS dissolve in the buffer solution and dilute to obtain a solution containing a known concentration of about 0.55 mg per ml.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel 3 to 10 µm,
- mobile phase: a mixture of 80 volumes of the buffer solution and 20 volumes of acetonitrile,
- flow rate. 1 ml per minute,
- spectrophotometer set at 225 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.8 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the content of C₁₉H₁₈ClN₃O₅S.

Cloxacillin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.40 Endotoxin Unit per mg of cloxacillin.

Cloxacillin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture at a temperature not exceeding 30°. If it is intended for use in the manufacture of parenteral preparations, the containers should be sterile and sealed so as to exclude micro-organisms.

Labelling. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

Cloxacillin Capsules

Cloxacillin Sodium Capsules

Cloxacillin Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of cloxacillin, C₁₉H₁₈ClN₃O₅S.

Identification

A. Determine on the contents of the capsules by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cloxacillin sodium RS or with the reference spectrum of cloxacillin sodium.

B. The contents of the capsules give reactions A and B of sodium salts (2.3.1).

Tests

Dissolution (2.5.2).

Apparatus. No 1

Medium. 900 ml of water

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Dilute the filtrate, if necessary, with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 271 nm (2.4.7). Similarly measure the absorbance of a standard solution of known concentration of cloxacillin sodium RS in place of the contents of the capsules. Calculate the content of C₁₉H₁₈ClN₃O₅S.

D. Not less than 75 per cent of the stated amount of C₁₉H₁₈ClN₃O₅S.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Buffer solution. Prepare a 0.02 M monobasic potassium phosphate solution and adjust the pH to 6.6 with 2 M sodium hydroxide.

Test solution. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 50 mg of Cloxacillin, dissolve in the buffer solution by stirring for 10 minutes, dilute to 100.0 ml with the buffer solution and filter.

Reference solution. Weigh accurately a suitable quantity of cloxacillin sodium RS dissolve in the buffer solution and dilute to obtain a solution containing a known concentration of about 0.55 mg per ml.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (3 to 10 µm),

Labelling. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.
mobile phase: a mixture of 80 volumes of the buffer solution and 20 volumes of acetonitrile,
flow rate. 1 ml per minute,
spectrophotometer set at 225 nm,
a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.8 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of C₁₉H₁₈ClN₃O₅S in the capsules.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of cloxacillin.

Cloxacillin Injection

Cloxacillin Sodium Injection

Cloxacillin Injection is a sterile material consisting of Cloxacillin Sodium with or without excipients. It is filled in a sealed container.

Inject alternately the test solution and the reference solution.

Calculate the content of C₁₉H₁₈ClN₃O₅S in the capsules.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of cloxacillin.

Cloxacillin Syrup

Cloxacillin Sodium Syrup; Cloxacillin Elixir; Cloxacillin Sodium Elixir; Cloxacillin Oral Solution; Cloxacillin Sodium Oral Solution

Cloxacillin Syrup is a mixture consisting of Cloxacillin Sodium with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of Water just before use.
Cloxacillin Syrup contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of C₁₉H₁₈ClN₃O₅S.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of cloxacillin, C₁₉H₁₈ClN₃O₅S.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

The constituted suspension complies with the tests stated under Oral liquids and with the following tests.

Tests

pH (2.4.24). 4.0 to 7.0.

Assay. Determine by liquid chromatography (2.4.14).

Buffer solution. Prepare a 0.02 M monobasic potassium phosphate solution and adjust to pH 6.6 with 1 M sodium hydroxide.

Test solution. Transfer an accurately weighed quantity of the oral suspension containing about 125 mg of the cloxacillin to a 250-ml volumetric flask and dissolve in the buffer solution by stirring for 15 minutes. Dilute to 250.0 ml with the buffer solution.

Reference solution. Weigh accurately a suitable quantity of cloxacillin sodium RS dissolve in the buffer solution and dilute to obtain a solution containing a known concentration of about 0.55 mg per ml.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (3 to 10 µm),
- mobile phase: a mixture of 80 volumes of the buffer solution and 20 volumes of acetonitrile,
- flow rate. 1 ml per minute,
- spectrophotometer set at 225 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.8 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Determine the weight per ml of the oral suspension (2.4.29) and calculate the content of C₁₉H₁₈ClN₃O₅S weight in volume.

Repeat the procedure using a portion of the constituted suspension that has been stored at the temperature and for the period stated on the label.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of cloxacillin.

Codeine Phosphate

Codeine Phosphate Hemihydrate

\[
\text{H}_3\text{CO} \quad \text{CH}_3
\]

C₁₈H₂₁NO₃•H₃PO₄•½H₂O \hspace{1cm} \text{Mol. Wt. 406.4}

Codeine Phosphate is (5R,6S)-7,8-didehydro-4,5-epoxy-3-methoxy-17-methylmorphinan-6-ol dihydrogen phosphate hemihydrate, an alkaloid occurring in Papaver somniferum.

Codeine Phosphate contains not less than 98.5 per cent and not more than 101.0 per cent of C₁₈H₂₁NO₃•H₃PO₄•½H₂O, calculated on the dried basis.

Description. Colourless crystals or a white, crystalline powder.

Identification

Test A may be omitted if tests B, C, D, E and F are carried out. Tests B, C, D and E may be omitted if tests A and F are carried out.

A. Dissolve 0.2 g in 4 ml of water, add 2 ml of 2 M sodium hydroxide and induce crystallisation, if necessary by scratching the wall of the tube with a glass rod and cooling in ice. The residue after washing with water and drying at 100° to 105° complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with codeine phosphate RS treated in the same manner or with the reference spectrum of codeine.

B. To 25 ml of a 0.04 per cent w/v solution add 25 ml of water and 10 ml of 1 M sodium hydroxide and dilute to 100 ml of water. When examined in the range 230 nm to 360 nm, the resulting solution shows an absorption maximum only at about 284 nm; absorbance at about 284 nm, about 0.38 (2.4.7).

C. On the surface of one drop of nitric acid place a little of the powder; a yellow but not red colour is produced (distinction from morphine).

D. Dissolve 0.1 g in 1 ml of sulphuric acid and 1 drop of ferric chloride test solution and warm gently; a bluish violet colour
is produced. Add 1 drop of dilute nitric acid; the colour changes to red.
E. Gives the reaction of alkaloids (2.3.1).
F. Gives reaction A of phosphates (2.3.1).

**Tests**

**Appearance of solution.** A 4.0 per cent w/v solution in carbon dioxide-free water prepared from distilled water is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

**pH** (2.4.24). 4.2 to 5.0, determined in a 4.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). –98.0° to –102°, determined in a 2.0 per cent w/v solution.

**Foreign alkaloids.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 72 volumes of ethanol, 30 volumes of cyclohexane and 6 volumes of strong ammonia solution.

**Test solution.** Dissolve 0.5 g of the substance under examination in sufficient of a mixture of 4 volumes of 0.1 M hydrochloric acid and 1 volume of ethanol to produce 10 ml.

**Reference solution (a).** Dilute 1.5 ml of the test solution to 100 ml with the same solvent.

**Reference solution (b).** Dilute 1 ml of the test solution to 100 ml with the same solvent.

Apply separately to the plate 10 µl of each solution. After development, dry the plate in air and spray with acidic potassium iodobismuthate solution. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot, with an Rf value higher than that of the principal spot, is more intense than the spot in the chromatogram obtained with reference solution (b).

Chlorides (2.3.12). 12.5 ml of a 2.0 per cent w/v solution in distilled water complies with the limit test for chlorides (0.1 per cent).

**Sulphates** (2.3.17). 7.5 ml of a 2.0 per cent w/v solution in distilled water complies with the limit test for sulphates (0.1 per cent).

**Morphine.** Dissolve 0.1 g in sufficient 0.1 M hydrochloric acid to produce 5 ml, add 2 ml of a 1 per cent w/v solution of sodium nitrite, allow to stand for 15 minutes and add 3 ml of 6 M ammonia. The resulting solution is not more intensely coloured than reference solution BS4 (2.4.1).

**Loss on drying** (2.4.19). Not more than 3.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.4 g of the substance under examination and dissolve in a mixture of 10 ml of anhydrous glacial acetic acid and 20 ml of dioxan. Titrate with 0.1 M perchloric acid, using a few drops of crystal violet solution as indicator. Carry out blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.03974 g of C₁₈H₂₁NO₃.H₃PO₄.

**Storage.** Store protected from light.

**Codeine Syrup**

**Codeine Phosphate Syrup**

Codeine Syrup is a 0.5 per cent w/v solution of Codeine Phosphate in a suitable flavoured vehicle.

Codeine Syrup contains not less than 0.48 per cent w/v and not more than 0.52 per cent w/v of codeine phosphate, C₁₈H₂₁NO₃.H₃PO₄.½H₂O.

**Identification**

To 10 ml of the syrup add sufficient dilute ammonia solution until the solution is alkaline and extract with three quantities, each of 10 ml, of chloroform. Evaporate the combined chloroform extracts to dryness on a water-bath and dry the residue at 80°. The residue complies with the following tests.

A. Dissolve 0.2 g in 4 ml of water; add 2 ml of 2 M sodium hydroxide and induce crystallisation, if necessary by scratching the wall of the tube with a glass rod and cooling in ice. The residue washed with water and dried at 100° to 105° complies with the following test.

B. Dissolve 0.1 g in 1 ml of sulphuric acid and 1 drop of ferric chloride test solution and warm gently; a bluish violet colour is produced. Add 1 drop of dilute nitric acid; the colour changes to red.

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 72 volumes of ethanol, 30 volumes of cyclohexane and 6 volumes of strong ammonia solution.

**Test solution.** To 20 ml of the syrup add 20 ml of water and 2 ml of strong ammonia solution and extract with two quantities, each of 20 ml, of chloroform. Dry the combined extracts with anhydrous sodium sulphate, filter, evaporate the filtrate to dryness and dissolve the residue in 1 ml of chloroform.
Reference solution (a). Dilute 1.5 ml of the test solution to 100 ml with the same solvent.

Reference solution (b). Dilute 1 ml of the test solution to 100 ml with the same solvent.

Apply separately to the plate 10 µl of each solution. After development, dry the plate in air and spray with acidic potassium iodobismuthate solution. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot, with an Rf value higher than that of the principal spot, is more intense than the spot in the chromatogram obtained with reference solution (b).

Other tests. Complies with the tests stated under Oral Liquids.

Assay. Weigh accurately about 10.0 g, add dilute ammonia solution until the solution is alkaline to litmus paper and extract with four quantities, each of 25 ml, of chloroform. Wash each extract successively with the same 10 ml of water; combine the chloroform extracts and evaporate to dryness on a water-bath. To the residue add 5 ml of ethanol (95 per cent) and again evaporate to dryness. Dissolve the residue in 5.0 ml of 0.05 M hydrochloric acid and titrate the excess of acid with 0.05 M sodium hydroxide using methyl red solution as indicator.

1 ml of 0.05 M hydrochloric acid is equivalent to 0.02032 g of C$_{18}$H$_{21}$NO$_3$.H$_3$PO$_4$.1/2.H$_2$O.

Determine the weight per ml of the syrup (2.4.29) and calculate the content of C$_{18}$H$_{21}$NO$_3$.H$_3$PO$_4$.1/2.H$_2$O, weight in volume.

Storage. Store protected from light.

Colchicine

\[
\begin{align*}
\text{H}_3\text{CO} & \quad \text{H}_3\text{CO} \\
\text{H}_3\text{CO} & \quad \text{H}_3\text{CO} \\
& \quad -\text{NHCOCOCH}_3 \\
\text{OCH}_3 & \quad \text{O} \\
\text{C}_22\text{H}_{25}\text{NO}_6 & \quad \text{Mol. Wt. 399.4}
\end{align*}
\]

Colchicine is (S)-N-(5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenz[a]heptalen-7-yl)acetamide, an alkaloid which occurs in the corm and seeds of various species of Colchicum.

Colchicine contains not less than 97.0 per cent and not more than 103.0 per cent of C$_{22}$H$_{25}$NO$_6$, calculated on the anhydrous and solvent-free basis.

Description. Pale yellow crystals, amorphous scales or a powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with colchicine RS or with the reference spectrum of colchicine.

B. When examined in the range 230 nm to 400 nm, a 0.001 per cent w/v solution in ethanol (95 per cent) shows absorption maxima, at about 243 nm and 350 nm; absorbance at about 243 nm, about 0.73 and at about 350 nm, about 0.42 (2.4.7).

C. Dissolve 50 mg in 1.5 ml of water; a yellow solution is produced which is intensified on adding mineral acids.

D. Mix 1 mg with 0.2 ml of sulphuric acid in a white dish; a yellow colour is produced which on the addition of 0.05 ml of nitric acid changes to greenish-blue and then rapidly becomes reddish and finally almost colourless. On addition of an excess of 5 M sodium hydroxide the colour changes to red.

Tests

Specific optical rotation (2.4.22). -235° to -250°, determined at 20° in a 0.5 per cent w/v solution in ethanol (95 per cent).

Colchicine. To 5 ml of a 1.0 per cent w/v solution add 0.1 ml of a 10.5 per cent w/v solution of ferric chloride. Any colour produced is not more intense than that obtained by mixing 2.0 ml of FCS with 1.0 ml of CCS and 2.0 ml of CSS (2.4.1).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel HF254.

Mobile phase. A mixture of 50 volumes of acetone, 25 volumes of 1,2-dichloroethane and 1 volume of strong ammonia solution.

Prepare the following solutions immediately before use.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of chloroform.

Reference solution (a). Dilute 1 ml of the test solution to 50 ml with the same solvent.

Reference solution (b). Dilute 1 volume of reference solution (a) with an equal volume of chloroform.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in chromatogram obtained with reference solution (b).
Solvent. Determine by gas chromatography (2.4.14).

Test solution. A 1.0 per cent w/v solution of the substance under examination and the same concentration of the internal standard as in the reference solution.

Reference solution. A solution in water containing 0.1 per cent v/v of ethanol-free chloroform, 0.1 per cent v/v of ethyl acetate and either 0.1 per cent v/v (for the determination of ethyl acetate) or 0.02 per cent v/v (for the determination of chloroform) of ethanol (internal standard).

Chromatographic system
- a glass or stainless steel column 1.5 m x 4 mm, packed with white diatomaceous earth (100 to 120 mesh) coated with 10 per cent w/w of polyethylene glycol 1000,
- temperature: column, 75°, inlet port, 130°, detector, 150°,
- a flame ionisation detector at 150°,
- flow rate. 30 ml per minute of the carrier gas.

Calculate the percentage w/w of ethyl acetate or chloroform, taking 0.901 g or 1.477 g, respectively, as the weight per ml at 20° (2.4.29).

The sum of the contents of chloroform or ethyl acetate and the percentage of Water is not more than 10 per cent.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 2.0 per cent, determined on 0.5 g.

Assay. Weigh accurately about 50 mg and dissolve in a mixture of 10 ml of acetic anhydride and 20 ml of toluene. Titrate with 0.02 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.02 M perchloric acid is equivalent to 0.007988 g of C$_{22}$H$_{25}$NO$_6$.

Storage. Store protected from light.

Colchicine Tablets

Colchicine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of colchicine, C$_{22}$H$_{25}$NO$_6$.

Identification

A. To a quantity of the powdered tablets containing 10 mg of Colchicine add 20 ml of water and mix well. Filter into a separating funnel and extract with 30 ml of chloroform. Evaporate the chloroform extract to dryness using moderate heat. The residue complies with the following test.

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Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with colchicine RS or with the reference spectrum of colchicine.

B. To a quantity of the powdered tablets containing 1 mg of Colchicine add 0.2 ml of sulphuric acid and mix; a yellow colour is produced. On adding a drop of nitric acid the colour changes to greenish-blue, then reddish and finally almost colourless. On the addition of an excess of 5 M sodium hydroxide the colour changes to red.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel HF254.

Mobile phase. A mixture of 50 volumes of acetone, 25 volumes of 1,2-dichloroethane and 1 volume of strong ammonia solution.

Prepare the following solutions immediately before use.

Test solution. Extract a quantity of the powdered tablets containing 5 mg of Colchicine with 5 ml of chloroform, filter and evaporate the filtrate to dryness in a current of air. Dissolve the residue as completely as possible in 0.1 ml of ethanol (95 per cent), centrifuge and use the supernatant liquid.

Reference solution (a). Dilute 1 volume of the test solution to 20 volumes with ethanol (95 per cent).

Reference solution (b). Dilute 1 volume of reference solution (a) with an equal volume of chloroform.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in chromatogram obtained with reference solution (b).

Uniformity of content. Comply with the test stated under Tablets.

Protect the solutions from light throughout the test.

Assay. Protect the solutions from light throughout the test.

Crush one tablet and transfer to a centrifuge tube with the aid of 10 ml of ethanol. Shake for 30 minutes, centrifuge and decant the supernatant liquid. Wash the residue with small quantities of ethanol, combine the extract and washings and add sufficient ethanol to produce a solution containing 0.001 per cent w/v of Colchicine. Measure the absorbance of the resulting solution at the maximum at about 350 nm (2.4.7). Calculate the content of C$_{22}$H$_{25}$NO$_6$ in the tablet taking 425 as the specific absorbance at 350 nm.

Other tests. Comply with the tests stated under Tablets.

Assay. Protect the solutions from light throughout the test.
Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.5 mg of Colchicine, add 10 ml of ethanol and shake for 30 minutes. Centrifuge and decant the supernatant liquid. Wash the residue with small quantities of ethanol and mix the extract and washings. Add sufficient ethanol to produce 50.0 ml and mix well. Measure the absorbance of the resulting solution at the maximum at about 350 nm (2.4.7). Calculate the content of \( \text{C}_{22}\text{H}_{25}\text{NO}_6 \) taking 425 as the specific absorbance at 350 nm.

**Storage.** Store protected from light.

### Colchicine and Probenecid Tablets

Colchicine and Probenecid Tablets contain not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of colchicine, \( \text{C}_{22}\text{H}_{25}\text{NO}_6 \), and not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of probenecid, \( \text{C}_{13}\text{H}_{19}\text{NO}_4\text{S} \).

#### Identification

**A.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 100 volumes of methanol and 1.5 volumes of strong ammonia solution.

**Test solution (a).** Shake a quantity of the powdered tablets containing 1 mg of Colchicine with 15 ml of water, mix, filter, extract the filtrate with 25 ml of chloroform and evaporate the chloroform extract to a volume of about 1 ml.

**Test solution (b).** Shake a quantity of the powdered tablets containing 10 mg of Probenecid with 10 ml of chloroform, allow to settle and decant the clear supernatant liquid.

**Reference solution (a).** A solution containing 0.1 per cent w/v of colchicine RS.

**Reference solution (b).** A solution containing 0.1 per cent w/v of probenecid RS.

Apply to the plate 5 \( \mu \)l of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with test solution (a) corresponds to that in the chromatogram obtained with reference solution (a). Similarly, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

**Uniformity of content.** For colchicine - Comply with the test stated under Tablets using the method described under Assay.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** For colchicine — Carry out the determination without delay, under subdued light, using low actinic glassware.

Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 1 mg of Colchicine, shake with 75 ml of ethanolic sodium carbonate for 30 minutes, add sufficient of ethanolic sodium carbonate to produce 100.0 ml and filter. Measure the absorbance of the resulting solution at the maximum at about 350 nm (2.4.7). Calculate the content of \( \text{C}_{22}\text{H}_{25}\text{NO}_6 \) taking 425 as the specific absorbance at 350 nm.

**For probenecid** — Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.25 g of Probenecid, shake with 100 ml of 0.1 M sodium hydroxide for 10 minutes, add sufficient of 0.1 M sodium hydroxide to produce 250.0 ml, mix and filter. Dilute 10.0 ml of the filtrate to 100.0 ml with 0.1 M sodium hydroxide. Dilute further 10.0 ml to 50.0 ml with the same solvent and measure the absorbance of the resulting solution at the maximum at about 248 nm (2.4.7). Calculate the content of \( \text{C}_{13}\text{H}_{19}\text{NO}_4\text{S} \) taking 332 as the specific absorbance at 248 nm.

**Storage.** Store protected from light.

### Cortisone Acetate

\[
\text{C}_{23}\text{H}_{30}\text{O}_6 \quad \text{Mol. Wt} \quad 402.5
\]

Cortisone Acetate is 17\( \alpha \),21-dihydroxyprog-4-ene-3,11,20-trione 21-acetate.

Cortisone Acetate contains not less than 96.0 per cent and not more than 104.0 per cent of \( \text{C}_{23}\text{H}_{30}\text{O}_6 \), calculated on the dried basis.

**Description.** A white or almost white, crystalline powder; odourless.

#### Identification

**Tests A and B may be omitted if tests C and D are carried out.**

**Tests C and D may be omitted if tests A and B are carried out.**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cortisone acetate RS or with the reference spectrum of cortisone acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.
Solvent mixture. A mixture of 90 volumes of acetone and 10 volumes of formamide.

Mobile phase. Chloroform.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of the same solvent mixture.

Reference solution (a). Dissolve 25 mg of cortisone acetate RS in 10 ml of the same solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with ethanolic sulphuric acid (20 per cent v/v). Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

D. 10 mg gives the reactions of acetyl groups (2.3.1).

Tests

Specific optical rotation (2.4.22). +211° to +220°, determined in a 1.0 per cent w/v solution in dioxan.

Light absorption (2.4.7). Dissolve 50 mg in sufficient ethanol (95 per cent) to produce 100 ml and dilute 2 ml to 100 ml with the same solvent. Absorbance of the resulting solution at the maximum at about 240 nm, 0.375 to 0.405.

Related substances. Determine by liquid chromatography (2.4.14).

Prepare the following solutions immediately before use.

Test solution. Dissolve 25.0 mg of the substance under examination in acetonitrile and dilute to 10.0 ml with the same solvent.

Reference solution (a). Dissolve 2 mg of cortisone acetate RS and 2 mg of hydrocortisone acetate RS in acetonitrile and dilute to 100.0 ml with the same solvent.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with acetonitrile.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 400 ml of acetonitrile and 550 ml of water; allowed to equilibrate sufficient water added to produce 1000 ml and mixed,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Equilibrate the column with the mobile phase for 30 minutes.

Inject reference solution (b). Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram is at least 50 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are: hydrocortisone acetate, about 10 minutes and cortisone acetate, about 12 minutes. The test is not valid unless the resolution between the peaks due to hydrocortisone acetate and cortisone acetate is at least 4.2.

Inject the test solution and reference solution (b). Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak other than the principal peak, is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all the peaks other than the principal peak, is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 0.5 g by drying in an oven at 105° for 3 hours.

Assay. Dissolve 0.1 g in ethanol and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of the solution to 100.0 ml with ethanol. Measure the absorbance at the maximum at about 237 nm (2.4.7).

Calculate the content of C_{23}H_{30}O_{6} taking 395 as the specific absorbance at 237 nm.

Storage. Store protected from light.

Cortisone Injection

Cortisone Injection is a sterile suspension of a very fine powder of Cortisone Acetate in Sodium Chloride Injection containing suitable dispersing agents.

D. 10 mg gives the reactions of acetyl groups (2.3.1).

Tests

Specific optical rotation (2.4.22). +211° to +220°, determined in a 1.0 per cent w/v solution in dioxan.

Light absorption (2.4.7). Dissolve 50 mg in sufficient ethanol (95 per cent) to produce 100 ml and dilute 2 ml to 100 ml with the same solvent. Absorbance of the resulting solution at the maximum at about 240 nm, 0.375 to 0.405.

Related substances. Determine by liquid chromatography (2.4.14).

Prepare the following solutions immediately before use.

Test solution. Dissolve 25.0 mg of the substance under examination in acetonitrile and dilute to 10.0 ml with the same solvent.

Reference solution (a). Dissolve 2 mg of cortisone acetate RS and 2 mg of hydrocortisone acetate RS in acetonitrile and dilute to 100.0 ml with the same solvent.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with acetonitrile.
Cortisone Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cortisone acetate, C_{23}H_{30}O_{6}.

**Description.** A white suspension, which settles on standing, but readily disperses on shaking.

**Identification**

Extract a volume of the injection containing 0.1 g of Cortisone Acetate with 6 ml of chloroform, filter and evaporate the chloroform. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cortisone acetate RS or with the reference spectrum of cortisone acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Solvent mixture.** A mixture of 90 volumes of acetone and 10 volumes of formamide.

**Mobile phase.** Chloroform.

**Test solution.** Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

**Reference solution (a).** Dissolve 25 mg of cortisone acetate RS in 10 ml of the solvent mixture.

**Reference solution (b).** Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with ethanolic sulphuric acid (20 per cent v/v). Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. 10 mg gives the reactions of acetyl groups (2.3.1).

**Tests**

**pH** (2.4.24). 5.0 to 7.2.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Mix a quantity of the suspension containing 25 mg of Cortisone Acetate with 15 ml of isopropyl alcohol, evaporate to dryness on a steam bath. To the residue add 10 ml of the mobile phase, shake, mix with the aid of ultrasound and filter (such as Whatman GF/C filter).

**Reference solution (a).** Dilute 1 volume of the test solution to 100 volumes with the mobile phase.

**Reference solution (b).** A solution containing 0.002 per cent w/v each of cortisone acetate RS and hydrocortisone acetate RS in the mobile phase.

**Chromatographic system**

- a stainless steel column 25 cm x 4.6 mm, packed with packed with octadecylsilyl silica gel (5 µm) (such as Hypersil ODS),
- mobile phase: a mixture of 400 ml of acetonitrile and 550 ml of water, allowed to equilibrate and sufficient water added to produce 1000 ml,  
- flow rate. 1 ml per minute,  
- spectrophotometer set at 254 nm,  
- a 20 µl loop injector.

Inject reference solution (b). The retention times are: hydrocortisone acetate, about 10 minutes and cortisone acetate, about 12 minutes. The test is not valid unless the resolution between the peaks due to hydrocortisone acetate and cortisone acetate is at least 4.2.

Inject the test solution and reference solution (a). Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not greater than half that of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent); the sum of the areas of all the secondary peaks is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Add 50 ml of methanol to a quantity of the injection containing about 10 mg of Cortisone Acetate, shake, mix with the aid of ultrasound for 2 minutes, dilute to 100.0 ml with water, shake, centrifuge and use the supernatant liquid.

**Reference solution.** Dilute 50 ml of a solution in methanol containing 0.02 per cent w/v each of cortisone acetate RS and prednisolone RS to 100.0 ml with water.

**Chromatographic system**

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm) (such as Hypersil ODS),
– mobile phase: methanol (60 per cent),
– flow rate, 1.5 ml per minute,
– spectrophotometer set at 240 nm,
– a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to cortisone acetate and prednisolone in the chromatogram obtained is at least 5.0.

Inject alternately the test solution and the reference solution. Calculate the content of C_{23}H_{30}O_{6} in the injection.

Storage. Store protected from light in single dose or multiple dose containers at a temperature not exceeding 30°. It should not be allowed to freeze.

Labelling. The label states (1) the name(s) of the dispersing agent(s) added; (2) that it is not meant to be given by intravenous injection; (3) that the container should be gently shaken before a dose is withdrawn.

Cortisone Tablets

Cortisone Acetate Tablets

Cortisone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cortisone acetate, C_{23}H_{30}O_{6}.

Identification

Extract a quantity of the powdered tablets containing 0.1 g of Cortisone Acetate with 5 ml of chloroform, filter and evaporate the chloroform. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cortisone acetate RS or with the reference spectrum of cortisone acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Solvent mixture. A mixture of 90 volumes of acetone and 10 volumes of dimethylformamide.

Mobile phase. Chloroform.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of the same solvent mixture.

Reference solution (a). Dissolve 25 mg of cortisone acetate RS in 10 ml of the same solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with ethanolic sulphuric acid (20 per cent v/v). Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. 10 mg gives the reactions of acetyl groups (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Mix a quantity of the powdered tablets containing 25 mg of Cortisone Acetate with 10 ml of the mobile phase, place in an ultrasonic bath for 10 minutes and filter (such as Whatman GF/C filter).

Reference solution (a). Dilute 1 volume of the test solution to 100 volumes with the mobile phase.

Reference solution (b). A solution containing 0.002 per cent w/v each of cortisone acetate RS and hydrocortisone acetate RS in the mobile phase.

Chromatographic system

– a stainless steel column 25 cm x 4.6 mm, packed with packed with octadecylsilyl silica gel (5 µm) (such as Hypersil ODS),
– mobile phase: a mixture of 400 ml of acetonitrile and 550 ml of water, allowed to equilibrate and sufficient water added to produce 1000 ml,
– flow rate. 1 ml per minute,
– spectrophotometer set at 254 nm,
– a 20 µl loop injector.

Inject reference solution (b). The retention times are: hydrocortisone acetate, about 10 minutes and cortisone acetate, about 12 minutes. The test is not valid unless the resolution between the peaks due to hydrocortisone acetate and cortisone acetate is at least 4.2.

Inject the test solution and reference solution (a). Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent); the sum of the areas of all
the secondary peaks is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Uniformity of content.** For tablets containing 10 mg or less.
Comply with the test stated under Tablets.

Powder one tablet, add 50 ml of ethanol, shake for 30 minutes and add sufficient ethanol to produce 100.0 ml. Centrifuge and dilute a suitable volume of the supernatant liquid containing 0.5 mg of Cortisone Acetate to 50.0 ml with ethanol. Measure the absorbance of the resulting solution at the maximum at about 240 nm (2.4.7). Calculate the content of C$_{23}$H$_{30}$O$_6$ taking 390 as the specific absorbance at 240 nm.

**Dissolution (2.5.2).**
Apparatus. No 1
Medium. 900 ml of a mixture of a 1 per cent v/v solution of hydrochloric acid and 30 volumes of 2-propanol
Speed and time. 100 rpm and 45 minutes.
Withdraw a suitable volume of the medium and filter through a membrane filter with an average pore diameter not greater than 1.0 µm. Measure the absorbance of the filtrate, suitably diluted if necessary with the medium, at the maximum at about 242 nm (2.4.7). Calculate the content of C$_{23}$H$_{30}$O$_6$ taking 399 as the specific absorbance at 242 nm.

D. Not less than 75 per cent of the stated amount of C$_{23}$H$_{30}$O$_6$.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh and powder 20 tablets. Add 50 ml of methanol to a quantity of the powder containing about 10 mg of Cortisone Acetate, shake, mix with the aid of ultrasound for 2 minutes, dilute to 100.0 ml with water, shake, centrifuge and use the supernatant liquid.

*Reference solution.* Dilute 50 ml of a solution in methanol containing 0.02 per cent w/v each of cortisone acetate RS and prednisolone to 100.0 ml with water.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm) (such as Hypersil ODS),
- mobile phase: methanol (60 per cent),
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 240 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to cortisone acetate and prednisolone in the chromatogram obtained is at least 5.0.

Inject alternately the test solution and the reference solution. Calculate the content of C$_{23}$H$_{30}$O$_6$ in the tablets.

**Storage.** Store protected from light.

**Absorbent Cotton**

**Absorbent Cotton Wool**

Absorbent Cotton consists of the new fibres or good quality new combers obtained from the seed coat of various species of the genus Gossypium Linn., cleaned, purified, bleached and carded. It does not contain any compensatory colouring matter.

**Description.** White, well-carded fibres of average staple length not less than 10 mm, containing not more than traces of leaf residue, seed coat and other impurities. It offers appreciable resistance when pulled and does not shed a significant quantity of dust when shaken gently; practically odourless.

**Identification**

A. When examined under a microscope, each fibre is seen to consist of a single cell, up to about 4 cm long and up to 40 µm wide, in the form of a flattened tube with thick and rounded walls and often twisted.

B. Treat with iodinated zinc chloride solution; the fibres become violet.

C. To 0.1 g add 10 ml of zinc chloride solution, heat to 40° and allowed to stand for 2 ½ hours, shaking occasionally; the fibres do not dissolve.

**Tests**

To 15.0 g add 150 ml of water, macerate for 2 hours in a closed vessel, decant the liquid, carefully squeezing out the residual liquid with a glass rod and mix. Reserve 10 ml for the test for surface-active substances and filter the remainder (solution S).

**Acidity or alkalinity.** To 25 ml of solution S add 0.1 ml of dilute phenolphthalein solution; to another 25 ml add 0.05 ml of methyl orange solution. Neither solution shows a pink colour.

**Surface-active substances.** Into a 25-ml graduated, ground-glass stoppered cylinder with external diameter of 18 to 22 mm, previously rinsed with sulphuric acid and then with water, add the 10-ml portion of solution S, shake vigorously 30 times in 10 seconds, allow to stand for 1 minute and shake again 30 times. After 5 minutes, the height of the froth does not exceed 2 mm above the surface of the liquid.

**Absorbency**

**Apparatus.** A dry, cylindrical wire basket, 80 mm high and 50 mm in diameter, fabricated from wire of diameter 0.4 mm and
having a mesh aperture of 15 to 20 mm; the basket weighs 2.4 to 3.0 g.

Sinking time. Not more than 10 seconds, determined by the following method.

Weigh the basket to the nearest 10 mg. Take five samples, each of approximately 1 g, from different places in the material being examined, pack loosely in the basket and weigh the packed basket to the nearest 10 mg. Hold the basket with its long axis in the horizontal position and drop it from a height of about 10 mm into water at 25° contained in a beaker at least 12 cm in diameter and filled to a depth of 10 cm. Measure with a stopwatch the time taken by the basket to sink below the surface of the water. Repeat the procedure on two further samples and calculate the average value.

Water-holding capacity. Not less than 23.0 g per g, determined by the following method.

After the sinking time has been recorded in test A, remove the basket from the water, allow it to drain for exactly 30 seconds with its long axis in the horizontal position, transfer it to a tared beaker and weigh to the nearest 10 mg. Calculate the weight of water retained by the sample. Repeat the procedure on two further samples and calculate the average value.

Foreign fibres. When examined under a microscope, it is seen to consist exclusively of typical cotton fibres, except that occasionally a few isolated foreign fibres may be seen.

Fluorescence. Examine a layer about 5 mm in thickness in ultraviolet light at 365 nm. It shows only a slight, brownish-violet fluorescence and a few yellow particles. Not more than a few isolated fibres show an intense blue fluorescence.

Colouring matter. Slowly extract 10 g in a narrow percolator with ethanol (95 per cent) until 50 ml of extract is obtained. The extract is not more intensely coloured than reference solution YS5 or GYS6 (2.4.1) or a solution prepared in the following manner. To 3.0 ml of CSS add 7.0 ml of a solution of hydrochloric acid containing 1 per cent v/v of hydrochloric acid and dilute 0.5 ml of the resulting solution to 10 ml with the same solution of hydrochloric acid.

Ether-soluble substances. Not more than 0.5 per cent determined by the following method. Extract 5 g with ether in a continuous extraction apparatus (2.1.8), for 4 hours in such a way that the rate is at least four extractions per hour. Evaporate the ether and dry the residue to constant weight at 105°.

Water-soluble substances. Not more than 0.5 per cent, determined by the following method. Boil 5 g with 500 ml of water for 30 minutes, stirring frequently and replacing the water lost by evaporation. Decant the liquid into a beaker, squeeze the residual liquid from the material carefully with a glass rod, mix the liquids and filter the extract whilst hot. Evaporate 400 ml of the filtrate and dry the residue to constant weight at 105°.

Neps. A thin layer approximately equivalent to 0.5 g for an area of 450 sq. cm. spread uniformly between two glass plates, and viewed by the naked eye under transmitted light, does not show more neps than about an average of 250 for three tests.

Sulphated ash (2.3.18). Not more than 0.5 per cent, determined on 5.0 g.

Loss on drying (2.4.19). Not more than 8.0 per cent, determined on 5.0 g by drying in an oven at 105°.

Packaging. Package in rolls of not more than 500 g of continuous lap, with a light-weight paper running under the entire lap, the paper being of such width that it may be folded over the edges of the lap, the two together being tightly and, evenly rolled, enclosed and sealed in a well-closed container.

Storage. Store protected from dust and moisture.

Cresol

Cresol is a mixture of cresols and other phenols obtained from coal tar.

Description. An almost colourless to pale brownish-yellow liquid, becoming darker on keeping or on exposure to light; odour, resembling that of phenol but more tarry.

Identification

To 0.5 ml add 300 ml of water, shake and filter. Divide the filtrate into two parts. To one part add ferric chloride test solution; a transient bluish colour is produced. To the other part add bromine solution; a pale yellow flocculent precipitate is produced.

Tests

Distillation range (2.4.8). Not more than 2.0 per cent v/v distils below 188° and not less than 80.0 per cent v/v between 195° and 205°.

Acidity. A 2 per cent w/v solution is neutral to bromocresol purple solution.

Weight per ml (2.4.29). 1.029 g to 1.044 g.

Hydrocarbons and volatile bases. Place 50 ml in a 500-ml round-bottomed flask, add about 83 ml of a 27 per cent w/v solution of sodium hydroxide and 100 ml of water and mix thoroughly. Connect the flask to a splash-bulb and air condenser about 60 cm long, with the end of the air-condenser fitting closely into the neck of a 250-ml pear-shaped separator and passing well into the separator, which has a cylindrical graduated portion above the stopcock. Fill the graduated portion of the
Moisten a piece of filter paper with a 10 per cent w/v solution of Sulphur compounds; not more than 0.15 per cent v/v of volatile bases; not more than a light yellow colour.

Hydrocarbons — Allow the separator to stand for a short time, measure the volume of hydrocarbon oil in the graduated portion and warm if necessary in order to keep the oil in the liquid state; subtract the volume of volatile bases in the hydrocarbon oil, as determined in the following test; not more than 0.5 per cent v/v of hydrocarbon oil is present.

Volatile bases — To the aqueous portion of the distillate obtained in the preceding test, add any aqueous liquid still remaining in the separator and neutralise it if necessary with 0.1 M hydrochloric acid, using phenolphthalein solution as indicator. Titrate with 1 M hydrochloric acid using methyl orange solution as indicator. Wash the oil from the separator into the titration flask with water and again titrate with 1 M hydrochloric acid. From the volume of additional 1 M hydrochloric acid calculate the proportion of volatile bases in the hydrocarbon oil. From the total volume of 1 M hydrochloric acid used in both titrations calculate the proportion of volatile bases in cresol.

1 ml of 1 M hydrochloric acid is equivalent to 0.08 ml of volatile bases; not more than 0.15 per cent v/v of volatile bases, calculated as pyridine, are present.

Sulphur compounds. Place about 20 ml in a small conical flask. Moisten a piece of filter paper with a 10 per cent w/v solution of lead acetate and fix it on the mouth of the flask; heat the flask on a water-bath for 5 minutes; the filter paper shows not more than a light yellow colour.

Non-volatile matter. Not more than 0.1 per cent w/v when evaporated on a water-bath and dried to constant weight at 105°C.

Storage. Store protected from light.

Cresol With Soap Solution

Lysol

Cresol with Soap Solution is prepared by the saponification of a mixture of Cresol with vegetable oils such as cotton seed, linseed, soyabean or similar oils but excluding coconut and palm kernel oils. Alternatively, the mixed fatty acids derived from these oils may be used.

Cresol with Soap Solution contains not less than 47.0 per cent v/v and not more than 53.0 per cent v/v of Cresol.

Description. An amber-coloured to reddish-brown liquid; odour, that of cresol; soapy to touch.

Tests

Appearance of solution. 5 ml mixed with 95 ml of water forms a clear solution without producing any opalescence on standing for 3 hours.

Alkalinity. Dilute 5 ml of ethanol (95 per cent) neutralised to phenol red solution and titrate with 1 M sulphuric acid, using phenol red solution as indicator; not more than 0.6 ml is required.

Hydrocarbons and volatile bases. Distil 120 ml until all the water and 50 ml of cresol have been collected. Place the cresol thus recovered in a 500-ml round-bottomed flask, add about 83 ml of a 27 per cent w/v solution of sodium hydroxide and 100 ml of water and mix thoroughly. Connect the flask to a splash-bulb and air condenser about 60 cm long, with the end of the air-condenser fitting closely into the neck of a 250-ml pear-shaped separator and passing well into the separator, which has a cylindrical graduated portion above the stopcock. Fill the graduated portion of the separator with water. Distil rapidly until 75 ml of distillate has been collected, cooling the separator in running water, if necessary. Allow the separator to stand in a vertical position until separation is complete and draw off the aqueous liquid into a titration flask.

Hydrocarbons — Allow the separator to stand for a short time, measure the volume of hydrocarbon oil in the graduated portion and warm if necessary in order to keep the oil in the liquid state; subtract the volume of volatile bases in the hydrocarbon oil, as determined in the following test; not more than 0.5 per cent v/v of hydrocarbon oil is present.

Volatile bases — To the aqueous portion of the distillate obtained in the preceding test, add any aqueous liquid still remaining in the separator and neutralise it if necessary with 0.1 M hydrochloric acid, using phenolphthalein solution as indicator. Titrate with 1 M hydrochloric acid using methyl orange solution as indicator. Wash the oil from the separator into the titration flask with water and again titrate with 1 M hydrochloric acid. From the volume of additional 1 M hydrochloric acid calculate the proportion of volatile bases in the hydrocarbon oil. From the total volume of 1 M hydrochloric acid used in both titrations calculate the proportion of volatile bases in cresol.

1 ml of 1 M hydrochloric acid is equivalent to 0.08 ml of volatile bases; not more than 0.15 per cent v/v of volatile bases, calculated as pyridine, are present.

Sulphur compounds. Complies with the test for Sulphur compounds described under Cresol.

Assay. To 50 ml, accurately measured, add 150 ml of kerosene, mix and add little powdered pumice stone and 3 g of sodium bicarbonate. Distil into a separator, the rate of distillation being not more than 2 drops per second until the kerosene and cresol have completely distilled. This is indicated by the
distillate being yellow in colour. Stop the distillation, add 50 ml of kerosene and collect a further 50 ml of the distillate. Discard the lower aqueous layer in the separator, dry the remainder with anhydrous calcium chloride and shake with 10 ml of sulphuric acid (50 per cent w/w). Set aside for 2 hours, reject the acid layer and to the kerosene layer add 40 ml of sodium hydroxide solution and shake for 5 minutes. Transfer the alkaline layer to a 100-ml volumetric flask and extract the kerosene layer with 20 ml of sodium hydroxide solution adding the alkaline layer to that in the 100-ml volumetric flask. Add sodium hydroxide solution from a burette to make the volume in the flask to 100 ml. The difference between the burette reading and 40.5 is equal to the volume of cresol in 50 ml of the sample.

Storage. Store protected from light.

**Croscarmellose Sodium**

Croscarmellose sodium (cross-linked sodium carboxymethylcellulose) is the sodium salt of a cross-linked, partly O-carboxymethylated cellulose.

**Description.** A white or greyish-white powder.

**Identification**

A. Shake 1 g with 100 ml of 0.00001 per cent w/v solution of methylene blue and allow to settle. The substance under examination absorbs the methylene blue and settles as a blue, fibrous mass.

B. Shake 1 g with 50 ml of water. Transfer 1 ml of the mixture to a test-tube, add 1 ml of water and 0.05 ml of a freshly prepared 4.0 per cent w/v solution of α-naphthol in methanol. Incline the test-tube and add carefully 2 ml of sulphuric acid down the side so that it forms a lower layer. A reddish-violet colour develops at the interface.

C. The solution prepared from the sulphated ash in the test for Heavy metals (see Tests) gives reaction (a) of sodium salts (2.3.1)

**Tests**

**pH** (2.4.24). 5.0 to 7.0, determined on 1.0 per cent w/v solution in carbon dioxide-free water.

**Degree of substitution.** Take 1.0 g in 500 ml conical flask, add 300 ml of a 10 per cent w/v solution of sodium chloride, 25.0 ml of 0.1 M sodium hydroxide, stopper the flask and allow to stand for 5 minutes, shaking occasionally. Add 0.05 ml of m-cresol purple solution and about 15 ml of 0.1 M hydrochloric acid from a burette. Insert the stopper and shake. If the solution is violet, add 0.1 M hydrochloric acid in 1 ml portions until the solution becomes yellow, shaking after each addition. Titrate with 0.1 M sodium hydroxide until the colour turns to violet.

Calculate the number of milliequivalents (M) of base required for the neutralisation equivalent to 1 g of dried substance. Calculate the degree of acid carboxymethyl substitution (A) from the expression:

\[
\frac{1150 M}{(7102 - 412 M - 80 C)}
\]

The degree of substitution is the sum of A + S and it is between 0.60 and 0.85, calculated on the dried basis.

**Sodium chloride and sodium glycollate.** The sum of the percentage contents of sodium chloride and sodium glycollate is not more than 0.5 per cent, calculated on the dried basis.

**Sodium chloride.** Place 5.0 g in a 250 ml conical flask, add 50 ml of water and 5 ml of strong hydrogen peroxide solution and heat on a water-bath for 20 minutes stirring occasionally to ensure total hydration. Cool, add 100 ml of water and 10 ml of nitric acid. Titrate with 0.05 M silver nitrate determining the end-point potentiometrically (2.4.25) using a silver indicator electrode and a double-junction reference electrode containing a 10 per cent w/v solution of potassium nitrate in the outer jacket and a standard filling solution in the inner jacket, and stirring constantly.

1 ml of 0.05 M silver nitrate is equivalent to 0.002922 g of NaCl.

**Sodium glycollate.** Place 0.5 g of the substance under examination in a 100 ml beaker. Add 5 ml of glacial acetic acid and 5 ml of water and stir to ensure total hydration (about 15 minutes). Add 50 ml of acetone and 1 g of sodium chloride. Stir for several minutes to ensure complete precipitation of the carboxymethylcellulose. Filter through a fast filter paper impregnated with acetone into a volumetric flask, rinse the beaker and filter with 30 ml of acetone and dilute the filtrate to 100.0 ml with the same solvent. Allow to stand for 24 hours without shaking. Use the clear supernatant to prepare the test solution.

**Reference solution.** Dissolve 0.1 g of glycollic acid in 100 ml of water. Use the solution within 30 days. Transfer 1.0 ml, 2.0 ml, 3.0 ml and 4.0 ml of the solution to separate volumetric flasks; dilute the contents of each flask to 5.0 ml with water, add 5 ml of glacial acetic acid, dilute to 100.0 ml with acetone and mix.

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Transfer 2.0 ml of the test solution and 2.0 ml of each of the reference solutions to separate 25 ml volumetric flasks. Heat the uncovered flasks for 20 minutes on a water-bath to eliminate acetone. Allow to cool and add 5.0 ml of 2,7-dihydroxynaphthalene solution to each flask. Mix, then add a further 15.0 ml of 2,7-dihydroxynaphthalene solution and mix again. Close the flasks with aluminium foil and heat on a water-bath for 20 minutes. Cool and dilute to 25.0 ml with sulphuric acid.

Measure the absorbance (2.4.7) of each solution at 540 nm.

Prepare a blank using 2.0 ml of a solution containing 5 per cent v/v each of glacial acetic acid and water in acetone. Prepare a standard curve using the absorbances obtained with the reference solutions. From the standard curve and the absorbance of the test solution, determine the mass, in milligrams, of glycollic acid in the substance under examination, and calculate the content of sodium glycollate from the expression:

\[
\frac{10 \times 1.29 \times a}{(100 - b)m}
\]

where:
- \(1.29\) = the factor converting glycollic acid to sodium glycollate
- \(b\) = loss on drying as a percentage
- \(m\) = mass of the substance under examination, in grams

**Water-soluble substances.** Not more than 10.0 per cent. Disperse 10.0 g in 800.0 ml of water and stir for 1 minute every 10 minutes during the first 30 minutes. Allow to stand for 1 hour and centrifuge, if necessary. Decant 200.0 ml of the supernatant liquid onto a filter paper in a vacuum filtration funnel, apply suction and collect 150.0 ml of the filtrate. Evaporate to dryness and dry the residue at 100° to 105° for 4 hours.

**Heavy metals** (2.3.13). To the residue obtained in sulphated ash add 1 ml of hydrochloric acid and evaporate on a water-bath. Take up the residue in 20 ml of water. 12 ml of the solution complies with the limit test for heavy metals, Method A (10 ppm). Prepare the reference solution using lead standard solution (1 ppm Pb).

**Settling volume**. 10.0 to 30.0 ml. Place 75 ml of water in a 100 ml graduated cylinder and add 1.5 g of the substance under examination in 0.5 g portions, shaking vigorously after each addition. Dilute to 100.0 ml with water and shake again until the substance is homogeneously distributed. Allow to stand for 4 hours.

**Microbial contamination** (2.2.9). Total microbial count is not more than \(10^3\) bacteria and \(10^2\) fungi per gram, determined by plate count. 10 g is free from *Escherichia coli*.

**Sulphated ash** (2.3.18). 14.0 to 28.0 per cent, determined on 2.0 g, using a mixture of equal volumes of sulphuric acid and water, and calculated on the dried basis.

**Loss on drying** (2.4.19). Not more than 10.0 per cent, determined on 1.0 g by drying in an oven at 105° for 6 hours.

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**Crosopovidone**

1-Ethyl-2-pyrrolidinone homopolymer; 1-Vinyl-2-pyrrolidinone homopolymer

Crosopovidone is a water-insoluble synthetic crosslinked homopolymer of N-vinyl-2-pyrrolidinone.

Crosopovidone contains not less than 11.0 per cent and not more than 12.8 per cent of nitrogen (N), calculated on the anhydrous basis.

**Description.** A white to creamy white hygroscopic powder having a faint odour.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6) on specimen previously dried in vacuum at 105° for 1 hour. Compare the spectrum with that obtained with crosopovidone RS.

B. Suspend 1 g in 10 ml of water, add 0.1 ml of 0.1 M iodine, and shake for 30 seconds. Add 1 ml of starch solution, and shake; no blue color develops.

**Tests**

**pH** (2.4.24). 5.0 and 8.0, determined in a 1.0 per cent w/v aqueous suspension.

**Water** (2.3.43). Not more than 0.4 per cent, determined on 2.0 g.

**Water-soluble substances.** Transfer 25.0 g to a 400 ml beaker, add 200 ml of water, and stir on a magnetic stirrer, using a 5-cm stirring bar, for 1 hour. Transfer to a 250 ml volumetric flask with the aid of about 25 ml of water, add water to volume, and mix. Allow the bulk of the solids to settle. Pass about 100 ml of the relatively clear supernatant through a membrane filter having a 0.45 mm porosity, protected against clogging by superimposing a membrane filter. Transfer 50.0 ml of the clear filtrate to a tared 100 ml beaker, evaporate to dryness, and dry at 110° for 3 hours: the weight of the residue does not exceed 75 mg (1.5 per cent).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Vinylpyrrolidinone.** Suspend 4.0 g in 20 ml of water, stir for 15 minutes, centrifuge the suspension, and filter the slightly
turbid upper layer through a sintered glass 10 mm filter. Stir the lower layer with 50 ml of water, centrifuge, and filter the upper layer through the same filter. Again stir the lower layer with 50 ml of water, and filter similarly. Add 0.5 g of sodium acetate to the combined filtrates and titrate with 0.1 M iodine until the color of iodine no longer fades, add 3.0 ml of 0.1 M iodine, allow to stand for 10 minutes, and titrate the excess of iodine with 0.1 M sodium thiosulphate, adding 3 ml of starch solution as the end-point is approached. Carry out a blank determination, using the same total volume of the same 0.1 M iodine, accurately measured, as was used for titrating the substance under examination. Before titrating the blank, adjust with acetic acid to the same pH as that of the substance under examination; not more than 0.72 ml of 0.1 M iodine is consumed, corresponding to not more than 0.1 per cent of vinylpyrrolidinone.

**Nitrogen.** Place 0.1 g of the substance under examination (m mg) in a combustion flask, add 5 g of a mixture of 1 g of copper sulphate, 1 g of titanium dioxide and 33 g of dipotassium sulphate, and 3 glass beads. Wash any adhering particles from the neck into the flask with a small quantity of water. Add 7 ml of sulphuric acid, allowing it to run down the sides of the flask, and mix the contents by rotation. Close the mouth of the flask loosely, for example by means of a glass bulb with a short stem, to avoid excessive loss of sulphuric acid. Heat gradually at first, then increase the temperature until there is vigorous boiling with condensation of sulphuric acid in the neck of the flask; precautions are to be taken to prevent the upper part of the flask from becoming overheated. Continue the heating for 45 minutes. Cool, dissolve the solid material by cautiously adding to the mixture 20 ml of water, cool again and place in a steam-distillation apparatus. Add 30 ml of strong sodium hydroxide solution through the funnel, rinse the funnel cautiously with 10 ml of water and distil immediately by passing steam through the mixture. Collect 80-100 ml of distillate in a mixture of 30 ml of a 4.0 per cent w/v solution of boric acid and 0.05 ml of bromoresol green-methyl red solution and enough water to cover the tip of the condenser. Towards the end of the distillation lower the receiver so that the tip of the condenser is above the surface of the acid solution and rinse the end part of the condenser with a small quantity of water. Titrate the distillate with 0.025 M sulphuric acid until the colour of the solution changes from green through pale greyish-blue to pale greyish-red-purple (n1 ml of 0.025 M sulphuric acid).

Repeat the test using about 100 mg of glucose in place of the substance under examination (n2 ml of 0.025 M sulphuric acid).

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\text{Percentage content of nitrogen} = \frac{0.7004(n_1 - n_2)}{m}
\]

**Storage.** Store protected from moisture.

**Labelling.** The label states the type (type A or type B).

### Cyanocobalamin

**Vitamin B\textsubscript{12}**

Cyanocobalamin is \(\text{CoC}[\alpha-(5,6\text{-dimethylbenzimidazolyl})]-\text{Co}\beta\)-cyanocobamide.

Cyanocobalamin contains not less than 96.0 per cent and not more than 102.0 per cent of \(\text{C}_{63}\text{H}_{88}\text{CoN}_{14}\text{O}_{14}\text{P}\), calculated on the dried basis.

**Description.** A dark red, crystalline powder; very hygroscopic.

**Identification**

A. When examined in the range 260 nm to 610 nm, a 0.0025 per cent w/v solution shows absorption maxima, at about 278 nm, 361 nm and 547 nm to 559 nm. The ratio of the absorbance at about 361 nm to that at about 547 nm to 559 nm is 3.15 to 3.45 and the ratio of the absorbance at about 361 nm to that at about 278 nm is 1.70 to 1.90 (2.4.7).

B. Determine by thin-layer chromatography (2.4.17), protected form light and coating the plate with silica gel G.

**Mobile phase.** A mixture of 60 volumes of chloroform, 40 volumes of methanol and 12 volumes of 6 M ammonia. Use an unlined tank.

**Test solution.** Dissolve 20 mg of the substance under examination in 10 ml of ethanol (50 per cent).

**Reference solution.** A 0.2 per cent w/v solution of cyanocobalamin RS in ethanol (50 per cent). Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine in day light. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.
C. Mix about 1 mg with 10 mg of potassium sulphate and 0.1 ml of 0.5 M sulphuric acid and heat carefully to redness. Allow to cool, add to the residue 0.1 ml of water, 0.5 ml of saturated solution of ammonium thiocyanate and 0.5 ml of benzyl alcohol and shake; a blue colour is formed and is taken into the benzyl alcohol layer.

Tests

Related substances. Determine by liquid chromatography (2.4.17).

The following solutions should be used within 1 hour of preparation.

Test solution. Dissolve 10 mg of the substance under examination in 10 ml of the mobile phase.

Reference solution (a). A 0.003 per cent w/v solution of the substance under examination in the mobile phase.

Reference solution (b). A 0.0001 per cent w/v solution of the substance under examination in the mobile phase.

Reference solution (c). Dissolve 5 mg of the substance under examination in 2 ml of water, warming if necessary, allow to cool, add 1 ml of a 0.1 per cent w/v solution of chloramine T and 0.1 ml of 0.05 M hydrochloric acid, dilute to 5 ml with water, shake and allow to stand for 5 minutes. Dilute 1 ml of this solution to 10 ml with the mobile phase. Use immediately.

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octylsilil silica gel (5 µm),
- mobile phase: a mixture of 147 volumes of a 1.0 per cent w/v solution of disodium hydrogen phosphate and 53 volumes of methanol adjusted to pH 3.5 with phosphoric acid (to be used within 2 days of preparation),
- flow rate. 0.8 ml per minute,
- spectrophotometer set at 361 nm,
- a 20 µl loop injector.

Inject the test solution and reference solutions (a), (b) and (c).

Allow the chromatography to proceed for three times the retention time of the peak due to cyanocobalamin.

In the chromatogram obtained with the test solution the sum of the areas of any secondary peaks is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a). Ignore any peak the area of which is less than that of the principal peak in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (c) exhibits two principal peaks, the resolution between these peaks is 2.5 or more and the chromatogram obtained with reference solution (a) exhibits one principal peak.

Loss on drying (2.4.19). Not more than 12.0 per cent, determined on 20.0 mg by drying in an oven at 105° at a pressure of 1.5 to 2.5 kPa for 2 hours.

Assay. Weigh accurately about 25 mg and dissolve in sufficient water to produce 1000.0 ml. Measure the absorbance of the solution at the maximum at about 361 nm (2.4.7). Calculate the content of C₆₃H₇₈CoN₁₄O₁₄P taking 207 as the specific absorbance at 361 nm.

Storage. Store protected from light and moisture.

Cyanocobalamin Injection

Vitamin B₁₂ Injection

Cyanocobalamin Injection is a sterile solution of Cyanocobalamin in Water for Injections containing sufficient Acetic Acid or Hydrochloric Acid to adjust the pH to about 4. It may contain suitable buffering agents.

Cyanocobalamin Injection contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous cyanocobalamin, C₆₃H₇₈CoN₁₄O₁₄P.

Identification

Measure the absorbance at about 278 nm, 361 nm and 550 nm. The ratio of the absorbance at about 278 nm to that at about 550 nm is 0.57 and the ratio of the absorbance at about 550 nm to that at about 361 nm is 0.30 (2.4.7).

Tests

pH (2.4.24). 3.8 to 5.5.

Related substances. Determine by liquid chromatography (2.4.14).

The following solutions should be used within 1 hour of preparation.

Test solution (a). Dilute a suitable volume of the injection, if necessary, with the mobile phase to produce a solution containing 0.0001 per cent w/v of Cyanocobalamin.

Test solution (b). Dilute a suitable volume of the injection, if necessary, with the mobile phase to produce a solution containing 0.05 per cent w/v of Cyanocobalamin.

Reference solution (a). Dilute a suitable volume of the injection, if necessary, with the mobile phase to produce a solution containing 0.003 per cent w/v of Cyanocobalamin.

Reference solution (b). Add 1 ml of a 0.1 per cent w/v solution of chloramine T and 0.1 ml of 0.05 M hydrochloric acid to a volume containing 5 mg of Cyanocobalamin, dilute to 10 ml with water, shake and allow to stand for 5 minutes. Dilute 2 ml of this solution to 10 ml with the mobile phase and use immediately.
Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octylsilyl silica gel (5 µm),
- mobile phase: a mixture of 147 volumes of a 1.0 per cent w/v solution of disodium hydrogen phosphate and 53 volumes of methanol, adjusted to pH 3.5 with phosphoric acid (to be used within 2 days of preparation),
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 361 nm,
- a 20 µl loop injector.

Allow the chromatography to proceed for three times the retention time of the peak due to cyanocobalamin.

In the chromatogram obtained with test solution (b) the sum of the areas of any secondary peaks is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a). Ignore any peak the area of which is less than that of the principal peak in the chromatogram obtained with test solution (a).

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Carry out the following procedure protected from light.

Dilute the injection, if necessary, with water to produce a solution containing not more than the equivalent of 0.0025 per cent w/v of anhydrous cyanocobalamin and measure the absorbance at the maximum at about 361 nm (2.4.7). Calculate the content of C₁₃H₈₈CoN₁₄O₁₄P taking 207 as the specific absorbance at 361 nm.

Storage. Store protected from light in single dose or multiple dose containers.

Labelling. The label states the strength in terms of the equivalent amount of anhydrous cyanocobalamin in a suitable dose-volume.

Cyclizine Hydrochloride

C₁₈H₂₂N₂, HCl
Mol. Wt. 302.9

Cyclizine Hydrochloride is 1-(diphenylmethyl)-4-methylpiperazine hydrochloride

Cyclizine Hydrochloride contains not less than 98.0 per cent and not more than 101.0 per cent of C₁₈H₂₂N₂, HCl, calculated on the dried basis.

Description. A white, crystalline powder; almost odourless.

Identification
A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cyclizine hydrochloride RS or with the reference spectrum of cyclizine hydrochloride.
B. When examined in the range 220 nm to 360 nm, a freshly prepared 0.002 per cent w/v solution in 0.05 M sulphuric acid shows absorption maximum only at about 225 nm; absorbance at about 225 nm, about 0.78 (2.4.7).
C. Dissolve 0.5 g in 10 ml of ethanol (95 per cent), warming if necessary, cool in ice, add 1 ml of 5 M sodium hydroxide and sufficient water to produce 20 ml. Stir well and filter; the precipitate, after washing with water and drying at 60° at a pressure not exceeding 0.7 kPa for 2 hours, melts at about 107° (2.4.21).
D. Gives the reactions of chlorides (2.3.1).

Tests
N-Methylpiperazine. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 90 volumes of chloroform, 8 volumes of methanol and 2 volumes of strong ammonia solution.

Prepare the following solutions freshly.
Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of methanol.
Reference solution. A 0.005 per cent w/v solution of N-methylpiperazine RS in methanol.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and expose to iodine vapours for 10 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the corresponding spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 130°.

Assay. Weigh accurately about 0.1 g, dissolve in 20 ml of anhydrous glacial acetic acid and add 50 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.
1 ml of 0.1 M perchloric acid is equivalent to 0.01514 g of C\textsubscript{18}H\textsubscript{22}N\textsubscript{2}HCl.

**Storage.** Store protected from light.

## Cyclizine Tablets

Cyclizine Hydrochloride Tablets

Cyclizine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of cyclizine hydrochloride, C\textsubscript{18}H\textsubscript{22}N\textsubscript{2}H\textsubscript{Cl}.

**Identification**

A. Extract a quantity of the powdered tablets containing 0.1 g of Cyclizine Hydrochloride with 10 ml of ethanol (95 per cent), filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cyclizine hydrochloride RS or with the reference spectrum of cyclizine hydrochloride.

B. Extract a quantity of the powdered tablets containing 0.5 g of Cyclizine Hydrochloride with 20 ml of water and filter. The filtrate gives reaction A of chlorides (2.3.1).

**Tests**

**N-Methylpiperazine.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

*Mobile phase.* A mixture of 90 volumes of chloroform, 8 volumes of methanol and 2 volumes of strong ammonia solution.

*Prepare the following solutions freshly.*

*Test solution.* Triturate a quantity of the powdered tablets containing 0.1 g of Cyclizine Hydrochloride with 10 ml of methanol and filter.

*Reference solution.* A 0.005 per cent w/v solution of N-methylpiperazine RS in methanol.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and expose to iodine vapours for 10 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the corresponding spot in the chromatogram obtained with the reference solution.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.125 g of Cyclizine Hydrochloride and shake with 400 ml of 0.5 M sulphuric acid for 15 minutes. Add sufficient 0.5 M sulphuric acid to produce 500.0 ml and filter. Dilute 5.0 ml of the filtrate to 100.0 ml with 0.5 M sulphuric acid and measure the absorbance of the resulting solution at the maximum at about 225 nm (2.4.7). Calculate the content of C\textsubscript{18}H\textsubscript{22}N\textsubscript{2}H\textsubscript{Cl} taking 390 as the specific absorbance at 225 nm.

**Storage.** Store protected from light.

## Cyclophosphamide

Cyclophosphamide is (RS)-2-bis(2-chloroethyl)aminoperhydro-1,3,2-oxazaphosphorinane 2-oxide monohydrate.

Cyclophosphamide contains not less than 98.0 per cent and not more than 102.0 per cent of C\textsubscript{7}H\textsubscript{15}Cl\textsubscript{2}N\textsubscript{2}O\textsubscript{2}P, calculated on the anhydrous basis.

**Description.** A white or almost white, crystalline powder.

**Identification**

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cyclophosphamide RS or with the reference spectrum of cyclophosphamide.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Dissolve 0.1 g in 10 ml of water and add 5 ml of silver nitrate solution; no precipitate is produced. Boil; a white precipitate is produced which is insoluble in dilute nitric acid but is soluble in dilute ammonia solution from which it can be reprecipitated by the addition of dilute nitric acid.

D. Dissolve 0.1 g in 3 ml of nitric acid and 1 ml of sulphuric acid, heat till brown fumes are evolved and the solution becomes colourless. Cool, add 10 ml of water, heat again up to 60° and add 10 ml of ammonium molybdate solution; a bright yellow precipitate is slowly formed.

**Tests**

**Appearance of solution.** A 2.0 per cent w/v solution in carbon dioxide-free water is clear (2.4.1), and not more intensely coloured than reference solution Y86 (2.4.1).
pH (2.4.24). 4.0 to 6.0, determined in a 2.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 80 volumes of 2-butanone, 12 volumes of water, 4 volumes of acetone and 2 volumes of anhydrous formic acid.

Test solution (a). Dissolve 0.2 g of the substance under examination in 10 ml of ethanol (95 per cent).

Test solution (b). Dilute 5 ml of test solution (a) to 50 ml with ethanol (95 per cent).

Reference solution (a). Dilute 5 ml of test solution (b) to 50 ml with ethanol (95 per cent).

Reference solution (b). A 0.2 per cent w/v solution of cyclophosphamide RS in ethanol (95 per cent).

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air and heat at 110° for 10 minutes. Place the plate while hot in a tank in which is placed a dish containing equal volumes of a 5 per cent w/v solution of potassium permanganate and hydrochloric acid, close the tank and allow to stand for 2 minutes. Remove the plate and place it in a current of cold air until excess chlorine is removed and an area of coating below the line of application gives not more than a faint blue colour with potassium iodide and starch solution; do not expose long to cold air. Spray the plate with potassium iodide and starch solution and allow to stand for 5 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). Ignore any spot remaining on the line of application.

Heavy metals (2.3.13). 1.0 g dissolved in 2 ml of dilute acetic acid and diluted to 25 ml with water complies with the limit test for heavy metals, Method A (20 ppm).

Chlorides (2.3.12). A freshly prepared solution of 0.75 g in sufficient water to produce 25 ml complies with the limit test for chlorides (330 ppm).

Water (2.3.43). 5.8 to 7.0 per cent, determined on 0.3 g.

Assay. Weigh accurately about 0.1 g and dissolve in 50 ml of a 0.1 per cent w/v solution of sodium hydroxide in ethylene glycol; boil under a reflux condenser for 30 minutes and allow to cool. Rinse the condenser with 25 ml of water, add 75 ml of 2-propanol, 15 ml of 2 M nitric acid, 10.0 ml of 0.1 M silver nitrate and 2 ml of ferric ammonium sulphate solution and titrate with 0.1 M ammonium thiocyanate.

1 ml of 0.1 M silver nitrate is equivalent to 0.01305 g of C7H15Cl2N2O2P.

Cyclophosphamide Phosphate intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.2 Endotoxin Unit per mg of cyclophosphamide.

Cyclophosphamide Phosphate intended for use in the manufacture of parenteral preparations without a further appropriate sterilization procedure complies with the following additional requirement.

Sterility. Complies with the test for sterility (2.2.11).

Storage. Store in a refrigerator (2° to 8°). Avoid long exposure to temperatures above 30°.

Labelling. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

Cyclophosphamide Injection

Cyclophosphamide for Injection is a sterile material consisting of 100 parts by weight of Cyclophosphamide and 45 parts by weight of Sodium Chloride. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cyclophosphamide Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of cyclophosphamide, C7H15Cl2N2O2P.

Description. A white or almost white powder.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. Shake a quantity containing 0.2 g of anhydrous cyclophosphamide with 2 ml of chloroform and filter. The solution complies with the following test.

B. Extract a quantity containing 0.2 g of anhydrous cyclophosphamide with ether and evaporate the extract to
dryness. Reserve a portion of the residue for identification test C. Dissolve 0.1 g in 10 ml of water and add 5 ml of silver nitrate solution; no precipitate is produced. Boil; a white precipitate is produced which is insoluble in dilute nitric acid but is soluble in dilute ammonia solution from which it can be reprecipitated by the addition of dilute nitric acid.

C. Dissolve 0.1 g of the residue from test B in 3 ml of nitric acid and 1 ml of sulphuric acid, heat till brown fumes are evolved and the solution becomes colourless. Cool, add 10 ml of water, heat again up to 60° and add 10 ml of ammonium molybdate solution; a bright yellow precipitate is slowly formed.

Tests

pH (2.4.24). 4.0 to 6.0, determined in a 2.0 per cent w/v solution immediately after preparation.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 80 volumes of 2-butanone, 12 volumes of water, 4 volumes of acetone and 2 volumes of anhydrous formic acid.

Test solution. Dissolve a quantity of the contents of the sealed container containing 0.2 g of anhydrous cyclophosphamide in sufficient ethanol (95 per cent) to produce 10 ml and filter.

Reference solution. Dilute 1 volume of solution (1) to 100 volumes with ethanol (95 per cent).

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air and heat at 110° for 10 minutes. Place the plate while hot in a tank in which is placed a dish containing equal volumes of a 5 per cent w/v solution of potassium permanganate and hydrochloric acid, close the tank and allow to stand for 2 minutes. Remove the plate and place it in a current of cold air until excess chlorine is removed and an area of coating below the line of application gives not more than a faint blue colour with potassium iodide and starch solution; do not expose long to cold air. Spray the plate with potassium iodide and starch solution and allow to stand for 5 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution. Ignore any spot remaining on the line of application.

Bacterial endotoxins (2.2.3). Not more than 0.2 Endotoxin Unit per mg of cyclophosphamide.

Assay. Determine the weight of the contents of 10 containers. Shake vigorously a quantity of the mixed contents of the 10 containers containing about 0.1 g of anhydrous cyclophosphamide in 30 ml of chloroform for 15 minutes, filter and wash the filter with 15 ml of chloroform. Evaporate the combined filtrate and washings to dryness and dissolve the residue in 50 ml of a 0.1 per cent w/v solution of sodium hydroxide in 1,2-ethanediol. Boil the solution under a reflux condenser for 30 minutes, allow to cool and rinse the condenser with 25 ml of water. Add 75 ml of 2-propanol, 15 ml of 2 M nitric acid, 10 ml of 0.1 M silver nitrate and 2 ml of ferric ammonium sulphate solution and titrate with 0.1 M ammonium thiocyanate. 1 ml of 0.1 M silver nitrate is equivalent to 0.01305 g of C7H15Cl2N2O2P.

Storage. Store in a refrigerator (2° to 8°). Avoid long exposure to temperatures above 30°. The solution should be used immediately after preparation as it deteriorates on storage.

Labelling. The label states (1) the quantity of Cyclophosphamide in terms of the equivalent amount of anhydrous cyclophosphamide; (2) the volume of Water for Injections to be added; (3) that the solution should be used immediately after preparation.

Cyclophosphamide Tablets

Cyclophosphamide Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of anhydrous cyclophosphamide, C7H15Cl2N2O2P. The tablets are coated.

Identification

A. Shake a quantity of the powdered tablets containing 0.2 g of anhydrous cyclophosphamide with 2 ml of chloroform and filter. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cyclophosphamide RS or with the reference spectrum of cyclophosphamide.

B. Extract a quantity of the powdered tablets containing 0.25 g of anhydrous cyclophosphamide with ether and evaporate the extract to dryness. Preserve a portion of the residue for identification test C. Dissolve 0.1 g in 10 ml of water and add 5 ml of silver nitrate solution; no precipitate is produced. Boil; a white precipitate is produced which is insoluble in dilute nitric acid but is soluble in dilute ammonia solution from which it can be reprecipitated by the addition of dilute nitric acid.

C. Dissolve 0.1 g of the residue from test B in 3 ml of nitric acid and 1 ml of sulphuric acid, heat till brown fumes are evolved and the solution becomes colourless. Cool, add 10 ml of water, heat again up to 60° and add 10 ml of ammonium molybdate solution; a bright yellow precipitate is slowly formed.
Tests

Acidity. Shake a quantity of the powdered tablets containing 0.25 g of anhydrous cyclophosphamide with 20 ml of carbon dioxide-free water; filter and titrate the filtrate with 0.1 M sodium hydroxide using phenolphthalein solution as indicator; not more than 0.2 ml of 0.1 M sodium hydroxide is required to change the colour of the solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 80 volumes of 2-butanone, 12 volumes of water, 4 volumes of acetone and 2 volumes of anhydrous formic acid.

Test solution. Shake vigorously a quantity of the powdered tablets containing 0.2 g of anhydrous cyclophosphamide with 50 ml of chloroform for 15 minutes, filter, evaporate the filtrate to dryness and dissolve the residue in 10 ml of ethanol (95 per cent).

Reference solution. Dilute 1 volume of solution (1) to 100 volumes with ethanol (95 per cent).

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air and heat at 110° for 10 minutes. Place the plate while hot in a tank in which is placed a dish containing equal volumes of a 5 per cent w/v solution of potassium permanganate and hydrochloric acid, close the tank and allow to stand for 2 minutes. Remove the plate and place it in a current of cold air until excess chlorine is removed and an area of coating below the line of application gives not more than a faint blue colour with potassium iodide and starch solution; do not expose long to cold air. Spray the plate with potassium iodide and starch solution and allow to stand for 5 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution. Ignore any spot remaining on the line of application.

Disintegration (2.5.1). Not more than 30 minutes.

Uniformity of content (for tablets containing 10 mg or less). Comply with the test stated under Tablets.

Place one tablet in a 10-ml volumetric flask, add about 7 ml of water, shake until the tablet is completely disintegrated, dilute with water to volume and filter. Wash the filter quantitatively with 10 ml of water and combine the filtrate and washings (test solution). In another volumetric flask dissolve an accurately weighed quantity of cyclophosphamide RS in water to obtain a solution of known concentration of about 500 µg per ml (reference solution). Place in separate test-tubes (170 mm x 25 mm) 2.0 ml of the test solution, 2.0 ml of the reference solution and 2.0 ml of water as the blank. Treat each tube in the following manner. Add 0.7 ml of a 2.35 per cent v/v solution of perchloric acid in water, mix and heat on a water-bath for 10 minutes. Cool, add 1 ml of 0.1 M sodium acetate and mix. Add 1.6 ml of a 0.75 per cent w/v solution of 4-(4-nitrobenzyl)pyridine in 1,2-ethanediol, mix and heat on a water-bath for 10 minutes. Cool, add 8.0 ml of a 2 per cent w/v solution of sodium hydroxide in ethanol (95 per cent). Measure the absorbances of the solutions against the blank within 4 minutes at the maximum at about 560 nm (2.4.7).

Calculate the content of C7H15Cl2N2O2P in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. To an accurately weighed quantity of the powder containing about 0.1 g of anhydrous cyclophosphamide add 30 ml of chloroform, shake vigorously for 15 minutes, filter and wash the filter with 15 ml of chloroform. Evaporate the combined filtrate and washings to dryness and dissolve the residue in 50 ml of a 0.1 per cent w/v solution of sodium hydroxide in 1,2-ethanediol. Boil the solution under a reflux condenser for 30 minutes, allow to cool and rinse the condenser with 25 ml of water. Add 75 ml of 2-propanol, 15 ml of 2 M nitric acid, 10 ml of 0.1 M silver nitrate and 2 ml of ferric ammonium sulphate solution and titrate with 0.1 M ammonium thiocyanate.

1 ml of 0.1 M silver nitrate is equivalent to 0.01305 g of C7H15Cl2N2O2P.

Storage. Store at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of anhydrous cyclophosphamide.

Cyclopropane

\[
\begin{align*}
\text{C}_3\text{H}_6 & \\
\text{H}_2 & \\
\text{C} & \\
\text{H}_2\text{C} & \text{CH}_2
\end{align*}
\]

C3H6

Mol. Wt. 42.1

Cyclopropane contains not less than 99.0 per cent v/v of C3H6.

Description. A colourless gas at atmospheric temperature and pressure; odour, characteristic; flammable.

NOTE — Mixtures of cyclopropane with oxygen or air at certain concentrations are explosive.

Tests

Acidity or alkalinity. Dilute 0.3 ml of methyl red solution with 400 ml of boiling water and boil the solution for 5 minutes. Cool to about 80° and pour 100 ml of solution into each of three matched Nessler cylinders marked A, B and C. To cylinder B add 0.2 ml of 0.01 M hydrochloric acid and to cylinder C
add 0.4 ml of 0.01 M hydrochloric acid. Stopper both the cylinders and cool to room temperature. Pass a volume of the gas equivalent to 2000 ml, measured at normal temperature and pressure, through the solution in cylinder B, the time occupied being about 30 minutes. The colour of the solution in cylinder B is not deeper red than that of the solution in cylinder C and not deeper yellow than that of the solution in cylinder A.

**Carbon dioxide.** Pass a volume of the gas equivalent to 1000 ml at normal temperature and pressure at a rate not exceeding 4000 ml per hour through 100 ml of a 3 per cent w/v solution of barium hydroxide contained in a vessel such that the depth of the solution is between 12 and 14 cm, using a delivery tube having a bore of about 1 mm and extending to within 2 mm of the bottom of the vessel. The turbidity produced is not more intense than that produced by adding 1 ml of a solution of 0.1 g of sodium bicarbonate in 100 ml of carbon dioxide-free water to 100 ml of a 3 per cent w/v solution of barium hydroxide.

**Ethanol and Water.** Pass a volume of the gas equivalent to 1000 ml, measured at normal temperature and pressure, through a weighed tube containing potassium hydroxide in small pieces, the time occupied being 40 to 60 minutes. The increase in weight of the tube is not more than 0.0056 g (equivalent to 0.3 per cent w/w of the Cyclopropane used).

**Unsaturated substances.** Pass the gas coming out of the tube of potassium hydroxide in the test for Ethanol and water through a gas washing trap provided with a sintered-glass bubbler containing 20.0 ml of iodine monochloride solution and connected in series with two gas washing bottles containing, respectively, 5.0 ml of iodine monochloride solution and 10 ml of potassium iodide solution. Mix the contents of the trap and washing bottles and titrate with 0.1 M sodium thiosulphate. Add 10 ml of potassium iodide solution to 25.0 ml of iodine monochloride solution and titrate with 0.1 M sodium thiosulphate. The difference between the titrations is not more than 1.8 ml (equivalent to 0.2 per cent w/w of unsaturated substances, calculated as propylene).

**Halogen-containing substances.** Pass a volume of the gas equivalent to 1000 ml, measured at normal temperature and pressure, with the necessary amount of air into a small mixing chamber and pass the resulting mixture through a heated quartz tube containing pieces of platiniised quartz or through a heated silica tube containing sintered silica plates or pieces of platiniised quartz, the time occupied being not less than 40 minutes. Absorb the products of combustion in 50 ml of a 3 per cent w/v solution of sodium peroxide. Boil the solution for about 10 minutes, cool, neutralise with a solution of nitric acid (containing about 30 per cent w/w of HNO₃) and add 5 ml of 2 M nitric acid (test solution). To 50 ml of the same solution of sodium peroxide which has been boiled, cooled, neutralised and acidified in the same manner, add 7.5 ml of 0.001 M potassium bromide (standard solution). Transfer the solutions to 100-ml matched Nessler cylinders, add 1.0 ml of 0.1 M silver nitrate to each, dilute to 100 ml with water, mix well and allow to stand in the dark for 15 minutes. Compare the turbidities of the two solutions by viewing them both transversely and vertically against a black background. The turbidity of the test solution is not more intense than that of the standard solution.

**Foreign odour.** Transfer 10 ml of the material liquefied under pressure to a cylinder cooled to a temperature not exceeding 40°, pour in successive small quantities onto a clean filter paper and allow it to evaporate spontaneously. No foreign odour is detectable at any stage of the evaporation.

**Assay.** In a suitable nitrometer containing mercury, place a volume of the material liquefied under pressure equivalent to 80 to 100 ml of the gas, measured at normal temperature and pressure, add 25 ml of sulphuric acid and allow to stand for 15 minutes. Not less than 99.0 per cent of its volume is absorbed.

**Storage.** Store under pressure in metal cylinders in a cool place.

**Labelling.** The metal cylinder is painted orange and on the shoulder is stencilled the name of the gas or the symbol C₃H₆.

**Cycloserine**

![Cycloserine structure](image)

C₃H₆N₂O₂

Mol. Wt. 102.1

Cycloserine is (R)-4-aminoisoxazolidin-3-one, an antimicrobial substance produced by the growth of certain strains of Streptomyces orchidaceus or S. garyphalus or obtained by synthesis.

Cycloserine contains not less than 98.0 per cent and not more than 100.5 per cent of C₃H₆N₂O₂, calculated on the dried basis.

**Description.** A white or pale yellow, crystalline powder; hygroscopic.

**Identification**

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. To 1 ml of a 0.01 per cent w/v solution in 0.1 M sodium hydroxide add 3 ml of 1 M acetic acid and 1 ml of a freshly
prepared mixture of equal volumes of a 4 per cent w/v solution of sodium nitroprusside and 5 M sodium hydroxide; a blue colour is produced slowly.

**Tests**

**pH** (2.4.24). 5.5 to 6.5, determined in a 10.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +108° to +114°, determined in a 5.0 per cent w/v solution in 2 M sodium hydroxide.

**Heavy metals** (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

**Condensation products.** Absorbance of a 0.04 per cent w/v solution in 0.1 M sodium hydroxide at about 285 nm, not more than 0.32 (2.4.7).

**Sulphated ash** (2.3.18). Not more than 0.5 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

**Assay.** Determine by liquid chromatography (2.4.14)

**Test solution.** Dissolve about 10 mg of the substance under examination in 20.0 ml of the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

**Reference solution.** Dissolve 10 mg of the cycloserine RS in 20.0 ml of the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilyl silica gel (5 µm) (such as Wakosil C8 RS),
- mobile phase: 0.1 per cent w/v of methane sulphonylic acid and 0.78 per cent w/v of sodium dihydrogen orthophosphate in water, the pH adjusted to 6.0 with dilute sodium hydroxide and filtered,
- flow rate. 1 ml per minute,
- spectrophotometer set at 227 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternatively the test solution and the reference solution.

Calculate the content of C₃H₆N₂O₂.

**Storage.** Store protected from moisture

**Cycloserine Capsules**

Cycloserine Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cycloserine, C₃H₆N₂O₂.

**Identification**

A. Shake a quantity of the contents of the capsules containing 10 mg of Cycloserine with 100 ml of 1 M sodium hydroxide and filter. To 1 ml of the filtrate add 3 ml of 1 M acetic acid and 1 ml of a freshly prepared mixture of equal volumes of a 4 per cent w/v solution of sodium nitroprusside and 5 M sodium hydroxide; a blue colour is produced slowly.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

**Tests**

**Dissolution** (2.5.2).

Apparatus. No 2

Medium. 900 ml of phosphate buffer pH 6.8.

Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

**Test solution.** The filtrate diluted to produce a 0.028 per cent w/v solution.

**Reference solution.** A 0.028 per cent w/v solution of cycloserine RS in the dissolution medium.

Use the chromatographic system described under Assay.

Calculate the content of C₃H₆N₂O₂.

D. Not less than 80 per cent of the stated amount of C₃H₆N₂O₂.

**Condensation products.** Weigh the contents of the capsules containing about 0.5 g of Cycloserine, dissolve in 250 ml of 0.1 M sodium hydroxide solution, disperse with the aid of ultrasound for 5 minutes. Dilute 5 ml of this solution to 25 ml with 0.1 M sodium hydroxide. Absorbance of the resulting solution at about 285 nm, not more than 0.32 (2.4.7).

**Loss on drying** (2.4.19). Not more than 2.0 per cent, determined on 1.0 g of the contents of the capsules, by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

**Other tests.** Comply with the tests stated under Capsules.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Mix the contents of 20 capsules. Weigh accurately a quantity of the mixed contents of the capsules containing about 250.0 mg of Cycloserine dissolve in phosphate buffer pH 6.8, dilute to 250.0 ml with the same solvent and filter. Dilute 5.0 ml of the filtrate to 25.0 ml with phosphate buffer pH 6.8.

**Reference solution.** A 0.02 per cent w/v solution of cycloserine RS in phosphate buffer pH 6.8.
Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilyl silica gel (5 µm) (such as Warkosil C8 RS),
- mobile phase: dissolve 1.0 g of methane sulphonic acid and 7.8 g of sodium dihydrogen orthophosphate dihydrate in 1000 ml of water and adjust pH to 6.0 with dilute sodium hydroxide, filter,
- flow rate. 1 ml per minute,
- spectrophotometer set at 227 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent. Inject alternately the test solution and the reference solution. Calculate the content of C₃H₆N₂O₂ in the capsules.

Storage. Store protected from moisture.

Cycloserine Tablets

Cycloserine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of cycloserine, C₃H₆N₂O₂.

Identification
A. Shake a quantity of the powdered tablets containing 0.5 g of Cycloserine with 25 ml of 1 M sodium hydroxide for 5 minutes and filter. The optical rotation of the filtrate is about +2.2° (2.4.22).

B. To 0.2 ml of the filtrate obtained in test A add 3 ml of 1 M acetic acid and 1 ml of a freshly prepared mixture of equal volumes of a 4 per cent w/v solution of sodium nitroprusside and 5 M sodium hydroxide; a blue colour is produced slowly.

Tests
Light absorption. Shake a quantity of the powdered tablets containing 0.25 g of Cycloserine with 80 ml of 0.1 M sodium hydroxide for 10 minutes, add sufficient 0.1 M sodium hydroxide to produce 100.0 ml, mix and filter. Dilute a suitable volume of the filtrate with sufficient 0.1 M hydrochloric acid to produce a solution containing 0.0025 per cent w/v of Cycloserine. Absorbance of the resulting solution, measured within 15 minutes of preparing the final solution, at the maximum at about 219 nm, 0.78 to 0.96 (2.4.7).

Disintegration (2.5.1). Not more than 30 minutes.

Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 1.0 g of the powdered tablets, by drying in an oven at about 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Cycloserine, shake with 150 ml of water for 30 minutes, add sufficient water to produce 200.0 ml and filter. To 10.0 ml of the filtrate add 10 ml of water and 25 ml of 0.2 M sodium hydroxide, dilute to 50.0 ml with water and mix. To 4.0 ml of the mixture add 10 ml of 1 M acetic acid and 4 ml of sodium nitroprusside solution, dilute to 20 ml with 1 M acetic acid, mix and allow to stand for 15 minutes. Measure the absorbance of the resulting solution at the maximum at about 625 nm (2.4.7), using as the blank a solution prepared by treating 4.0 ml of 0.1 M sodium hydroxide in the same manner beginning at the words “add 10 ml of 1 M acetic acid...” Calculate the content of C₁₃H₂₁N₂O₂ from the absorbance obtained by repeating the operation using cycloserine RS in place of the powdered tablets.

Storage. Store at a temperature not exceeding 30°.

Cyproheptadine Hydrochloride

\[
\text{Cyproheptadine Hydrochloride} \quad \text{C}_{21}\text{H}_{21}\text{N\cdot HCl,1½H}_{2}\text{O} \quad \text{Mol. Wt. 332.9}
\]

Cyproheptadine Hydrochloride is 4-(5H-dibenzo[a,d]-cyclohepten-5-ylidene)-1-methylpiperidine hydrochloride.

Cyproheptadine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of C₂₁H₂₁N, calculated on the dried basis.

Description. A white or slightly yellow, crystalline powder.

Identification
Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Dissolve 0.1 g in 10 ml of water, make alkaline with 1 M sodium hydroxide, extract with 5 ml of dichloromethane, dry over anhydrous sodium sulphate and remove the solvent with the aid of a current of nitrogen. The oily residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cyproheptadine hydrochloride RS treated in the same manner or with the reference spectrum of cyproheptadine.
B. When examined in the range 230 nm to 360 nm, a 0.002 per cent w/v solution in ethanol (95 per cent) shows an absorption maximum only at about 286 nm; absorbance at about 286 nm, about 0.67 (2.4.7).

C. Determine by thin-layer chromatography (2.4.17) coating the plate with silica gel GF254.

Mobile phase. A mixture of 75 volumes of cyclohexane, 20 volumes of ether and 5 volumes of diethylamine.

Test solution. Dissolve 0.1 g of the substance under examination in 100 ml of methanol.

Reference solution (a). A 0.1 per cent w/v solution of cyproheptadine hydrochloride RS in methanol.

Reference solution (b). A solution containing 0.05 per cent w/v of each of imipramine hydrochloride RS and cyproheptadine hydrochloride RS in methanol.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any spot corresponding to the substance in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with the test solution shows two clearly separated principal spots.

D. A saturated solution gives reaction A of chlorides (2.3.1).

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17) coating the plate with silica gel GF254.

Mobile phase. A mixture of 90 volumes of dichloromethane and 10 volumes of methanol.

Solvent mixture. A mixture of 9 volumes of dichloromethane and 1 volumes of methanol.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml with solvent mixture.

Reference solution (a). A 0.001 per cent w/v solution of the substance under examination in the same solvent mixture.

Reference solution (b). A solution containing 0.002 per cent w/v of dibenzocycloheptatriene RS in the same solvent mixture.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any spot corresponding to dibenzocycloheptatriene in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b) and any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

**Loss on drying (2.4.19).** 7.0 to 9.0 per cent, determined on 1.0 g by drying in an oven at 100° at a pressure not exceeding 0.7 kPa.

**Assay.** Weigh accurately about 0.5 g, dissolve in 0.5 ml of acetic anhydride and 20 ml of anhydrous glacial acetic acid and add 10 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid, using crystal violet solution as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.03239 g of C21H21N,HCl.

**Storage.** Store protected from light.

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**Cyproheptadine Syrup**

Cyproheptadine Hydrochloride Syrup

Cyproheptadine Syrup contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cyproheptadine hydrochloride, C21H21N,HCl.

**Identification**

To 5 ml add 5 ml of a 1 per cent w/v solution of sodium bicarbonate and extract with three quantities, each of 15 ml, of 2,2,4-trimethylpentane. Wash the combined 2,2,4-trimethylpentane extracts with 5 ml of the sodium bicarbonate solution and discard the washings. Evaporate the 2,2,4-trimethylpentane solution to dryness on a water-bath and dissolve the residue in 100 ml of ethanol (95 per cent). When examined in the range 230 nm to 360 nm, the resulting solution shows an absorption maximum only at about 286 nm (2.4.7).

**Tests**

**pH (2.4.24).** 3.5 to 4.5.

**Other tests.** Comply with the tests stated under Oral Liquids.

**Assay.** To an accurately measured volume of the syrup containing about 2 mg of Cyproheptadine Hydrochloride add 20 ml of a 1 per cent w/v solution of sodium bicarbonate and extract with two quantities, each of 25 ml, of 2,2,4-trimethylpentane. Wash the combined 2,2,4-trimethylpentane extracts with 5 ml of the sodium bicarbonate solution and discard the washings. Extract the 2,2,4-trimethylpentane solution with 50 ml of 0.05 M sulphuric acid and collect the aqueous extract in a 100-ml volumetric flask. Dilute to volume with 0.05 M sulphuric acid and mix. Filter a portion of the solution through a dry filter paper and discard the first 20 ml of the filtrate. Measure the absorbance of the filtrate at the maximum at about 286 nm (2.4.7), using 0.05 M sulphuric acid.
as the blank. Calculate the content of C$_{21}$H$_{21}$N,HCl taking 355 as the specific absorbance at 286 nm.

**Cyproheptadine Tablets**

Cyproheptadine Hydrochloride Tablets

Cyproheptadine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cyproheptadine hydrochloride, C$_{21}$H$_{21}$N,HCl.

**Identification**

A. To a quantity of the powdered tablets containing 20 mg of Cyproheptadine Hydrochloride add 10 ml of water and 2.5 ml of 0.1 M sodium hydroxide, extract with 10 ml of dichloromethane, filter through anhydrous sodium sulphate placed over absorbent cotton moistened with dichloromethane and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cyproheptadine hydrochloride RS treated in the same manner or with the reference spectrum of cyproheptadine.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Extract a quantity of the powdered tablets containing 20 mg of Cyproheptadine Hydrochloride with 7 ml of water, filter, add 0.3 ml of 5 M ammonia to the filtrate and filter again. The filtrate gives reaction A of chlorides (2.3.1).

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), using a precoated silica gel plate.

**Mobile phase.** A mixture of 90 volumes of dichloromethane and 10 volumes of methanol.

**Test solution (a).** Shake mechanically for 10 minutes a quantity of the powdered tablets containing 50 mg of Cyproheptadine Hydrochloride with 5 ml of the mobile phase and filter (such as Whatman GF/C filter paper).

**Test solution (b).** Dilute 1 volume of test solution (a) to 10 volumes with the mobile phase.

**Reference solution (a).** Dilute 1 volume of test solution (a) serially in two steps to 1000 volumes with the mobile phase.

**Reference solution (b).** A solution containing 0.1 per cent w/v of cyproheptadine hydrochloride RS in the mobile phase.

**Reference solution (c).** A solution containing 0.002 per cent w/v of dibenzocycloheptatriene RS in the mobile phase.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with ethanolic sulphuric acid (20 per cent). Heat at 110° for 30 minutes and examine in ultraviolet light at 365 nm. In the chromatogram obtained with test solution (a) any spot corresponding to dibenzocycloheptatriene is not more intense than the spot in the chromatogram obtained with reference solution (c) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Uniformity of content.** Comply with the test stated under Tablets.

Powder one tablet, warm with 20 ml of ethanol (95 per cent) and centrifuge. Repeat the extraction with three further quantities, each of 20 ml, of ethanol (95 per cent). Cool the combined extracts and add sufficient ethanol (95 per cent) to produce 200.0 ml. Measure the absorbance of the resulting solution at the maximum at about 286 nm (2.4.7). Calculate the content of C$_{21}$H$_{21}$N,HCl taking 355 as the specific absorbance at 286 nm.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 1.5 mg of Cyproheptadine Hydrochloride, add sufficient ethanol (95 per cent) to produce 100.0 ml, mix well and filter. Measure the absorbance of the filtrate at the maximum at about 286 nm (2.4.7). Calculate the content of C$_{21}$H$_{21}$N,HCl taking 355 as the specific absorbance at 286 nm.

**Cytarabine**

β-Cytosine Arabinoside

![Cytarabine](image)

C$_{9}$H$_{13}$N$_{3}$O$_{5}$  Mol. Wt. 243.2

Cytarabine is 1-β-D-arabinofuranosylcytosine.

Cytarabine contains not less than 99.0 per cent and not more than 100.5 per cent of C$_{9}$H$_{13}$N$_{3}$O$_{5}$, calculated on the dried basis.

**Description.** A white or almost white, crystalline powder.
CAUTION — Cytarabine is very poisonous. Great care should be taken to avoid inhaling the particles of cytarabine and exposing the skin to the dried substance.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cytarabine RS or with the reference spectrum of cytarabine.

B. When examined in the range 230 nm to 360 nm, a 0.001 per cent w/v solution in 0.1 M hydrochloric acid shows an absorption maximum only at about 280 nm; absorbance at about 280 nm, about 0.55 (2.4.7).

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

Tests

Specific optical rotation (2.4.22). +154° to +160°, determined in a 1.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 65 volumes of 2-butanone, 20 volumes of acetone and 15 volumes of water.

Test solution (a). A 5 per cent w/v solution of the substance under examination in water.

Test solution (b). A 0.02 per cent w/v solution of the substance under examination in water.

Reference solution (a). A 0.0025 per cent w/v solution of the substance under examination in water.

Reference solution (b). A solution containing 0.02 per cent w/v solution of cytarabine RS in water.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven over phosphorus pentoxide at 60° for 3 hours at a pressure of 0.2 kPa to 0.7 kPa.

Assay. Weigh accurately about 0.5 g and dissolve in 40 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, using 1-naphtholbenzein solution as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02432 g of C9H13N3O5.

Cytarabine intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.07 Endotoxin Unit per mg.

Cytarabine intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility. Complies with the test for sterility (2.2.11).

Storage. Store protected from light. If it is intended for use in the manufacture of parenteral preparations, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

Labeling. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

Cytarabine Injection

β-Cytosine Arabinoside Injection

Cytarabine for Injection is a sterile material consisting of Cytarabine with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cytarabine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of cytarabine, C9H13N3O5.

Description. A white or almost white powder.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

Mix 0.1 g of the substance under examination with 10 ml of hot ethanol (95 per cent), filter, allow the filtrate to cool and induce crystallisation if necessary. Filter, wash the crystals with 2 ml of ethanol (95 per cent) and dry at 60° at a pressure of 0.7 kPa. The residue complies with the following test.
Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with cytarabine RS or with the reference spectrum of cytarabine.

**Tests**

**pH** (2.4.24). 4.0 to 6.0, determined in a 2.0 per cent w/v solution in the solvent stated on the label.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

*Mobile phase.* A mixture of 65 volumes of 2-butanone, 20 volumes of acetone and 15 volumes of water.

*Test solution.* A 4 per cent w/v solution of the substance under examination in water.

*Reference solution (a).* A 0.02 per cent w/v solution of the substance under examination water.

*Reference solution (b).* A solution containing 0.04 per cent w/v solution of uridine water.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution with an Rf value of about 1.1 relative to the spot in the chromatogram obtained with reference solution (b) is not more intense than the spot in the chromatogram obtained with reference solution (b). Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Water** (2.3.43). Not more than 3.0 per cent, determined on 0.8 g.

**Bacterial endotoxins** (2.2.3). Not more than 0.07 Endotoxin unit per mg.

**Assay.** Determine the weight of the contents of 10 containers. Weigh accurately about 0.5 g of the mixed contents of the 10 containers and dissolve by heating, if necessary, in 80 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, using 1-naphtholbenzein solution as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02432 g of C₉H₁₃N₃O₅.

**Storage.** Store protected from light.
D

Danazol
Danazol Capsules
Dapsone
Dapsone Tablets
Dehydroacetic Acid
Dehydroemetine Hydrochloride
Dehydroemetine Injection
Dequalinium Chloride
Desferrioxamine Mesylate
Desferrioxamine Injection
Deslanoside
Deslanoside Injection
Desoxycortone Acetate
Desoxycortone Acetate Injection
Dexamethasone
Dexamethasone Tablets
Dexamethasone Sodium Phosphate
Dexamethasone Injection
Dextran 40 Injection
Dextran 70 Injection
Dextran 110 Injection
Dextrin
Dextromethorphan Hydrobromide
Dextromethorphan Hydrobromide Syrup
Dextrose
Dextrose Injection
Diazepam
Diazepam Capsules
Diazepam Injection
Diazepam Tablets
Dibutyl Phthalate
Diclofenac Sodium
Diclofenac Injection
Diclofenac Tablets
Dicyclomine Hydrochloride
Dicyclomine Injection
Dicyclomine Oral Solution
Dicyclomine Tablets
Didanosine
Didanosine Capsules
Didanosine Tablets
Dienoestrol
Dienoestrol Tablets
Diethylcarbamazine Citrate
Diethylcarbamazine Tablets
Diethyl Phenyl Acetamide
Diethyl Phthalate
Diethyltoluamide
Digitoxin
Digitoxin Tablets
Digoxin
Digoxin Injection
Digoxin Paediatric Solution
Digoxin Tablets
Diiodohydroxyquinoline
Diiodohydroxyquinoline Tablets
Diloxanide Furoate
Diloxanide Tablets
Diltiazem Hydrochloride
Diltiazem Tablets
Dimercaprol
Dimercaprol Injection
Activated Dimethicone
Diphenhydramine Hydrochloride
Diphenhydramine Capsules
Diphenoxylate Hydrochloride
Disodium Edetate
Disodium Edetate Injection
Disulfiram
Disulfiram Tablets
Dithranol
Dithranol Ointment
Docusate Sodium
Domperidone Maleate
Domperidone Tablets
Donepezil Hydrochloride
Donepezil Tablets
Dothiepin Hydrochloride
Dothiepin Capsules
Doxepin Hydrochloride
Doxepin Capsules
Doxorubicin Hydrochloride
Doxorubicin Injection
Doxycycline Hydrochloride
Doxycycline Capsules
Dydrogesterone
Dydrogesterone Tablets
Danazol

C$_{22}$H$_{27}$NO$_2$  Mol. Wt. 337.5
Danazol is 17α-pregna-2,4-diene-20-yno[2,3-d]isoxazol-17-ol.
Danazol contains not less than 97.0 per cent and not more than 102.0 per cent of C$_{22}$H$_{27}$NO$_2$, calculated on the dried basis.

Description. A white to pale yellow, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with danazol RS or with the reference spectrum of danazol.
B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum only at about 285 nm.
C. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Solvent mixture. A mixture of 90 volumes of chloroform and 10 volumes of methanol.

Mobile phase. A mixture of 70 volumes of cyclohexane and 30 volumes of ethyl acetate.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

Reference solution (a). Dissolve 25 mg of danazol RS in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the liquid to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 1 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with ethanolic sulphuric acid (20 per cent v/v). Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

Tests

Specific optical rotation (2.4.22). +21.0° to +27.0°, determined in a 1.0 per cent w/v solution in chloroform.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 70 volumes of cyclohexane and 30 volumes of ethyl acetate.

Test solution. Dissolve 0.5 g in a mixture of 9 volumes of chloroform and 1 volume of methanol.

Reference solution (a). Dissolve 50 mg of danazol RS in 100 ml of the same solvent mixture.

Reference solution (b). Dilute 10 ml of reference solution (a) to 20 ml with the same solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in warm air and examine in ultraviolet light at 254 nm. Expose the plate to the vapour of iodine for 5 minutes and examine the plate again. By both methods of visualisation, any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 2.7 kPa.

Assay. Weigh accurately about 0.1 g, previously dried, dissolve in 50 ml of ethanol (95 per cent), swirling until dissolved, and dilute to 100.0 ml with ethanol (95 per cent). Dilute 2.0 ml of this solution to 100.0 ml with ethanol (95 per cent). Measure the absorbance of the resulting solution at the maximum at about 285 nm (2.4.7). Calculate the content of C$_{22}$H$_{27}$NO$_2$ from the absorbance obtained by repeating the procedure using a solution containing 0.002 per cent w/v of danazol RS in place of the substance under examination.

Storage. Store protected from light.

Danazol Capsules

Danazol Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of danazol, C$_{22}$H$_{27}$NO$_2$.

Identification

A. Extract the contents of the capsules containing about 50 mg of Danazol with 50 ml of chloroform, filter and evaporate
the filtrate to dryness on a water-bath in a stream of nitrogen. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with danazol RS or with the reference spectrum of danazol.

Tests

Other tests. Comply with the tests stated under Capsules.

Assay. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 0.1 g of Danazol, dissolve in 50 ml of chloroform, shake well for 5 minutes, dilute to 100.0 ml with chloroform and filter. Dilute 2.0 ml of the filtrate to 100.0 ml with chloroform and measure the absorbance of the resulting solution at the maximum at about 285 nm (2.4.7).

Calculate the content of C22H27NO2 from the absorbance obtained by repeating the procedure using a solution containing 0.002 per cent w/v of danazol RS in place of the substance under examination.

Storage. Store protected from light.

Dapsone

C12H12N2O2S Mol. Wt. 248.3

Dapsone is the bis(4-aminophenyl)sulphone.

Dapsone contains not less than 99.0 per cent and not more than 101.0 per cent of C12H12N2O2S, calculated on the dried basis.

Description. A white or creamy-white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with dapsone RS or with the reference spectrum of dapsone.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0005 per cent w/v solution in methanol shows absorption maxima at about 260 nm and 295 nm; absorbance at about 260 nm, about 0.36 and at about 295 nm, about 0.6.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).

D. 2 ml of a 0.005 per cent w/v solution in 0.1 M hydrochloric acid gives the reaction of primary aromatic amines (2.3.1).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 20 volumes of n-heptane, 20 volumes of ethyl acetate, 6 volumes of methanol and 1 volume of strong ammonia solution.

Test solution (a). Dissolve 0.1 g of the substance under examination in 10 ml of methanol.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with methanol.

Reference solution (a). Dilute 1 ml of test solution (b) to 10 ml with methanol.

Reference solution (b). Dilute 1 ml of reference solution (a) to 50 ml with methanol.

Reference solution (c). A 0.1 per cent w/v solution of dapsone RS in methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with a 0.1 per cent w/v solution of 4-dimethylaminocinnamaldehyde in a mixture of 99 volumes of ethanol (95 per cent) and 1 volume of hydrochloric acid and examine in daylight. Any secondary spot in the chromatogram obtained with the test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.3 g and dissolve in a mixture of 20 ml of water and 20 ml of hydrochloric acid. Cool the solution to about 15° and determine by the nitrite titration (2.3.31). Carry out a blank titration. 1 ml of 0.1 M sodium nitrite is equivalent to 0.01242 g of C12H12N2O2S.

Storage. Store protected from light.

Dapsone Tablets

Dapsone Tablets contain not less than 93.0 per cent and not more than 107.0 per cent of the stated amount of dapsone, C12H12N2O2S.
Identification

A. Shake a quantity of the powdered tablets containing 0.1 g of Dapsone with 10 ml of acetone, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with dapsone RS or with the reference spectrum of dapsone.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 20 volumes of n-heptane, 20 volumes of ethyl acetate, 6 volumes of methanol and 1 volume of strong ammonia solution.

Test solution (a). Shake a quantity of the powdered tablets containing 0.1 g of Dapsone with 10 ml of methanol and filter.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with methanol.

Reference solution (a). Dilute 1 ml of test solution (b) to 10 ml with methanol.

Reference solution (b). Dilute 2 ml of reference solution (a) to 10 ml with methanol.

Reference solution (c). A 0.1 per cent w/v solution of dapsone RS in methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with a 0.1 per cent w/v solution of 4-dimethylaminocinnamaldehyde in a mixture of 99 volumes of ethanol (95 per cent) and 1 volume of hydrochloric acid and examine in daylight. Any secondary spot in the chromatogram obtained with the test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (b).

Dissolution (2.5.2).

Apparatus. No 1

Medium. 900 ml of a 2 per cent w/v solution of hydrochloric acid.

Speed and time. 100 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate. Transfer an accurately measured volume of the filtrate containing about 0.2 mg of Dapsone to a 25-ml volumetric flask, add 5 ml of 1 M sodium hydroxide, dilute to volume with water and mix. Measure the absorbance of the resulting solution at the maximum at about 290 nm (2.4.7). Calculate the content of C_{12}H_{12}N_{2}O_{2}S from the absorbance obtained from a solution prepared by adding 5 ml of 1 M sodium hydroxide to 20 ml of a 2 per cent v/v solution of hydrochloric acid containing 0.2 mg of dapsone RS and adding sufficient water to produce 25.0 ml.

D. Not less than 75 per cent of the stated amount of C_{12}H_{12}N_{2}O_{2}S.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.25 g of Dapsone and dissolve in a mixture of 15 ml of water and 15 ml of 2 M hydrochloric acid. Cool the solution to about 15° and determine by the nitrite titration (2.3.31). Carry out a blank titration.

1 ml of 0.1 M sodium nitrite is equivalent to 0.01242 g of C_{12}H_{12}N_{2}O_{2}S.

Storage. Store protected from light.

Dehydroacetic Acid

\[
\begin{align*}
\text{C}_{6}\text{H}_{5}\text{O}_4 & \quad \text{Mol. Wt. 168.1} \\
\text{Dehydroacetic Acid} & \text{is a tautomeric mixture of 3-acetyl-6-methyl-2H-pyran-2,4(3H)-dione and 3-acetyl-4-hydroxy-6-methyl-2H-pyran-2-one}.
\end{align*}
\]

Dehydroacetic Acid contains not less than 98.0 per cent and not more than 100.5 per cent of C_{6}H_{5}O_{4}, calculated on the anhydrous basis.

Description. A white or almost white, crystalline powder; odourless or practically odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with dehydroacetic acid RS.

B. Melts at 109° to 111° (2.4.21).
DEHYDROEMETINE HYDROCHLORIDE

IP 2007

Tests
Arsenic (2.3.10). Heat gently 3.3 g with 2 ml of nitric acid and
0.5 ml of sulphuric acid in a long-necked flask until the first
reaction has subsided, cool, add carefully and in small portions,
15 ml of nitric acid and 6 ml of sulphuric acid, taking care to
avoid excessive foaming. Continue heating, adding further
small portions of nitric acid, if necessary, until white fumes
are evolved and the solution becomes colourless or almost
colourless. Cool, add carefully 10 ml of water, evaporate until
white fumes are evolved. Repeat the addition of water and
evaporation until all the nitric acid has been removed, cool,
dilute to 50 ml with water and add 10 ml of stannated
hydrochloric acid AsT. The resulting solution complies with
the limit test for arsenic (3 ppm).
Heavy metals (2.3.13). 2.0 g complies with the limit test for
heavy metals, Method B (10 ppm).
Sulphated ash (2.3.18). Not more than 0.1 per cent, determined
on 2.0 g.

Description. A white to yellowish-white, crystalline powder;
odourless.

Identification
A. When examined in the range 230 nm to 360 nm (2.4.7), a
0.005 per cent w/v solution in 0.1 M hydrochloric acid shows
an absorption maximum only at about 282 nm; absorbance at
about 282 nm, about 0.62.
B. Sprinkle 5 mg on the surface of a 5 per cent w/v solution of
ammonium molybdate in sulphuric acid; a green colour
develops.
C. Gives reaction A of chlorides (2.3.1).

Tests
Appearance of solution. A 5.0 per cent w/v solution is clear
(2.4.1), and not more intensely coloured than reference solution
YS5 or BYS6 (2.4.1).
pH (2.4.24). 3.5 to 5.0, determined in a 3.0 per cent w/v solution.

Water (2.3.43). Not more than 1.0 per cent, determined on
2.0 g.

Heavy metals (2.3.13). 1.0 g complies with the limit test for
heavy metals, Method B (20 ppm).

Assay. Weigh accurately about 0.5 g, dissolve in 75 ml of
previously neutralised ethanol (95 per cent), add
phenolphthalein solution and titrate with 0.1 M sodium
hydroxide to a pink end-point that persists for not less than
30 seconds.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.01681 g of
C8H8O4.

Assay. Weigh accurately about 0.4 g, dissolve in 40 ml of
anhydrous glacial acetic acid and add 15 ml of mercuric
acetate solution. Titrate with 0.1 M perchloric acid, using
crystal violet solution as indicator. Carry out a blank titration.

Loss on drying (2.4.19). Not more than 7.0 per cent, determined
on 1.0 g by drying in an oven at 100° at a pressure not exceeding
0.7 kPa for 4 hours.

1 ml of 0.1 M perchloric acid is equivalent to 0.02758 g of
C29H38N2O4,2HCl.

Dehydroemetine Hydrochloride

Storage. Store protected from light.

H3CO
H3CO

N
H

Dehydroemetine Injection
CH3

Dehydroemetine Hydrochloride Injection

,2HCl
HN

Dehydroemetine Injection is a sterile solution of
Dehydroemetine Hydrochloride in Water for Injections.

OCH3

Dehydroemetine Injection contains not less than 95.0 per cent
and not more than 105.0 per cent of the stated amount of
dehydroemetine hydrochloride, C29H38N2O4,2HCl.

OCH3
C29H38N2O4,2HCl

Mol. Wt. 551.6

Description. A clear, almost colourless solution.

Dehydroemetine Hydrochloride is 2,3-didehydro-6′,7′,10, 11tetramethoxyemetan dihydrochloride

Identification

Dehydroemetine Hydrochloride contains not less than
98.5 per cent and not more than 101.5 per cent of
C29H38N2O4,2HCl, calculated on the dried basis.

A. To a volume containing 30 mg of Dehydroemetine
Hydrochloride add 1 ml of 0.05 M iodine; a yellowish-brown
precipitate is produced.

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B. To a volume containing 15 mg of Dehydroemetine Hydrochloride add 1 ml of potassium mercuri-iodide solution; a white precipitate is produced.

Tests

pH (2.4.24). 2.8 to 5.0.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. To an accurately measured volume containing 60 mg of Dehydroemetine Hydrochloride add sufficient 0.1 M hydrochloric acid to produce 100.0 ml. Dilute 5.0 ml to 100.0 ml with 0.1 M hydrochloric acid, mix and measure the absorbance of the resulting solution at the maximum at about 282 nm (2.4.7). Calculate the content of C_{29}H_{38}N_{2}O_{4},2HCl taking 123 as the specific absorbance at the maximum at about 282 nm.

Storage. Store protected from light, in single dose container.

Dequalinium Chloride

\[
\text{H}_2\text{N} \overset{\text{N}}{\text{N}} \text{-(CH}_2\text{)}_{10} \overset{\text{N}}{\text{N}} \overset{2\text{Cl}^-}{\text{NH}_2} \\
\text{CH}_3 \quad \text{H}_3\text{C}
\]

C_{30}H_{40}Cl_{2}N_{4} \quad \text{Mol. Wt. 527.7}

Dequalinium Chloride is 4,4′'-diamino-2,2′'-dimethyl-N,N'-decamethylenediamine (quinolinium chloride).

Dequalinium Chloride contains not less than 95.0 per cent and not more than 101.0 per cent of C_{30}H_{40}Cl_{2}N_{4}, calculated on the dried basis.

Description. A creamy white powder; odourless or almost odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with dequalinium chloride RS or with the reference spectrum of dequalinium chloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0008 per cent w/v solution shows absorption maxima at about 240 nm, 326 nm and 335 nm; absorbance at about 240 nm, about 0.65, at about 326 nm, about 0.4 and at about 335 nm, about 0.35.

C. Gives reaction A of chlorides (2.3.1).

Tests

Acidity or alkalinity. Shake 0.1 g for 10 minutes with 100 ml of carbon dioxide-free water and add 0.5 ml of bromocresol purple solution. Not more than 0.2 ml of 0.1 M hydrochloric acid or 0.1 M sodium hydroxide is required to change the colour of the solution.

Non-quaternised amines. Not more than 1.0 per cent, calculated as 4-aminoquinidine, C_{10}H_{10}N_{2}, on the dried basis and determined by the following method. Shake 1.0 g with 45 ml of water for 5 minutes, add 5 ml of dilute nitric acid and shake for 10 minutes. Filter through cotton wool. Transfer 20.0 ml of the filtrate to a separator, add 20 ml of 1 M hydrochloric acid, extract with two quantities, each of 50 ml, of ether, washing each extract in turn with the same 5 ml of water, and then extract each ether extract successively with 20 ml, 20 ml and 5 ml of 1 M hydrochloric acid. Combine the acid extracts, dilute to 50.0 ml with 1 M hydrochloric acid and measure the absorbance of the resulting solution at the maximum at about 319 nm and 326.5 nm (2.4.7). The ratio of the absorbance at 319 nm to that at 326.5 nm is not less than 1.0. Calculate the percentage of C_{10}H_{10}N_{2} from the expression 0.387a - 0.306b, where a and b are the specific absorbances at about 319 nm and 326.5 nm respectively.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours at a pressure not exceeding 0.7 kPa.

Assay. Weigh accurately about 0.5 g, dissolve in a mixture of 80 ml of anhydrous glacial acetic acid and 20 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid, using crystal violet solution as indicator. Carry out a blank titration. 1 ml of 0.1 M perchloric acid is equivalent to 0.02638 g of C_{30}H_{40}Cl_{2}N_{4}.

Desferrioxamine Mesylate

Desferrioxamine Mesylate; Deferoxamine Mesilate

\[
\text{H}_2\text{N} \overset{\text{N}}{\text{N}} \text{-(CH}_2\text{)}_{5} \overset{\text{N}}{\text{N}} \overset{\text{CH}_3\text{SO}_3\text{H}}{\text{OH}} \\
\text{H}_2\text{N} \overset{\text{N}}{\text{N}} \text{-(CH}_2\text{)}_{5} \overset{\text{OH}}{\text{OH}}
\]

C_{25}H_{48}N_{6}O_{8},CH_{4}SO_{3} \quad \text{Mol. Wt. 656.8}

Desferrioxamine Mesylate is 30-amino-3,14,25-trihydroxy-3,9,14,20,25-pentaazatriacontane-2,10,13,21,24-pentaone methanesulphonate.
Desferrioxamine Mesylate contains not less than 98.0 per cent and not more than 102.0 per cent of C$_{25}$H$_{48}$N$_6$O$_8$,CH$_4$SO$_3$, calculated on the anhydrous basis.

**Description.** A white or almost white powder.

**Identification**

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with desferrioxamine mesylate RS or with the reference spectrum of desferrioxamine mesylate.

B. The titrated solution (solution A) obtained in the Assay is reddish brown. The colour is extracted by benzyl alcohol but not by ether.

C. Dissolve 5 mg in 5 ml of water, add 2 ml of a 0.5 per cent w/v solution of tribasic sodium phosphate, mix and then add 0.5 ml of a 2.5 per cent w/v solution of sodium 1,2-naphthoquinone-4-sulphonate; a blackish brown colour is produced.

D. Dissolve 0.1 g in 5 ml of 2 M hydrochloric acid and add 1 ml of barium chloride solution; the solution remains clear. In a porcelain crucible mix 0.1 g with 1 g of anhydrous sodium carbonate, heat and ignite over a Bunsen flame. Allow to cool, dissolve the residue in 10 ml of water by heating if necessary and filter; the filtrate gives reaction A of sulphates (2.3.1).

**Tests**

**Appearance of solution.** A 10.0 per cent w/v solution is not more opalescent than opalescence standard OS2 (2.4.1), and the absorbance of the solution at about 425 nm is not more than 0.10 (2.4.7).

**pH** (2.4.24). 3.7 to 5.5, determined in a freshly prepared 10.0 per cent w/v solution.

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Chlorides** (2.3.12). 0.75 g complies with the limit test for chlorides (330 ppm).

**Sulphates** (2.3.17). 0.25 g complies with the limit test for sulphates (600 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 2.0 per cent, determined on 1.0 g.

**Assay.** Weigh accurately about 0.5 g, dissolve in 25 ml of water and add 4 ml of 0.05 M sulphuric acid. Titrate slowly with 0.1 M ferric ammonium sulphate, determining the end-point potentiometrically using a platinum indicator electrode and a calomel reference electrode (2.4.25). Towards the end of the titration, titrate uniformly and at a rate of about 0.2 ml per minute. Retain the titrated solution (solution A) for Identification test B.

1 ml of 0.1 M ferric ammonium sulphate is equivalent to 0.06568 g of C$_{25}$H$_{48}$N$_6$O$_8$,CH$_4$SO$_3$.

Desferrioxamine Mesylate intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

**Bacterial endotoxins** (2.2.3). Not more than 0.025 Endotoxin Unit per mg of desferrioxamine.

Desferrioxamine Mesylate intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage.** Store protected from light in a refrigerator (2° to 8°). Do not freeze. If the substance is sterile, store in sterile, airtight, tamper-evident containers sealed so as to exclude microorganisms.

**Labelling.** The label states where applicable, that the substance is sterile.

**Desferrioxamine Injection**

Desferrioxamine Mesylate Injection; Deferoxamine Injection

Desferrioxamine Mesylate Injection is a sterile material consisting of Desferrioxamine Mesylate with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage.** The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Desferrioxamine Mesylate Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of desferrioxamine mesylate, C$_{25}$H$_{48}$N$_6$O$_8$,CH$_4$SO$_3$.

**Description.** A white or almost white powder; very hygroscopic.
The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with desferrioxamine mesylate RS or with the reference spectrum of desferrioxamine mesylate.

B. The titrated solution (solution A) obtained in the Assay is reddish brown. The colour is extracted by benzyl alcohol but not by ether.

**Tests**

**Bacterial endotoxins** (2.2.3). Not more than 0.025 Endotoxin Unit per mg of desferrioxamine.

**Sterility** (2.2.11). Comply with the test for sterility.

**Assay.** Determine the weight of the mixed contents of the 10 containers. Weigh accurately a quantity of the mixed contents of the 10 containers containing about 0.5 g of Desferrioxamine Mesylate, dissolve in 25 ml of water and add 4 ml of 0.05 M sulphuric acid. Titrate slowly with 0.1 M ferric ammonium sulphate, determining the end-point potentiometrically using a platinum indicator electrode and a calomel reference electrode (2.4.25). Towards the end of the titration, titrate uniformly and at a rate of about 0.2 ml per minute. Retain the titrated solution (solution A) for Identification test B.

1 ml of 0.1 M ferric ammonium sulphate is equivalent to 0.06568 g of C$_{25}$H$_{48}$N$_6$O$_{8}$,CH$_4$SO$_3$.

**Storage.** Store protected from light in a refrigerator (2° to 8°). Do not freeze.

**Deslanoside**

Deslanoside is 3-[(O-β-D-glucopyranosyl)-(1→4)-O-2,6-dideoxy-β-D-ribo-hexopyranosyl-(1→4)-O-2,6-dideoxy-β-D-ribo-hexopyranosyl-(1→4)-O-2,6-dideoxy-β-D-ribo-hexopyranosyl]oxy]-12,14-dihydroxy-3β,5β,12β-card-20 (22)-enolide.

Deslanoside contains not less than 95.0 per cent and not more than 105.0 per cent of C$_{47}$H$_{74}$O$_{19}$, calculated on the dried basis.

**Description.** White crystals or a fine, crystalline powder; hygroscopic. It loses water in an atmosphere of low relative humidity.

**Identification**

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Prepare a dispersion of the substance under examination by dissolving 1 mg in 0.3 ml of methanol and triturating with 0.4 g of dry, finely powdered potassium bromide IR until a uniform and dry mixture is obtained. The powder complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with deslanoside RS treated in the same manner. When comparing the spectra attention should be given to the absence of a distinct maximum at 1260 cm$^{-1}$ and to the intensity of the maximum at 1740 cm$^{-1}$.

B. In the test for Related substances, the principal band in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. Suspend 0.5 mg in 0.5 ml of ethanol (60 per cent) and add 0.1 ml of dinitrobenzoic acid solution and 0.1 ml of 2 M sodium hydroxide; the suspension becomes violet.

D. Dissolve 5 mg in 5 ml of glacial acetic acid, add 0.1 ml of ferric chloride test solution, mix and cautiously add 2 ml of sulphuric acid so as to form a separate layer; a brown ring is formed at the junction of the liquids and the upper layer develops a green colour which becomes blue on standing.

**Tests**

**Appearance of solution.** A 2.0 per cent w/v solution in a mixture of equal volumes of chloroform and methanol is clear (2.4.1), and colourless (2.4.1).

**Specific optical rotation** (2.4.22). +7.0° to +8.5°, determined in a 2.0 per cent w/v solution in dehydrated pyridine.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 130 volumes of dichloromethane, 36 volumes of methanol and 3 volumes of water.

**Solvent mixture.** A mixture of equal volumes of chloroform and methanol.
Test solution (a). Dissolve 0.2 g of the substance under examination in 10 ml with solvent mixture.

Test solution (b). Dilute 5 ml of test solution (a) to 50 ml with the same solvent mixture.

Reference solution (a). A 0.2 per cent w/v solution of deslanoside RS in the same solvent mixture.

Reference solution (b). Dilute 5 ml of reference solution (a) to 20 ml with the same solvent mixture.

Reference solution (c). Dilute 5 ml of reference solution (a) to 50 ml with the same solvent mixture.

Apply separately to the plate, as 1-cm bands, 5 µl of each solution. After development, dry the plate in a current of warm air, spray with ethanolic sulphuric acid (5 per cent v/v), heat at 140° for 15 minutes and examine in daylight. Any secondary band in the chromatogram obtained with test solution (a) is not more intense than the band in the chromatogram obtained with reference solution (b) and not more than two such bands are more intense than the band in the chromatogram obtained with reference solution (c).

Sulphated ash (2.3.18). Not more than 0.2 per cent, determined on the residue obtained in the test for Loss on drying.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 0.5 g by drying in an oven at 105° at a pressure of 1.5 to 2.5 kPa.

Assay. Protect the solutions from light throughout the assay and maintain at a constant temperature of 20° ± 1°.

Weigh accurately about 30 mg and dissolve in sufficient methanol to produce 100.0 ml. Dilute 10.0 ml of this solution to 100.0 ml with methanol. To 10.0 ml of the resulting solution add 6 ml of alkaline picric acid solution and dilute to 25.0 ml with water. Allow to stand for 1 hour and measure the absorbance of the resulting solution at the maximum at about 490 nm (2.4.7), using as the blank a mixture of 10 ml of methanol and 6 ml of alkaline picric acid solution diluted to 25.0 ml with water. Calculate the content of C47H74O19 from the absorbance obtained by simultaneously carrying out the operation using 30 mg of undried deslanoside RS instead of the substance under examination.

Storage. Store protected from light in a refrigerator (2° to 8°). Do not freeze.

Deslanoside Injection

Deslanoside Injection is a sterile solution of Deslanoside in Water for Injections containing suitable buffering agents.

Deslanoside Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of deslanoside, C47H74O19.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 130 volumes of dichloromethane, 36 volumes of methanol and 3 volumes of water.

Test solution. Transfer a volume of the injection containing about 2 mg of Deslanoside to a separator and extract with 25 ml of a mixture of 7 volumes of chloroform and 3 volumes of ethanol (95 per cent). Transfer the extract to a 10-ml flask and evaporate to dryness on a water-bath. Dissolve the residue in 1 ml of a mixture of equal volumes of chloroform and methanol.

Reference solution. A 0.2 per cent w/v solution of deslanoside RS in the same solvent mixture.

Apply separately to the plate, as 1-cm bands, 5 µl of each solution. After development, dry the plate in a current of warm air, spray with ethanolic sulphuric acid (5 per cent v/v), heat at 140° for 15 minutes and examine in daylight. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 5.5 to 7.0.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Protect the solution from light throughout the assay and maintain at a constant temperature of 20° ± 1°.

To an accurately measured volume of the injection containing 3 mg of Deslanoside, add 10 ml of water and extract with five quantities, each of 20 ml, of a mixture of 60 volumes of chloroform and 40 volumes of 2-propanol with the addition of sodium chloride if necessary to disperse any emulsions that may form. Wash each extract with the same quantities of 20 ml and then of 10 ml of water. Filter the combined extracts through a plug of cotton wool and evaporate the filtrate to dryness at about 35° at a pressure not exceeding 0.7 kPa. Transfer the residue to a flask with methanol and add sufficient methanol to produce 20.0 ml. To 10.0 ml of the resulting solution add 6 ml of alkaline picric acid solution and dilute to 25.0 ml with water. Allow to stand for 1 hour and measure the absorbance of the resulting solution at the maximum at about 490 nm (2.4.7), using as the blank a mixture of 10.0 ml of methanol and 6 ml of alkaline picric acid solution diluted to 25.0 ml with water. Calculate the content of C47H74O19 from the absorbance obtained by simultaneously carrying out the operation using a solution prepared by dissolving 30 mg of deslanoside RS in sufficient methanol to produce 50.0 ml, diluting 25.0 ml to 100.0 ml with methanol and continuing as
described above beginning at the words “To 10.0 ml of the resulting solution...”.

**Storage.** Store protected from light.

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**Desoxycortone Acetate**

Desoxycorticosterone Acetate; Deoxycortone Acetate

![Desoxycortone Acetate molecule](image)

C\(_{23}\)H\(_{32}\)O\(_4\)  Mol. Wt. 372.5

Desoxycortone Acetate is 3,20-dioxo-4-pregnen-21-yl acetate.

Desoxycortone Acetate contains not less than 96.0 per cent and not more than 104.0 per cent of C\(_{23}\)H\(_{32}\)O\(_4\), calculated on the dried basis.

**Description.** A white or creamy-white, crystalline powder; odourless.

**Identification**

*Test A* may be omitted if tests *B, C, D* and *E* are carried out. *Tests B, D* and *E* may be omitted if tests *A* and *C* are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with desoxycortone acetate RS.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in ethanol (95 per cent) shows an absorption maximum at about 240 nm; absorbance at about 240 nm, 0.43 to 0.46.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Solvent mixture.** A mixture of 90 volumes of acetone and 10 volumes of 1,2-propanediol.

**Mobile phase.** A mixture of equal volumes of cyclohexane and light petroleum (40° to 60°).

**Test solution.** Dissolve 25 mg of the substance under examination in 10 ml of the same solvent mixture.

**Reference solution (a).** Dissolve 25 mg of desoxycortone acetate RS in 10 ml of the same solvent mixture.

**Reference solution (b).** Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the liquid to ascend to the top, remove the plate from the tank and allow the solvents to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with ethanolic sulphuric acid (20 per cent v/v). Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

D. Dissolve 40 mg in 1 ml of methanol, warm and add 1 ml of alkaline cupritartrate solution; a red precipitate is formed.

E. Dissolve 5 mg in 0.5 ml of methanol, add 0.5 ml of ammoniacal silver nitrate solution; a black precipitate is slowly produced in the cold but is rapidly produced on warming.

**Tests**

**Specific optical rotation** (2.4.22). +171.0° to +179.0°, determined in a 1.0 per cent w/v solution in dioxan.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 10 ml with the mobile phase.

**Reference solution (a).** Dissolve 2 mg of desoxycortone acetate RS and 2 mg of betamethasone 17-valerate RS in the mobile phase and dilute to 200 ml with the mobile phase.

**Reference solution (b).** Dilute 1 ml of the test solution to 200 ml with the mobile phase.

**Chromatographic system**

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 350 ml of water and 600 ml of acetonitrile, allowed to equilibrate, diluted to 1000 ml with water and mixed again,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Equilibrate the column with the mobile phase for about 30 minutes.
Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is not less than 50 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are: betamethasone 17-valerate, about 7.5 minutes and desoxycortone acetate about 9.5 minutes. The test is not valid unless the resolution between the peaks corresponding to betamethasone 17-valerate and desoxycortone acetate is at least 4.5. If necessary, adjust the concentration of acetonitrile in the mobile phase.

Inject the test solution and reference solution (b). Continue the chromatography for three times the retention time of the principal peak. In the chromatogram obtained with the test solution, the sum of the areas of all the peaks other than the principal peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.1 times that of the principal peak in the chromatogram obtained with reference solution (b).

**Sulphated ash (2.3.18).** Not more than 0.1 per cent.

**Loss on drying (2.4.19).** Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.1 g, dissolve in sufficient ethanol to produce 100.0 ml. Dilute 2.0 ml of this solution to 100.0 ml with ethanol and mix. Measure the absorbance of the resulting solution (2.4.7) at the maximum at about 240 nm. Calculate the content of C_{23}H_{32}O_{4} taking 450 as the specific absorbance at 240 nm.

**Storage.** Store protected from light.

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**Desoxycortone Acetate Injection**

Desoxycorticosterone Acetate Injection; Desoxycortone Acetate Injection

Desoxycortone Acetate Injection is a sterile solution of Desoxycortone Acetate in Ethyl Oleate or other suitable ester, in a suitable fixed oil, or in any mixture of these. It may contain suitable alcohols.

Desoxycortone Acetate Injection contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of desoxycortone acetate, C_{23}H_{32}O_{4}.

**Identification**

Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 70 volumes of n-heptane and 30 volumes of acetone.

**Test solution.** Dilute the injection with carbon tetrachloride to give a solution containing 0.25 per cent w/v of Desoxycortone Acetate.

**Reference solution.** A 0.25 per cent w/v solution of desoxycortone acetate RS in carbon tetrachloride.

Apply to the plate 1 µl of each solution. After development, dry the plate in air until the odour of solvent is no longer detectable, spray with ethanolic sulphuric acid (10 per cent v/v), heat at 105° for 30 minutes and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution. Ignore any spots due to the vehicle.

**Tests**

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** To an accurately measured volume containing 10 mg of Desoxycortone Acetate add sufficient ethanol to produce 100.0 ml. Dilute 2.0 ml of this solution to 100.0 ml with ethanol and mix. Measure the absorbance of the resulting solution (2.4.7) at the maximum at about 240 nm. Calculate the content of C_{23}H_{32}O_{4} taking 450 as the specific absorbance at 240 nm.

**Storage.** Store protected from light.

**Labelling.** The label states (1) the composition of the solvent; (2) that it is meant for intramuscular injection only; (3) that any sediment should be dissolved by warming before use.

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**Dexamethasone**

C_{22}H_{29}FO_{5}  
Mol. Wt. 392.5

Dexamethasone is 9α-fluoro-11β,17α,21-trihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione.

Dexamethasone contains not less than 96.0 per cent and not more than 104.0 per cent of C_{22}H_{29}FO_{5}, calculated on the dried basis.

**Description.** White or almost white crystals or a crystalline powder; odourless.
Identification

Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with dexamethasone RS or with the reference spectrum of dexamethasone.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with a suitable silica gel containing a fluorescent indicator with an optimal intensity at about 254 nm.

Mobile phase. A mixture of 85 volumes of ether, 10 volumes of toluene and 5 volumes of 1-butanol saturated with water.

Solvent mixture. A mixture of 9 volumes of chloroform and 1 volume of methanol.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml with solvent mixture.

Reference solution (a). A 0.25 per cent w/v solution of dexamethasone RS in the same solvent mixture.

Reference solution (b). A solution containing 0.125 per cent w/v each of the substance under examination and dexamethasone RS in the same solvent mixture.

Reference solution (c). A solution containing 0.125 per cent w/v each of dexamethasone RS and betamethasone RS in the same solvent mixture.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, spray with ethanolic sulphuric acid (20 per cent v/v), heat at 120° for 10 minutes or until spots appear, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution is similar in colour in day-light, fluorescence in ultraviolet light at 365 nm, in position and size to that in the chromatogram obtained with reference solution (a) and the chromatogram obtained with reference solution (b) shows only one spot. The test is not valid unless the chromatogram obtained with reference solution (c) shows two spots that are close to one another but separated.

C. Place 2 ml of a 0.01 per cent w/v solution in ethanol in a stoppered tube, add 10 ml of phenylhydrazine-sulphuric acid solution, mix, place in a water-bath at 60° for 20 minutes and cool immediately. Absorbance of the resulting solution at the maximum at about 419 nm, not less than 0.4 (2.4.7).

D. To 2 ml of sulphuric acid add 2 mg and shake to dissolve; a faint reddish brown colour is produced within 5 minutes. Add 10 ml of water and mix; the colour is discharged.

Tests

Specific optical rotation (2.4.22). +75.0° to +80.0°, determined in a 1.0 per cent w/v solution in dioxan.

Light absorption (2.4.7). Absorbance of a 0.001 per cent w/v solution in ethanol (95 per cent) at the maximum at about 240 nm, 0.38 to 0.41.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. To 25 mg of the substance under examination add 1.5 ml of acetonitrile and 5 ml of mobile phase A. Mix with the aid of ultrasonic sound until the solids are completely dissolved and add sufficient of the mobile phase to produce 10 ml and mix well.

Reference solution (a). Dissolve 2 mg of dexamethasone RS and 2 mg of methylprednisolone RS in sufficient of the mobile phase to produce 10 ml.

Reference solution (b). Dilute 1 ml of the test solution to 100 ml with mobile phase A.

Chromatographic system

– a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm),
– column temperature. 45°,
– mobile phase: A. 250 volumes of acetonitrile and 700 volumes of water mixed, allowed to equilibrate and adjusted to 1000 volumes with water and mixed,

B. acetonitrile,

– flow rate. 2 ml per minute,
– a linear gradient programme using the conditions given below,
– spectrophotometer set at 254 nm,
– a 20 µl loop injector.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
<td>isocratic</td>
</tr>
<tr>
<td>15</td>
<td>100 → 0</td>
<td>0 → 100</td>
<td>begin linear gradient</td>
</tr>
<tr>
<td>40</td>
<td>0</td>
<td>100</td>
<td>end chromatogram, return to 100 A</td>
</tr>
<tr>
<td>41</td>
<td>100</td>
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<td>being equilibration with A</td>
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<tr>
<td>46=0</td>
<td>100</td>
<td>0</td>
<td>end equilibration, being next chromatogram</td>
</tr>
</tbody>
</table>

Equilibrate the column for at least 30 minutes with mobile phase B and then with mobile phase A for 5 minutes. For subsequent operations use the conditions described from 40 to 46 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is at least 50 per cent of the full scale of the recorder.
Inject reference solution (a). When the chromatograms are recorded, the retention times are; methylprednisolone about 11.5 minutes, and dexamethasone about 13 minutes. The test is not valid unless the resolution between the peaks corresponding to methylprednisolone and dexamethasone is at least 2.8; if necessary, adjust the concentration of acetonitrile in mobile phase A.

Inject mobile phase A as the blank, the test solution and reference solution (b). Record the chromatogram of the test solution for twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak other than the principal peak, is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all the peaks other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent). Ignore any peak due to the blank and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. Weigh accurately about 0.1 g and dissolve in sufficient ethanol to produce 100.0 ml and mix. Dilute 2.0 ml of this solution to 100.0 ml with ethanol and mix well. Determine the absorbance of the resulting solution (2.4.7) at the maximum at about 238 nm. Calculate the content of C_{22}H_{29}FO_{5} taking 394 as the specific absorbance at 238 nm.

Storage. Store protected from light.

Dexamethasone Tablets

Dexamethasone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dexamethasone, C_{22}H_{29}FO_{5}.

Identification

Shake a quantity of the powdered tablets containing 20 mg of Dexamethasone with 50 ml of chloroform for 30 minutes, filter and evaporate the filtrate to dryness at 105° for 2 hours. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with dexamethasone RS or with the reference spectrum of dexamethasone.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with a suitable silica gel containing a fluorescent indicator with an optimal intensity at about 254 nm.

Mobile phase. A mixture of 85 volumes of ether, 10 volumes of toluene and 5 volumes of 1-butanol saturated with water.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of a mixture of 9 volumes of chloroform and 1 volume of methanol.

Reference solution (a). A 0.25 per cent w/v solution of dexamethasone RS in the same solvent mixture.

Reference solution (b). A solution containing 0.125 per cent w/v each of the substance under examination and dexamethasone RS in the same solvent mixture.

Reference solution (c). A solution containing 0.125 per cent w/v each of dexamethasone RS and betamethasone RS in the same solvent mixture.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, spray with ethanolic sulphuric acid (20 per cent v/v), heat at 120° for 10 minutes or until spots appear, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution is similar in colour in day-light, fluorescence in ultraviolet light at 365 nm, in position and size to that in the chromatogram obtained with reference solution (a) and the chromatogram obtained with reference solution (b) shows only one spot. The test is not valid unless the chromatogram obtained with reference solution (c) shows two spots that are close to one another but separated.

C. To 2 ml of sulphuric acid add 2 mg and shake to dissolve; a faint reddish brown colour is produced within 5 minutes. Add 10 ml of water and mix; the colour is discharged.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. To a weighed quantity of the tablets containing 2.5 mg of Dexamethasone add 10 ml of acetonitrile, mix with the aid of ultrasound and filter through a 0.45 µm filter. Dilute 4 ml of the filtrate to 10 ml with water.

Reference solution (a). Dilute 1 ml of the test solution to 100 ml with mobile phase A.

Reference solution (b). Dissolve 2 mg of dexamethasone RS and 2 mg of methylprednisolone RS in mobile phase A and dilute to 100 ml with the same solvent.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecysilyl silica gel (5 µm) (such as Hypersil ODS),
- column temperature 45°,
- mobile phase: A. 15 per cent v/v acetonitrile,
- B. acetonitrile,
- flow rate. 2.5 ml per minute,
- a linear gradient programme using the conditions given below,
– spectrophotometer set at 254 nm,
– a 20 µl loop injector.

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<tr>
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<td>100</td>
<td>0</td>
<td>being equilibration with A</td>
</tr>
<tr>
<td>46-0</td>
<td>100</td>
<td>0</td>
<td>end equilibration, being next chromatogram</td>
</tr>
</tbody>
</table>

Inject reference solution (b). When the chromatograms are recorded, the retention times are; methylprednisolone about 13 minutes, and dexamethasone about 16 minutes. The test is not valid unless the resolution between the peaks corresponding to methylprednisolone and dexamethasone is at least 2.8; if necessary, adjust the concentration of acetonitrile in mobile phase A.

Inject mobile phase A, the test solution and reference solution (a). In the chromatogram obtained with the test solution, the area of any secondary peak is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent); the sum of the areas of all secondary peaks is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak due to mobile phase A and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Uniformity of content.** Comply with the test stated under Tablets.

**Test solution.** Finely crush one tablet, add sufficient quantity of a 0.002 per cent w/v solution of hydrocortisone in methanol (50 per cent) to produce a solution containing 0.0025 per cent w/v solution of Dexamethasone, shake for 10 minutes and filter through a glass-fibre filter paper (such as Whatman GF/C).

**Reference solution.** A solution containing 0.0025 per cent w/v of dexamethasone RS and 0.002 per cent w/v of hydrocortisone (internal standard) in methanol (50 per cent).

**Chromatographic system**

– a stainless steel column 20 cm x 5 mm, packed with octadecylsilyl silica gel (5 µm) (such as Spherisorb ODS 1),
– mobile phase: a mixture of 53 volumes of water and 47 volumes of methanol,
– flow rate. 1.4 ml per minute,
– spectrophotometer set at 238 nm,
– a 20 µl loop injector.

Calculate the content of C_{22}H_{29}FO_{5} in the tablet.

**Other tests.** Comply with the tests stated under Tablets.

**Assay — For tablets containing 2 mg or more of dexamethasone**

Determine by liquid chromatography (2.4.14), protected from light.

*Test solution (a).* Weigh and powder 20 tablets. To a quantity of the powder containing about 2.5 mg of Dexamethasone add 20.0 ml of methanol (50 per cent), shake for 20 minutes and filter through a glass-fibre filter paper (such as Whatman GF/C).

*Test solution (b).* Prepare in the same manner as test solution (a) but use 20.0 ml of a 0.01 per cent w/v solution of hydrocortisone in methanol (50 per cent) in place of the 20.0 ml of methanol (50 per cent).

*Reference solution.* A solution containing 0.0125 per cent w/v of dexamethasone RS and 0.01 per cent w/v of hydrocortisone (internal standard) in methanol (50 per cent).

**Chromatographic system**

– a stainless steel column 20 cm x 5 mm, packed with octadecylsilyl silica gel (10 µm) (such as Spherisorb ODS 1),
– mobile phase: a mixture of 53 volumes of water and 47 volumes of methanol,
– flow rate. 1.4 ml per minute,
– spectrophotometer set at 238 nm,
– a 20 µl loop injector.

Calculate the content of C_{22}H_{29}FO_{5} in the tablets.

*For tablets containing less than 2 mg of dexamethasone.*

Use the average of the ten individual results obtained in the test for Uniformity of content.

**Storage.** Store protected from light.

**Dexamethasone Sodium Phosphate**

![Dexamethasone Sodium Phosphate](image)

C_{22}H_{28}FNa_{2}O_{8}P  Mol. Wt. 516.4
Dexamethasone Sodium Phosphate is disodium 9α-fluoro-11β,17α-dihydroxy-16α-methyl-3,20-dioxo-1,4-pregnadien-21-yl phosphate.

Dexamethasone Sodium Phosphate contains not less than 97.0 per cent and not more than 103.0 per cent of C22H28FNa2O8P.

Description. A white or slightly yellow, crystalline powder; almost odourless; very hygroscopic. It shows polymorphism.

**Identification**

Test A may be omitted if tests B, C and, D are carried out. Tests B and D may be omitted if Tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with dexamethasone sodium phosphate RS or with the reference spectrum of dexamethasone sodium phosphate.

B. Dissolve 10 mg in 5 ml of water and dilute to 100 ml with ethanol. To 2 ml of the resulting solution in a glass-stoppered tube add 10 ml of phenylhydrazine-sulphuric acid solution, mix, heat in a water-bath at 60° for 20 minutes and cool immediately. Absorbance of the resulting solution at the maximum at about 419 nm, not less than 0.20 (2.4.7).

C. In the test for Related substances, the principal peak in the chromatogram obtained with reference solution (b) corresponds to the peak in the chromatogram obtained with reference solution (c).

D. Heat gently 40 mg with 2 ml of sulphuric acid until white fumes are evolved, add nitric acid dropwise until oxidation is complete and cool. Add 2 ml of water, heat until white fumes are evolved again, cool, add 10 ml of water and neutralise to litmus paper with 5 M ammonia. The solution gives reaction A of sodium salts and reaction B of phosphates (2.3.1).

**Tests**

pH (2.4.24). 7.5 to 9.5, determined in a 1.0 per cent w/v solution.

Specific optical rotation (2.4.22). +75.0° to +83.0°, determined in a 1.0 per cent w/v solution.

Inorganic phosphates. Not more than 0.5 per cent, calculated as P2O5, determined by the following method. Weigh accurately about 25 mg, dissolve in 10 ml of water, add 4 ml of dilute sulphuric acid, 1 ml of ammonium molybdate solution and 2 ml of methylaminophenol with sulphite solution and allow to stand for 15 minutes. Add sufficient water to produce 25.0 ml, allow to stand for further 15 minutes and measure the absorbance of the resulting solution at the maximum at about 730 nm (2.4.7). Calculate the content of phosphate from a calibration curve prepared by treating suitable aliquots of a 0.00143 per cent w/v solution of potassium dihydrogen phosphate in a similar manner.

Free dexamethasone. Not more than 1 per cent w/w, determined by the following method. Weigh accurately about 25 mg in a glass-stoppered 50-ml tube, add 5 ml of water and shake to dissolve. Add 25.0 ml of dichloromethane, insert the stopper and mix by gentle shaking. Allow to stand until the dichloromethane layer is clear. Measure the absorbance of the dichloromethane solution at the maximum at about 236 nm (2.4.7), using dichloromethane as the blank. Calculate the content of dexamethasone taking 390 as the specific absorbance at about 236 nm.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (a). Dissolve 2 mg of dexamethasone sodium phosphate RS and 2 mg of betamethasone sodium phosphate RS in the mobile phase and dilute to 100 ml with the mobile phase.

Reference solution (b). Dilute 1 ml of the test solution to 100 ml with the mobile phase.

Reference solution (c). A 0.0025 per cent w/v solution of dexamethasone RS in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 1.360 g of potassium dihydrogen phosphate and 0.60 g of hexylamine allowed to stand for 10 minutes and then dissolved in 182.5 ml of water and 67.5 ml of acetonitrile, mixed and filtered,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Equilibrate the column with the mobile phase for about 45 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is at least 50 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are: betamethasone sodium phosphate, about 12.5 minutes and dexamethasone sodium phosphate about 14 minutes. The test is not valid unless the resolution between the peaks corresponding to betamethasone sodium phosphate and dexamethasone sodium phosphate is at least 2.2. If necessary, adjust the concentration of acetonitrile or increase the concentration of water in the mobile phase.
Inject the test solution and reference solution (b). Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with the test solution: the area of any peak other than the principal peak, is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all such peaks is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent). Ignore any peak with an area less than 0.05 times that of the principal peak in the chromatogram obtained with reference solution (b).

**Ethanol.** Not more than 3.0 per cent w/w, determined by gas chromatography (2.4.13).

**Internal standard.** A 1.0 per cent v/v solution of 1-propanol.

**Test solution (a).** A 10.0 per cent w/v solution of the substance under examination in water.

**Test solution (b).** A solution containing 10.0 per cent w/v of the substance under examination and 1.0 per cent v/v of the internal standard.

**Reference solution.** A solution containing 1.0 per cent v/v of 1-propanol and 1.0 per cent v/v of ethanol. Adjust the content of ethanol to produce a peak of similar height to the corresponding peak in the chromatogram obtained with test solution (a).

Chromatographic system
- a glass column 1 m x 3.2 mm, packed with porous polymer beads (150 to 180 µm),
- temperature:
  - column. 150°,
  - inlet port. 250°,
  - detector. 280°,
- flow rate. 30 ml per minute of the carrier gas.

Inject 2 µl of each solution.

Calculate the percentage w/w of ethanol assuming the weight per ml at 25° to be 0.787 g.

**Water** (2.3.43). Determine on 0.2 g.

**Total ethanol and water.** Not more than 16.0 per cent w/w, calculated from the sum of the percentage of ethanol determined by the method described above and the percentage of water.

**Assay.** Weigh accurately about 0.1 g and dissolve in sufficient water to produce 100.0 ml. Dilute 5.0 ml of this solution to 250.0 ml with water and measure the absorbance of the resulting solution at the maximum at about 240 nm (2.4.7). Calculate the content of $C_{22}H_{30}FO_8P$, taking 297 as the specific absorbance at 240 nm.

**Storage.** Store protected from light.

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**Dexamethasone Injection**

Dexamethasone Sodium Phosphate Injection

Dexamethasone Injection is a sterile solution of Dexamethasone Sodium Phosphate in Water for Injections.

Dexamethasone Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of dexamethasone phosphate, $C_{22}H_{30}FO_8P$.

**Identification**

Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 50 volumes of chloroform, 50 volumes of acetone and 1 volume of water.

**Test solution.** Dilute a quantity of the injection containing about 5 mg of dexamethasone phosphate with 25 ml of water and extract with two quantities, each of 25 ml, of dichloromethane. Discard the dichloromethane each time and transfer the aqueous layer to a 50-ml volumetric flask, dilute to volume with water and mix. Pipette 5 ml into a 50-ml glass-stoppered tube and incubate at 37° for 45 minutes with 5 ml of alkaline phosphatase solution. Extract with 25 ml of dichloromethane, evaporate 15 ml of the dichloromethane extract to dryness and dissolve the residue in 1 ml of dichloromethane.

**Reference solution.** Dissolve 3 mg of dexamethasone RS in sufficient dichloromethane to produce 10 ml.

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of solvent is no longer detectable, spray with a 50 per cent w/v solution of sulphuric acid, heat at 105° until brown and black spots appear. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

**Tests**

**pH** (2.4.24). 7.5 to 8.5.

**Free dexamethasone.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dilute a volume of the injection with the mobile phase to produce a solution containing the equivalent of 0.25 per cent w/v of dexamethasone phosphate.

**Reference solution (a).** A 0.00125 per cent w/v solution of dexamethasone RS in the mobile phase.

**Reference solution (b).** A solution containing 0.25 per cent w/v of dexamethasone phosphate RS, 0.01 per cent w/v of propyl hydroxybenzoate and 0.001 per cent w/v of dexamethasone RS in the mobile phase.
Reference solution (c). A solution containing 0.01 per cent w/v of *propyl hydroxybenzoate* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadeccylsilil silica gel (5 µm) (such as Hypersil ODS),
- mobile phase: a mixture of 1.360 g of *potassium dihydrogen phosphate* and 0.60 g of *hexylamine* allowed to stand for 10 minutes and then dissolved in 182.5 ml of water and 67.5 ml of *acetonitrile*, mixed and filtered,
  - flow rate. 1 ml per minute,
  - spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Equilibrate the column with the mobile phase for about 45 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is at least 50 per cent of the full scale of the recorder.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to betamethasone sodium phosphate and dexamethasone phosphate is at least 2.2. If necessary, adjust the concentration of acetonitrile or increase the concentration of water in the mobile phase.

Inject alternately the test solution and reference solution (a). Calculate the content of C_{22}H_{30}FO_{8}P in the injection.

Storage. Store protected from light at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of dexamethasone phosphate in a suitable dose-volume.

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**Dextran 40 Injection**

Dextran 40 Intravenous Infusion

Dextran 40 Injection is a sterile solution, in Dextrose Injection or in Sodium Chloride Injection, of dextrans of average molecular weight of about 40,000, derived from the dextrans produced by the fermentation of sucrose by means of a certain strain of *Leuconostoc mesenteroides*. The dextrans are polymers of dextrose in which the linkages between the dextrose units are almost entirely of the α-1→6 type.

Dextran 40 Injection contains not less than 9.0 per cent and not more than 11.0 per cent w/v of dextrans.

Description. An almost colourless, slightly viscous solution.

Tests

**pH** (2.4.24). 3.5 to 6.5 for solutions in Dextrose Injection; 4.0 to 7.0 for solutions in Sodium Chloride Injection.

**Molecular size**. For solutions in Dextrose Injection, before proceeding with tests A, B and C add 4 volumes of ethanol (95 per cent), centrifuge and dissolve the residue in a volume of Sodium Chloride Injection sufficient to restore the original volume.

A (2.4.28). Determine the viscosity ratios by Method A, using size C U-tube viscometer at 37°, of solutions in saline solution containing about 3.5, 2.5, 1.5 and 0.75 per cent w/v of dextrans, accurately determined. For each solution, plot (viscosity ratio – 1.00)/concentration (in per cent w/v) against concentration (in per cent w/v). The intercept on the viscosity axis of the straight line joining the points represents the intrinsic viscosity; the intrinsic viscosity is 0.16 to 0.20.

B. Place in each of five stoppered flasks 100 ml of a solution in saline solution containing 6 per cent w/v of dextrans and add
slowly, with continuous stirring, sufficient ethanol to produce a faint cloudiness (about 45 ml is usually required). Add 0.5, 1.0, 1.5, 2.0 and 2.5 ml of ethanol to the separate flasks, stopper the flasks and immerse in a water-bath at about 35° with occasional shaking until clear solutions are obtained. Transfer the flasks to a water-bath maintained at 25.0° ± 0.1° and allow to stand overnight or until two clear liquid phases are formed. Reject the supernatant liquids, dissolve separately the syrupy to stand overnight or until two clear liquid phases are formed. Separate the flasks and immerse in a water-bath at about 35° with 1.0, 1.5, 2.0 and 2.5 ml of ethanol by evaporation at a pressure of about 2 kPa, dilute to 25.0 ml with water and determine the optical rotation (2.4.22). From the optical rotations calculate the amount of dextrans precipitated as described in the Assay. Choose that fraction containing as nearly as possible but not more than 10 per cent of the dextrans present in the injection and determine its intrinsic viscosity by the method described under test A; the intrinsic viscosity is not more than 0.27.

C. Place in each of four stoppered flasks 100 ml of a solution in saline solution containing 6 per cent w/v of dextrans and add slowly, with continuous stirring, 80, 90, 100 and 110 ml respectively of ethanol. Stopper the flasks, transfer to a water-bath maintained at 25.0° ± 0.1° and allow to stand overnight or until two clear liquid phases are formed. Separate the supernatant solution from the syrupy residues. Remove the ethanol from each supernatant solution separately by evaporation at a pressure of 2 kPa, dialyse in cellophane tubing against water to remove sodium chloride, adjust the volume to 25.0 ml with water, add sufficient sodium chloride to produce solutions containing 0.9 per cent w/v and determine the optical rotation (2.4.22). From the optical rotations, calculate the amounts of dextrans present as described in the Assay. Choose that fraction containing as nearly as possible but not more than 10 per cent of the dextrans present in the injection and determine the intrinsic viscosity by the method in test A above; the intrinsic viscosity is not less than 0.08.

Content of dextrose. For solutions in Dextrose Injection, 4.5 to 5.5 per cent w/v, determined by the following method. Dilute 15.0 ml to 50.0 ml with water. To 5.0 ml in a stoppered flask add 25 ml of a buffer solution containing 14.3 per cent w/v of sodium carbonate and 4.0 per cent w/v of potassium iodide and 25.0 ml of 0.05 M iodine. Stopper the flask and allow to stand for exactly 30 minutes at 20°, add 30 ml of dilute hydrochloric acid and titrate immediately with 0.1 M sodium thiosulphate. Repeat the operation beginning at the words “add 25 ml of a buffer solution...” but using 5 ml of water in place of 5 ml of the preparation under examination. The difference between the titrations represents the amount of iodine required to oxidise the dextrose.

1 ml of 0.05 M iodine is equivalent to a 0.00901 g of dextrose.

Acetone. To 10 ml add sufficient ammonium sulphate to give a saturated solution, add 1 ml of sodium nitroprusside solution and 5 ml of strong ammonia solution, and allow to stand for 10 minutes. Any purple colour produced is not more intense than that produced by treating in the same manner 10 ml of a 0.02 per cent v/v solution of acetone.

Ethanol. Distil 100 ml, collect the first 45 ml of distillate and dilute to 50 ml with water. Mix 10 ml of 0.0167 M potassium dichromate and 10 ml of sulphuric acid in a stoppered boiling tube, immediately add 5 ml of the distillate, mix, stopper the tube, and allow to stand for 5 minutes. Transfer to a 500-ml flask, dilute to about 300 ml with carbon dioxide-free water, add 2 g of potassium iodide and 1 ml of a 10 per cent w/v solution of potassium thiocyanate, allow to stand for 5 minutes and titrate the liberated iodine with 0.1 M sodium thiosulphate using starch solution, added towards the end of the titration, as indicator. Repeat the determination beginning at the words “Mix 10 ml of 0.0167 M potassium dichromate...” but using 5 ml of water in place of 5 ml of the distillate. The difference between the titrations is not more than 4.2 ml.

Heavy metals (2.3.13). To 4.0 ml add 5 ml of dilute acetic acid and sufficient water to produce 25.0 ml. The resulting solution complies with the limit test for heavy metals, Method A (5 ppm).

Nitrogen (2.3.30). Determine by Method B, using 50 ml. For solutions in Dextrose Injection, use 30 ml of nitrogen-free sulphuric acid. For solutions in Sodium Chloride Injection use 20 ml of nitrogen-free sulphuric acid.

Not more than 0.35 ml of 0.05 M sulphuric acid is required.

Sulphated ash. Titrates 25 ml with 0.1 M silver nitrate using potassium chromate solution as indicator. Deduct the theoretical value of the sulphated ash due to the sodium chloride present.

1 ml of 0.1 M silver nitrate is equivalent to 0.007102 g of sulphated ash (0.05 per cent w/v)

Foreign protein. Inject 0.5 ml on three occasions at intervals of 2 days into the peritoneal cavity of each of six healthy guinea-pigs weighing not less than 250 g, which have not previously been treated with any material that will interfere with the test. Inject 0.2 ml intravenously into each of the three guinea-pigs 14 days after the first intra-peritoneal injection, and into each of the other three guinea-pigs 21 days after the first intra-peritoneal injection. Observe the guinea-pigs for 30 minutes after each intravenous injection and again 24 hours later; the animals exhibit no signs of anaphylaxis such as coughing, bristling of hair or respiratory distress.

Bacterial endotoxins (2.2.3). Not more than 1.25 Endotoxin Units per ml.

Other tests. Complies with the tests stated under Parenteral Preparations (Infusions).
**Assay.** For solutions in Dextrose Injection — Add a drop of dilute ammonia solution to the required volume and determine the optical rotation (2.4.22). Calculate the content of dextrans from the following expression \(0.5076(\alpha - 0.328D)\), where \(\alpha\) is the observed angular rotation and \(D\) the content of dextrose per cent w/v, determined in the test for Content of dextrose.

For solutions in Sodium Chloride Injection — Measure the optical rotation (2.4.22), and multiply the value obtained by 0.5076.

**Storage.** Store at a temperature not exceeding 30°. The injection should not be exposed to undue fluctuations of temperature.

**Labelling.** The label states (1) the strength as the percentage w/v of dextrans; (2) the name of the solvent; (3) the strain of Leuconostoc mesenteroides used; (4) that the injection should not be used if it is cloudy or if a deposit is present.

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**Dextran 70 Injection**

Dextran 70 Intravenous Infusion

Dextran 70 Injection is a sterile solution, in Dextrose Injection or in Sodium Chloride Injection, of dextrans of average molecular weight about 70,000, derived from the dextrans produced by the fermentation of sucrose by means of a certain strain of Leuconostoc mesenteroides. The dextrans are polymers of dextrose in which the linkages between the dextrose units are almost entirely of the \(\alpha-1\rightarrow6\) type.

Dextran 70 Injection contains not less than 5.5 per cent and not more than 6.5 per cent w/v of dextrans.

**Description.** An almost colourless, slightly viscous solution.

**Tests**

**pH** (2.4.24). 3.5 to 6.5 for solutions in Dextrose Injection; 5.0 to 7.0 for solutions in Sodium Chloride Injection.

**Molecular size.** For solutions in Dextrose Injection, before proceeding with tests A, B and C, add 4 volumes of ethanol (95 per cent), centrifuge and dissolve the residue in sufficient Sodium Chloride Injection to restore the original volume.

A (2.4.28). Determine the viscosity ratios by Method A, using a size C U-tube viscometer at 37°, of solutions in saline solution containing about 3.5, 2.5, 1.5 and 0.75 per cent w/v of dextrans, accurately determined. For each solution, plot (viscosity ratio –1.00)/concentration (in percentage w/v) against concentration (in per cent w/v). The intercept on the viscosity ratio axis of a straight line through the points represents the intrinsic viscosity. The intrinsic viscosity is 0.22 to 0.27.

B. Place 100 ml in each of five stoppered flasks and adjust the temperature to 25.0 ± 0.1°. Maintaining this temperature, add slowly with continuous stirring sufficient ethanol to produce a faint cloudiness (about 45 ml). To the separate flasks add 0.5, 1.0, 1.5, 2.0 and 2.5 ml of ethanol, stopper the flasks and immerse in a water-bath at about 35°, shaking occasionally, until clear solutions are obtained. Transfer the flasks to a water-bath maintained at 25.0° ± 0.1° and allow to stand overnight or until two clear liquid phases are formed. Discard the supernatant liquids, dissolve separately the syrupy residues in sufficient saline solution to produce 25.0 ml, remove the ethanol by evaporation at a pressure of 2 kPa, dilute to 25.0 ml with water and determine the optical rotation (2.4.22). From the optical rotations, calculate the amounts of dextrans precipitated as described in the Assay. Choose that fraction containing as nearly as possible but not more than 10 per cent of the dextrans present in the injection and determine its intrinsic viscosity by the method described under test A using a U-tube viscometer (size A). The intrinsic viscosity is not more than 0.36.

C. Place in each of four stoppered flasks 100 ml of a solution in saline solution containing 6 per cent w/v of dextrans and add slowly, with continuous stirring, 80, 90, 100 and 110 ml respectively of ethanol. Stopper the flasks, transfer to a water-bath maintained at 25.0° ± 0.1° and allow to stand overnight or until two clear liquid phases are formed. Separate the supernatant solutions from the syrupy residues. Remove the ethanol from each supernatant solution separately by evaporation at a pressure of 2 kPa, dialyse in cellophane tubing against water to remove sodium chloride, adjust the volume to 25.0 ml with water, add sufficient sodium chloride to produce solutions containing 0.9 per cent w/v of sodium chloride and determine the optical rotation (2.4.22). From the optical rotations, calculate the amounts of dextrans present as described in the Assay. Choose that fraction containing as nearly as possible but not more than 10 per cent of the dextrans present in the injection and determine the intrinsic viscosity by the method in test A. The intrinsic viscosity is not less than 0.13.

**Content of dextrose (if present).** For solutions in Dextrose Injection, between 4.5 and 5.5 per cent w/v, determined by the following method. Dilute 15.0 ml to 50.0 ml with water. To 5.0 ml in a stoppered flask, add 25 ml of a buffer solution containing 14.3 per cent w/v of sodium carbonate and 4.0 per cent w/v of potassium iodide, and 25.0 ml of 0.05 M iodine. Stopper the flask, allow to stand for exactly 30 minutes at 20°, add 35 ml of dilute hydrochloric acid and titrate immediately with 0.1 M sodium thiosulphate. Repeat the operation using 5 ml of water and beginning at the words ‘add 25 ml of a buffer solution…….’. The difference between the titrations represents the amount of iodine required to oxidise the dextrose.

1 ml of 0.05 M iodine is equivalent to 0.00901 g of C\(_6\)H\(_{12}\)O\(_6\).

**Acetone.** To 10 ml add sufficient ammonium sulphate to give a saturated solution, add 1 ml of sodium nitroprusside solution.
and 5 ml of strong ammonia solution, and allow to stand for 10 minutes. Any purple colour produced is not more intense than that produced by treating in the same manner 10 ml of a 0.02 per cent v/v solution of acetone in the same solvent.

Content of sodium chloride (if present). For solutions in Sodium Chloride Injection, 0.81 to 0.99 per cent w/v, determined by the following method. Titrate an accurately measured volume containing 0.1 g of sodium chloride with 0.1 M silver nitrate using potassium chromate solution as indicator.

1 ml of 0.1 M silver nitrate is equivalent to 0.005844 g of NaCl.

5-Hydroxymethylfurfural and related substances (if dextrose is present). Dilute a volume containing 1.0 g of Dextrose to 500.0 ml with water and measure the absorbance of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.25 (2.4.7).

Ethanol. Distil 100 ml, collect the first 45 ml of the distillate and dilute to 50 ml with water. Mix 10 ml of 0.0167 M potassium dichromate and 10 ml of sulphuric acid in a stoppered boiling tube, immediately add 5.0 ml of the distillate, mix, stopper the tube, and allow to stand for 5 minutes. Transfer to a 500-ml flask, dilute to about 300 ml with carbon dioxide-free water, add 2 g of potassium iodide and 1 ml of 10 per cent w/v solution of potassium thiocyanate, allow to stand for 5 minutes and titrate the liberated iodine with 0.1 M sodium thiosulphate using starch solution, added towards the end of the titration, as indicator. Repeat the determination beginning at the words ‘Mix 10 ml of 0.0167 M potassium dichromate…..’ but using 5.0 ml of water in place of 5.0 ml of the distillate. The difference between the titrations is not more than 4.2 ml.

Heavy metals (2.3.13). Not more than 5 ppm, determined by Method A, on 4.0 ml to which 5 ml of dilute acetic acid and sufficient water are added to produce 25.0 ml.

Nitrogen. Carry out Method B for the determination of nitrogen (2.3.30), using 50.0 ml. For solutions in Dextrose Injection, use 30 ml of nitrogen-free sulphuric acid. For solutions in Sodium Chloride Injection, use 20 ml of nitrogen-free sulphuric acid. Not more than 0.35 ml of 0.05 M sulphuric acid is required.

Sulphated ash (2.3.18). Not more than 0.05 per cent w/v, determined by titrating 25.0 ml with 0.1 M silver nitrate using potassium chromate solution as indicator and deducting the theoretical value of the sulphated ash present due to the sodium chloride.

1 ml of 0.1 M silver nitrate is equivalent to 0.007102 g of Sulphated ash.

Foreign protein.Inject 0.5 ml on three occasions at intervals of 2 days into the peritoneal cavity of each of six healthy guinea-pigs, weighing not less than 250 g, which have not previously been treated with any material, which will interfere with the test. Inject 0.2 ml intravenously into each of the three guinea-pigs 14 days after the first intra-peritoneal injection, and into each of the other three guinea-pigs 21 days after the first intraperitoneal injection. Observe the guinea-pigs for 30 minutes after each intravenous injection and again 24 hours later; the animals exhibit no signs of anaphylaxis such as coughing, bristling of hair or respiratory distress.

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Other tests. Complies with the tests stated under Parenteral Preparations (Infusions).

Assay. For solutions in Dextrose Injection — Add a drop of dilute ammonia solution to 25.0 ml of the injection under examination and determine the optical rotation (2.4.22). Calculate the content of dextrose from the expression

\[ 0.5076(\alpha - 0.528) D \]

where \( \alpha \) is the observed angular rotation and D the content of dextrose, in per cent w/v, determined in the test for Content of dextrose.

For solutions in Sodium Chloride Injection — Measure the optical rotation (2.4.22), and multiply the value obtained by 0.5076.

Storage. Store in single dose containers in a cool place. The injection should not be exposed to undue fluctuations of temperature.

Labelling. The label states (1) the strength as the percentage w/v of dextrans; (2) the name of the solvent; (3) the strain of Leuconostoc mesenteroides used; (4) that the injection should not be used if it is cloudy or if a deposit is present.

Dextran 110 Injection

Dextran 110 Intravenous Infusion

Dextran 110 Injection is a sterile solution, in Dextrose Injection or in Sodium Chloride Injection, of dextrans of average molecular weight of about 110,000, derived from the dextrans produced by the fermentation of sucrose by means of a certain strain of Leuconostoc mesenteroides. The dextrans are polymers of dextrose in which the linkages between the dextrose units are almost entirely of the \( \alpha \)-type. Not more than 5 ppm, determined by

\[ (\alpha - 1 \rightarrow 6) \]
(95 per cent), centrifuge and dissolve the residue in a volume of Sodium Chloride Injection sufficient to restore the original volume.

A (2.4.28). Determine the viscosity ratios by Method A, using size C U-tube viscometer at 37°, of solutions in saline solution containing about 2.0, 1.0, 0.5 and 0.25 per cent w/v of dextrans, accurately determined. For each solution, plot (viscosity ratio - 1.00)/concentration (in per cent w/v) against concentration (in per cent w/v). The intercept on the viscosity ratio axis of the straight line joining the points represents the intrinsic viscosity; the intrinsic viscosity is 0.27 to 0.32.

B. Place 100 ml in each of five stoppered flasks and adjust the temperature to 25.0° ± 0.1°. With precautions to maintain this temperature, add slowly with continuous stirring sufficient ethanol to produce a faint cloudiness (about 45 ml is usually required). Add 0.5, 1.0, 1.5, 2.0 and 2.5 ml of ethanol to the separate flasks, stopper the flasks and immerse in a water-bath at about 35° with occasional shaking until clear solutions are obtained. Transfer the flasks to a water-bath maintained at 25.0° ± 0.1° and allow to stand overnight or until two clear liquid phases are formed. Reject the supernatant liquids, dissolve separately the syrupy residues in sufficient saline solution to produce 25.0 ml, remove the ethanol by evaporation at a pressure of about 2 kPa, dilute to 25.0 ml with water and determine the optical rotation (2.4.22). From the optical rotations calculate the amount of dextrans precipitated as described in the Assay. Choose that fraction containing as nearly as possible but not more than 10 per cent of the dextrans present in the injection and determine its intrinsic viscosity by the method described under test A; the intrinsic viscosity is not more than 0.40.

Content of dextrose. For solutions in Dextrose Injection, between 4.5 and 5.5 per cent w/v determined by the following method. Dilute 15.0 ml to 50.0 ml with water. To 5.0 ml in a stoppered flask add 25 ml of a buffer solution containing 14.3 per cent w/v of sodium carbonate and 4.0 per cent w/v of potassium iodide and 25.0 ml of 0.05 M iodine. Stopper the flask and allow to stand for exactly 30 minutes at 20°, add 30 ml of dilute hydrochloric acid and titrate immediately with 0.1 M sodium thiosulphate. Repeat the operation beginning at the words “add 25 ml of a buffer solution...” but using 5 ml of water in place of 5 ml of the preparation under examination. The difference between the titrations represents the amount of iodine required to oxidise the dextrose.

1 ml of 0.05 M iodine is equivalent to a 0.00901 g of dextrose.

Acetone. To 10 ml add sufficient ammonium sulphate to give a saturated solution, add 1 ml of sodium nitroprusside solution and 5 ml of strong ammonia solution, and allow to stand for 10 minutes. Any purple colour produced is not more intense than that produced by treating in the same manner 10 ml of a 0.02 per cent v/v solution of acetone.

Ethanol. Distil 100 ml, collect the first 45 ml of distillate and dilute to 50 ml with water. Mix 10 ml of 0.0167 M potassium dichromate and 10 ml of sulphuric acid in a stoppered boiling tube, immediately add 5 ml of the distillate, mix, stopper the tube, and allow to stand for 5 minutes. Transfer to a 500-ml flask, dilute to about 300 ml with carbon dioxide-free water, add 2 g of potassium iodide and 1 ml of a 10 per cent w/v solution of potassium thiocyanate, allow to stand for 5 minutes and titrate the liberated iodine with 0.1 M sodium thiosulphate using starch solution, added towards the end of the titration, as indicator. Repeat the determination beginning at the words “Mix 10 ml of 0.0167 M potassium dichromate...” but using 5 ml of water in place of 5 ml of the distillate. The difference between the titrations is not more than 4.2 ml.

Heavy metals (2.3.13). To 4.0 ml add 5 ml of dilute acetic acid and sufficient water to produce 25.0 ml. The resulting solution complies with the limit test for heavy metals, Method A (5 ppm).

Nitrogen (2.3.30). Determine by Method B, using 50 ml. For solutions in Dextrose Injection, use 30 ml of nitrogen-free sulphuric acid. For solutions in Sodium Chloride Injection, use 20 ml of nitrogen-free sulphuric acid.

Not more than 0.35 ml of 0.05 M sulphuric acid is required.

Sulphated ash. Titrte 25 ml with 0.1 M silver nitrate using potassium chromate solution as indicator. Deduct the theoretical value of the sulphated ash due to the sodium chloride present.

1 ml of 0.1 M silver nitrate is equivalent to 0.007102 g of sulphated ash (0.05 per cent w/v)

Foreign protein. Inject 0.5 ml on three occasions at intervals of 2 days into the peritoneal cavity of each of six healthy guinea-pigs weighing not less than 250 g that have not previously been treated with any material that will interfere with the test. Inject 0.2 ml intravenously into each of the three guinea-pigs 14 days after the first intra-peritoneal injection, and into each of the other three guinea-pigs 21 days after the first intra-peritoneal injection. Observe the guinea-pigs for 30 minutes after each intravenous injection and again 24 hours later; the animals exhibit no signs of anaphylaxis such as coughing, bristling of hair or respiratory distress.

Bacterial endotoxins (2.2.3). Not more than 1.25 Endotoxin Units per ml.

Other tests. Complies with the tests stated under Parenteral Preparations (Infusions).

Assay. For solutions in Dextrose Injection — Add a drop of dilute ammonia solution to the required volume and determine the optical rotation (2.4.22). Calculate the content of dextrans from the following expression 0.5076(α - 0.528D), where α is
the observed angular rotation and D the content of dextrose per cent w/v, determined in the test for Content of dextrose.

For solutions in Sodium Chloride Injection — Measure the optical rotation (2.4.22), and multiply the value obtained by 0.5076.

Storage. Store at a temperature not exceeding 30°. The injection should not be exposed to undue fluctuations of temperature.

Labelling. The label states (1) the strength as the percentage w/v of dextrans; (2) the name of the solvent; (3) the strain of Leuconostoc mesenteroides used; (4) that the injection should not be used if it is cloudy or if a deposit is present.

Dextrin

Dextrin is starch partially hydrolysed by heat with or without the aid of suitable acids and buffers.

Description. A white or pale yellow powder; odour, slight and characteristic.

Identification

A. Microscopic characteristics - Granules have similar appearance to the starch from which the dextrin has been prepared. In dextrin prepared from maize starch many of the granules show concentric striations and in dextrin prepared from potato starch concentric striations are not clearly visible; the hilum may be bicleft and some of the granules may be distorted.

B. Boil 1 g in 50 ml of water, cool. To 5 ml of the cloudy suspension, add a drop of iodine solution and mix; a purple colour is produced.

C. To 5 ml of the suspension produced in test B add 2 ml of 2 M sodium hydroxide, mix, add dropwise with shaking 0.5 ml of cupric sulphate solution and boil; a red precipitate is produced.

Tests

Acidity. Add 10 g to 100 ml of ethanol (70 per cent), previously neutralised to phenolphthalein solution, shake for 1 hour, filter and titrate 50 ml of the filtrate with 0.1 M sodium hydroxide using phenolphthalein solution as indicator. Not more than 1 ml of 0.1 M sodium hydroxide is required to change the colour of the solution.

Heavy metals (2.3.13). 0.5 g complies with the limit test for heavy metals, Method A (40 ppm).

Chlorides (2.3.12). Dissolve 2.5 g in 50 ml of boiling water, cool, dilute to 100 ml with water and filter. 5 ml of the filtrate diluted to 15 ml complies with the limit test for chlorides (0.2 per cent).

Ethanol-soluble substances. Not more than 1 per cent, determined by the following method. Boil under a reflux condenser 1 g with 20 ml of ethanol (95 per cent) for 5 minutes and filter while hot. Evaporate 10 ml of the filtrate on a waterbath, dry the residue at 105° and weigh.

Protein. Not more than 0.5 per cent, determined by the following method. Carry out Method A for the determination of nitrogen (2.3.30), using 5 g, accurately weighed, and 30 ml of nitrogen-free sulphuric acid. Calculate the content of protein by multiplying the percentage of nitrogen in the substance under examination by 6.25.

Reducing substances. Not more than 10 per cent, calculated as dextrose, C₆H₁₂O₆, determined by the following method. Weigh accurately a quantity containing 2 g of the dried substance, add 100 ml of water, shake for 30 minutes, dilute to 200.0 ml with water and filter. To 10 ml of cupri-tartaric solution add 20.0 ml of the filtrate, mix and heat at a rate such that the solution is brought to boil in 3 minutes. Boil for a further 2 minutes and cool quickly. Add 5 ml of a 30 per cent w/v solution of potassium iodide and 10 ml of 1 M sulphuric acid, mix and titrate immediately with 0.1 M sodium thiosulphate using starch solution, added towards the end of the titration, as indicator. Repeat the procedure using 20.0 ml of a 0.1 per cent w/v solution of dextrose in place of the filtrate beginning at the words “To 10 ml of...”. Carry out a blank titration using 20 ml of water in place of 20.0 ml of the sample filtrate. The titre obtained with the sample filtrate is not greater than the titre obtained with the dextrose solution.

Ash (2.3.19). Not more than 1 per cent, determined on 1.0 g.

Loss on drying (2.4.19). Not more than 12 per cent, determined on 1.0 g by drying in an oven at 110°.

Storage. Store protected from moisture.

Dextromethorphan Hydrobromide

Dextromethorphan Hydrobromide is ent-3-methoxy-9α-methylmorphinan hydrobromide monohydrate.

Dextromethorphan Hydrobromide contains not less than 99.0 per cent and not more than 101.0 per cent of C₁₈H₂₅NO,HBr calculated on the anhydrous basis.
**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with dextromethorphan hydrobromide RS or with the reference spectrum of dextromethorphan hydrobromide.

B. Evaporate the chloroform solution obtained from test A on a water-bath to dryness, dissolve and dilute the residue in 10 ml of methanol. Evaporate the combined extracts at 50° under nitrogen to dryness, dissolve and dilute the residue in 10 ml of 2 M sodium hydroxide; the solution is dextrorotatory (2.4.22). Retain the chloroform solution for test B.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). 4.0 to 5.5 per cent, determined on 0.2 g.

**Assay.** Weigh accurately about 0.3 g and dissolve in 20 ml of ethanol (95 per cent) and titrate with 0.1 M sodium hydroxide, determining the end-point potentiometrically (2.4.25). 1 ml of 0.1 M sodium hydroxide is equivalent to 0.03523 g of C₁₈H₂₅NO,HBr.

**Storage.** Store protected from light.

**Dextromethorphan Hydrobromide Syrup**

Dextromethorphan Hydrobromide Syrup is a solution of Dextromethorphan Hydrobromide in a suitable flavoured vehicle.

Dextromethorphan Hydrobromide Syrup contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of dextromethorphan hydrobromide, C₁₈H₂₅NO,HBr,H₂O.

**Identification**

A. To 50 ml, add 20 ml of water, 5 ml of 2.5 M sodium hydroxide and extract with three quantities, each of 40 ml of hexane, collect the hexane layer and filter through anhydrous sodium sulphate placed over absorbent cotton wetted with hexane. Evaporate the combined extracts at 50° under nitrogen to dryness, dissolve and dilute the residue in 10 ml of chloroform; the solution is dextrorotatory (2.4.22). Retain the chloroform solution for test B.

B. Evaporate the chloroform solution obtained from test A on a water-bath to dryness, dissolve the residue in 2 ml of 1 M sulphuric acid and add 1 ml of a solution prepared freshly by dissolving 700 mg of mercuric nitrate in 4 ml of water, adding 100 mg of sodium nitrate, mixing and filtering; the solution gives no colour, but after heating, a yellow to red colour develops in about 15 minutes.
Tests

Other tests. Complies with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the syrup containing about 10 mg of Dextromethorphan Hydrobromide to 100.0 ml with water.

Reference solution. A 0.01 per cent w/v solution of dextromethorphan hydrobromide RS.

Chromatographic system – a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm), – mobile phase: a filtered and degassed solution of 0.007 M ammonium nitrate in a mixture of 70 volumes of acetonitrile and 30 volumes of water adjusted to pH 3.4 with glacial acetic acid, – flow rate. 1 ml per minute, – spectrophotometer set at 280 nm, – a 20 µl loop injector.

Calculate the content of C_{18}H_{25}NO.HBr,H_{2}O.

Storage. Store protected from light.

Dextrose

Glucose; D-Glucose

\[
\begin{align*}
\text{C}_6\text{H}_{12}\text{O}_6 & \quad \text{Mol. Wt. 180.2 (anhydrous)} \\
\text{C}_6\text{H}_{12}\text{O}_6\text{H}_2\text{O} & \quad \text{Mol. Wt. 198.2 (monohydrate)}
\end{align*}
\]

Dextrose is D-(+)-glucopyranose or D-(+)-glucopyranose monohydrate.

Description. A white crystalline powder.

Identification.

A. When heated, it melts, swells up and burns, and an odour of burnt sugar is perceptible.

B. Dissolve 0.1 g in 10 ml of water, add 3 ml of potassium cupri-tartrate solution; the solution is blue and clear. Heat to boiling; a copious red precipitate is formed.

Tests

Appearance and odour of solution. Dissolve 10.0 g in 15 ml of water. The solution is clear (2.4.1), not more intensely coloured than reference solution BYS7 (2.4.1), and is odourless.

Acidity or alkalinity. Dissolve 6.0 g in 25 ml of carbon dioxide-free water and add 0.3 ml of phenolphthalein solution. The solution is colourless and not more than 0.15 ml of 0.1 M sodium hydroxide is required to change the colour of the solution to pink.

Specific optical rotation (2.4.22). +52.5° to +53.3°, determined in a solution prepared by dissolving 10.0 g in 80 ml of water, adding 0.2 ml of 5 M ammonia, mixing well, allowing to stand for 30 minutes and diluting to 100.0 ml with water.

Arsenic (2.3.10). Dissolve 10.0 g in 50 ml of water and add 10 ml of stannated hydrochloric acid AsT. The resulting solution complies with the limit test for arsenic (1 ppm).

Heavy metals (2.3.13). A solution prepared by dissolving 4.0 g in 10 ml of water, 2 ml of dilute acetic acid and sufficient water to produce 25 ml, complies with the limit test for heavy metals, Method A (5 ppm).

Chlorides (2.3.12). 20 ml of a 10.0 per cent w/v solution (solution A) complies with the limit test for chlorides (125 ppm).

Sulphates (2.3.17). 7.5 ml of solution A diluted to 15 ml with distilled water complies with the limit test for sulphates (200 ppm).

Sulphite. Dissolve 5.0 g in 40 ml of water, add 2.0 ml of 0.1 M sodium hydroxide and dilute to 50.0 ml with water. To 10.0 ml of the solution add 1 ml of a 31 per cent w/v solution of hydrochloric acid, 2.0 ml of decolorised magenta solution and 2.0 ml of a 0.5 per cent v/v solution of formaldehyde solution. Allow to stand for 30 minutes and measure the absorbance of the resulting solution at the maximum at about 583 nm (2.4.7). The absorbance is not more than that of a standard prepared in the following manner. Dissolve 76 mg of sodium metabisulphite in sufficient water to produce 50.0 ml, dilute 5.0 ml of this solution to 100.0 ml and to 3.0 ml of resulting solution add 4.0 ml of 0.1 M sodium hydroxide and dilute to 100.0 ml with water. Immediately treat 10.0 ml of the resulting solution in the same manner as the test solution beginning at the words “add 1 ml of a 31 per cent w/v solution.....”. Use as the blank for both measurements a solution prepared in the same manner using 10 ml of water.

Barium. To 10 ml of solution A add 1 ml of 1 M sulphuric acid. Examine exactly after 1 hour; any opalescence in the solution is not more intense than that in a mixture of 10 ml of solution A and 1 ml of water.

Foreign sugars, soluble starch and dextrins. Boil 1.0 g in 30 ml of ethanol (90 per cent) to dissolve. The appearance of the solution does not change on cooling.

Sulphated ash (2.3.18). Not more than 0.1 per cent determined by the following method. Dissolve 5.0 g in 5 ml of water, add 2 ml of sulphuric acid, evaporate to dryness and ignite to constant weight. If necessary, repeat the heating with the sulphuric acid.
Water (2.3.43). Not more than 1.0 per cent (anhydrous form) and 7.0 to 9.5 per cent (monohydrate), determined on 0.5 g.

Dextrose intended for use in the manufacture of parenteral preparations complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Units per ml for preparations containing 5 per cent w/v or less of Dextrose. Dilute injections containing more than 5 per cent w/v of Dextrose with sufficient water BET so as to contain 5 per cent w/v of Dextrose.

Other tests. Complies with the tests stated under Parenteral Preparations (Infusions).

Assay. To an accurately measured volume containing between 2 g and 5 g of Dextrose, add 0.2 ml of 5 M ammonia and sufficient water to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, C₆H₁₂O₆, in the volume taken for assay.

Storage. Store in single dose containers at a temperature not exceeding 30°.

Labelling. The label states (1) the strength as the percentage w/v of anhydrous dextrose, C₆H₁₂O₆; (2) that the injection should not be used if it contains visible solid particles.

Dextrose Injection

Dextrose Intravenous Infusion; Glucose Intravenous Infusion

Dextrose Injection is a sterile solution of Dextrose in Water for Injection.

Dextrose Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of anhydrous dextrose, C₆H₁₂O₆.

Description. A clear, colourless solution. Solutions containing 20.0 per cent w/v or more of Dextrose may be not more than faintly straw-coloured.

Identification

A. To 1 ml add 0.05 ml of potassium cupri-tartrate solution; the solution is blue and clear. Heat to boiling; a copious red precipitate is formed.

B. The solution prepared as directed in the Assay is dextrorotatory.

Tests

pH (2.4.24). 3.5 to 6.5, determined in a solution diluted, if necessary, with water for injections to contain not more than the equivalent of 5 per cent w/v of Dextrose and to which 0.30 ml of a saturated solution of potassium chloride has been added for each 100 ml of solution.

5-Hydroxymethylfurfural and related substances. Dilute a volume containing 1.0 g of Dextrose to 250.0 ml with water. Absorbance of the resulting solution at the maximum at about 284 nm, not more than 0.25 (2.4.7).

Heavy metals (2.3.13). A solution prepared by evaporating a volume containing 4 g of Dextrose to 10 ml and adding 2 ml of dilute acetic acid and sufficient water to produce 25 ml complies with the limit test for heavy metals, Method A (5 ppm).

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Units per ml for preparations containing 5 per cent w/v or less of Dextrose. Dilute injections containing more than 5 per cent w/v of Dextrose with sufficient water BET so as to contain 5 per cent w/v of Dextrose.

Diazepam

Diazepam is 7-chloro-1,3-dihydro-1-methyl-5-phenyl-1,4-benzodiazepin-2-one.

Diazepam contains not less than 98.5 per cent and not more than 101.0 per cent of C₁₆H₁₃ClN₂O₂, calculated on the dried basis.

Description. A white or almost white to pale yellow, crystalline powder; odourless or almost odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with diazepam RS or with the reference spectrum of diazepam.

B. Measure the absorbances in subdued light immediately after preparation of the solution.
When examined in the range 230 nm to 360 nm (2.4.7), a 0.0005 per cent w/v solution in 0.05 M methanolic sulphuric acid shows absorption maxima at about 241 nm and 284 nm; absorbance at about 241 nm, about 0.5 and at about 284 nm, about 0.23.

C. Measure the absorbance in subdued light immediately after preparation of this solution.

When examined in the range 325 nm to 400 nm (2.4.7), a 0.0025 per cent w/v solution in 0.05 M methanolic sulphuric acid shows an absorption maximum only at about 366 nm; absorbance at about 366 nm, between 0.35 and 0.39.

D. Determine by the oxygen-flask method (2.3.34), using 20 mg of the substance under examination and 5 ml of dilute sodium hydroxide solution as the absorbing liquid. When the process is complete, acidify the solution with dilute sulphuric acid and boil gently for 2 minutes; the solution gives the reactions of chlorides (2.3.1).

Tests

Related substances and decomposition products. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of equal volumes of hexane and ethyl acetate.

Prepare the following solutions freshly.

Test solution. Dissolve 1 g of the substance under examination in sufficient acetone to produce 10 ml.

Reference solution. Dilute 1 ml of the test solution to 100 ml with acetone.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven over phosphorus pentoxide at 60° at a pressure of 1.5 to 2.5 kPa for 4 hours.

Assay. Weigh accurately about 0.25 g, dissolve in 80 ml of anhydrous glacial acetic acid with the aid of heat, if necessary and cool. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration. 1 ml of 0.1 M perchloric acid is equivalent to 0.02847 g of C16H13ClN2O.

Storage. Store protected from light.

Diazepam Capsules

Diazepam Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of diazepam, C16H13ClN2O.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 100 volumes of chloroform and 10 volumes of methanol.

Test solution. Shake a quantity of the contents of the capsules with sufficient methanol to produce a solution containing 0.5 per cent w/v of Diazepam, allow to settle and decant the supernatant liquid.

Reference solution. A 0.5 per cent w/v solution of diazepam RS in methanol.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, spray with a 10 per cent v/v solution of sulphuric acid in ethanol, heat at 105° for 10 minutes and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows two absorption maxima at about 242 nm and 284 nm.

Tests

Related substances and decomposition products. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of equal volumes of hexane and ethyl acetate.

Prepare the following solutions freshly.

Test solution. Shake a quantity of the contents of the capsules containing 50 mg of Diazepam with 5 ml of acetone and filter.

Reference solution. Dilute 1 volume of the test solution to 50 volumes with acetone.

Apply to the plate 20 µl of the test solution and 5 µl of the reference solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Dissolution (2.5.2).

Apparatus. No. 1 Medium. 900 ml of 0.1 M hydrochloric acid.
Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with 0.1 M hydrochloric acid. Measure the absorbance of the resulting solution at the maximum at about 242 nm (2.4.7). Calculate the content of diazepam, C₁₆H₁₃ClN₂O in the medium from the absorbance obtained from a solution of known concentration of diazepam RS.

D. Not less than 85 per cent of the stated amount of C₁₆H₁₃ClN₂O.

Uniformity of content. Comply with the test stated under Capsules using the following method of analysis. Weigh an intact capsule. Open the capsule without losing any part of the shell and transfer the contents as completely as possible to a 100-ml volumetric flask. Weigh the shell, remove any retained contents and reweigh the shell. To the flask add 1 ml of water, mix and allow to stand for 15 minutes. Add 80 ml of a 0.5 per cent w/v solution of sulphuric acid in methanol, shake for 15 minutes, add sufficient of the methanolic sulphuric acid to produce 100.0 ml and filter. Dilute suitably, if necessary and measure the absorbance at the maximum at about 284 nm (2.4.7). Calculate the content of C₁₆H₁₃ClN₂O taking 450 as the specific absorbance at 284 nm, making an appropriate adjustment for any retained capsule content.

Other tests. Comply with the tests stated under Capsules.

Assay. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 10 mg of Diazepam, add 5 ml of water and complete the test as described under Uniformity of content beginning at the words “mix and allow to stand for 15 minutes...”. Calculate the content of C₁₆H₁₃ClN₂O taking 450 as the specific absorbance at 284 nm.

Storage. Store protected from light.

Diazepam Injection

Diazepam Injection is a sterile solution of Diazepam in Water for Injections or other suitable solvent.

Diazepam Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of diazepam, C₁₆H₁₃ClN₂O.

Description. A clear, colourless or almost colourless solution.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Diazepam Tablets

Diazepam Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of diazepam, C₁₆H₁₃ClN₂O.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.
Mobile phase. A mixture of 100 volumes of chloroform and 10 volumes of methanol.

Test solution. Shake a quantity of the powdered tablets with sufficient methanol to produce a solution containing 0.5 per cent w/v of Diazepam, allow to settle and decant the supernatant liquid.

Reference solution. A 0.5 per cent w/v solution of diazepam RS in methanol.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, spray with a 10 per cent v/v solution of sulphuric acid in ethanol, heat at 105° for 10 minutes and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. When examined in the range 230 to 360 nm (2.4.7), the solution obtained in the Assay shows absorption maxima at about 242 and at about 284 nm.

Tests

Related substances and decomposition products. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of equal volumes of hexane and ethyl acetate.

Test solution. Prepare freshly by shaking a quantity of the powdered tablets containing 50 mg of Diazepam with 5 ml of ethanol (95 per cent) and filtering.

Reference solution. Dilute 1 ml of the test solution to 50 ml with ethanol (95 per cent).

Apply to the plate 20 µl of the test solution and 5 µl of the reference solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Dissolution (2.5.2).

Apparatus. No 1
Medium. 900 ml of 0.1 M hydrochloric acid.
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with 0.1 M hydrochloric acid. Measure the absorbance of the resulting solution at the maximum at about 242 nm (2.4.7). Calculate the content of diazepam, C₁₆H₁₃ClN₂O in the medium from the absorbance obtained from a solution of known concentration of diazepam RS.

D. Not less than 85 per cent of the stated amount of C₁₆H₁₃ClN₂O.

Uniformity of content. Comply with the test stated under Tablets.

Powder one tablet, add 1 ml of water, mix and allow to stand for 15 minutes. Add 80 ml of a 0.5 per cent w/v solution of sulphuric acid in methanol, shake for 15 minutes, add sufficient of the methanolic sulphuric acid to produce 100.0 ml and filter. Dilute suitably, if necessary and measure the absorbance at the maximum at about 284 nm (2.4.7). Calculate the content of C₁₆H₁₃ClN₂O in the tablet taking 450 as the specific absorbance at 284 nm.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 10 mg of Diazepam, add 5 ml of water and complete the test as described under Uniformity of content beginning at the words “mix and allow to stand for 15 minutes....”. Calculate the content of C₁₆H₁₃ClN₂O taking 450 as the specific absorbance at 284 nm.

Storage. Store protected from light.

Dibutyl Phthalate

\[
\text{C}_{16}\text{H}_{22}\text{O}_4, \text{Mol. Wt. 278.4}
\]

Dibutyl Phthalate is dibutyl benzene-1,2-dicarboxylate.

Dibutyl Phthalate contains not less than 99.0 per cent and not more than 101.0 per cent of C₁₆H₂₂O₄, calculated on the anhydrous basis.

Description. A clear, colourless or very slightly yellow, oily liquid.

Identification

Test A may be omitted if tests B, C, D and E are carried out.
Tests B, C and D may be omitted if tests A and E are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with dibutyl phthalate RS.
B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 70 volumes of ether and 30 volumes of n-heptane.

**Test solution.** Dissolve 50 mg of the substance under examination in sufficient ether to produce 10 ml.

**Reference solution.** Dissolve 50 mg of dibutyl phthalate RS in sufficient ether to produce 10 ml.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. To about 0.1 ml add 0.25 ml of sulphuric acid and 50 mg of resorcinol, heat in a water-bath for 5 minutes, allow to cool and add 10 ml of water and 1 ml of 10 M sodium hydroxide; the solution becomes yellow or brownish yellow and shows a green fluorescence.

D. **Relative density** (2.4.29). 1.043 to 1.048.

E. **Refractive index** (2.4.27). 1.490 to 1.495, determined at 20°.

**Tests**

**Appearance of solution.** The liquid under examination is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

**Acidity.** Dissolve 20.0 g in 50 ml of ethanol (95 per cent) previously neutralised to phenolphthalein solution and add 0.2 ml of phenolphthalein solution. Not more than 0.5 ml of 0.1 M sodium hydroxide is required to change the colour of the solution.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 0.2 per cent, determined on 10.0 g.

**Assay.** Weigh accurately about 1.5 g into a hard-glass flask, dissolve in 5 ml of ethanol (95 per cent), previously boiled thoroughly to expel carbon dioxide and neutralised to phenolphthalein solution. Neutralise any free acid in the solution with 0.1 M ethanolic potassium hydroxide using 0.2 ml of phenolphthalein solution as indicator. Add 25.0 ml of 0.5 M ethanolic potassium hydroxide and boil under a reflux condenser on a water-bath for 1 hour. Add 20 ml of water and titrate the excess of alkali with 0.5 M hydrochloric acid using a further 0.2 ml of phenolphthalein solution as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the alkali required to saponify the ester.

1 ml of 0.5 M ethanolic potassium hydroxide is equivalent to 0.06959 g of C16H22O4.

**Diclofenac Sodium**

\[
\text{C}_{14}\text{H}_{10}\text{Cl}_2\text{NNaO}_2 \quad \text{Mol. Wt. 318.1}
\]

Diclofenac Sodium is sodium 2-[(2,6-dichlorophenyl)-amino]phenylacetate.

Diclofenac Sodium contains not less than 98.5 per cent and not more than 101.0 per cent of C14H10Cl2NNaO2, calculated on the dried basis.

**Description.** A white to slightly yellowish crystalline powder; slightly hygroscopic.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with diclofenac sodium RS or with the reference spectrum of diclofenac sodium.

B. To 1 ml of a 0.4 per cent w/v solution in methanol add 1 ml of nitric acid; a dark red colour develops.

C. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. A 1 per cent w/v solution gives the reaction of sodium salts (2.3.1).

**Tests**

**Appearance of solution.** A 5.0 per cent w/v solution in methanol is clear (2.4.1), and not more intensely coloured than reference solution BYS6 (2.4.1).

**pH** (2.4.24). 6.5 to 8.5, determined on a 1.0 per cent w/v solution.

**Light absorption** (2.4.7). Absorbance of a 5.0 per cent w/v solution in methanol at about 440 nm, not more than 0.050.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 50 mg of the substance under examination in methanol and dilute to 50 ml with the same solvent.

**Reference solution.** Dilute 2 ml of the test solution to 100 ml with methanol. Dilute 1 ml of this solution to 10 ml with methanol.
Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with end-capped octylsilyl silica gel (5 µm),
- mobile phase: a mixture of 34 volumes of a solution containing 0.5 g per litre of phosphoric acid and 0.8 g per litre of sodium dihydrogen phosphate adjusted to pH 2.5 with phosphoric acid, and 66 volumes of methanol,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution: the area of any peak other than the principal peak is not greater than the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent); the sum of the areas of all peaks other than the principal peak is not greater than 2.5 times that of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with the reference solution.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Weigh accurately about 0.2 g and dissolve in 50 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.03181 g of C₁₄H₁₀Cl₂NNaO₂.

Storage. Store protected from light.

Diclofenac Injection

Diclofenac Injection is a sterile solution of Diclofenac Sodium in Water for Injections. It may contain Propylene Glycol, Benzyl Alcohol and sufficient Sodium Hydroxide to adjust the pH of the solution.

Diclofenac Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of diclofenac sodium, C₁₄H₁₀Cl₂NNaO₂.

Description. A clear, colourless to yellowish liquid.

Identification
Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 90 volumes of chloroform, 5 volumes of acetone and 5 volumes of formic acid in a saturated chamber.

Test solution. Dilute a suitable volume of the injection containing 25 mg of Diclofenac Sodium to 10 ml with methanol.

Reference solution. A 0.25 per cent w/v solution of diclofenac sodium RS in methanol.

Apply to the plate 2 µl of each solution. After development, dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. Alternatively, spray with a 0.5 per cent w/v solution of potassium dichromate in sulphuric acid (20 per cent). By both methods of visualisation, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 8.1 to 9.0.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a suitable volume of the injection containing 25 mg of Diclofenac Sodium to 10.0 ml with the mobile phase.

Reference solution. A 0.25 per cent w/v solution of diclofenac sodium RS in the mobile phase.

Chromatographic system
- a stainless steel column 12.5 cm x 4.6 mm, packed with octylsilyl silica gel (5 µm),
- mobile phase: a mixture of 60 volumes of methanol and 40 volumes of 0.1 M sodium acetate solution,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 10 µl loop injector.

Inject alternately the test solution and the reference solution and record the chromatograms for 2.5 times the retention time of the principal peak. If necessary adjust the concentration of methanol in the mobile phase to obtain the resolution of the peak due to diclofenac sodium.

Calculate the content of C₁₄H₁₀Cl₂NNaO₂ in the injection.

Diclofenac Tablets

Diclofenac Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of diclofenac sodium, C₁₄H₁₀Cl₂NNaO₂. The tablets may be enteric-coated.
Identification
Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel 60 F254 or using a precoated silica gel 60 F254 plate.

Mobile phase. A mixture of 100 volumes of toluene, 10 volumes of hexane and 10 volumes of anhydrous formic acid.

Test solution. Shake a quantity of the powdered tablets containing 50 mg of Diclofenac Sodium with 5 ml of methanol, centrifuge and use the supernatant liquid.

Reference solution. A 1 per cent w/v solution of diclofenac sodium RS in methanol.

Apply separately to the plate 1 µl of each solution. After development, dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. Alternatively, spray the plate with a 0.5 per cent w/v solution of potassium dichromate in sulphuric acid (20 per cent). By both methods of visualisation, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Diclofenac Sodium, shake with 60 ml of methanol in a 200-ml volumetric flask and dilute to volume with methanol. Dilute 5.0 ml of this solution to 100.0 ml with methanol and measure the absorbance of the resulting solution at the maximum at about 285 nm (2.4.7). Calculate the content of C₁₉H₁₉Cl₂NNaO₂ from the absorbance obtained by repeating the procedure using diclofenac sodium RS in place of the substance under examination.

Storage. Store protected from light.

Dicyclomine Hydrochloride

Dicycloverine Hydrochloride

Dicyclomine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of C₁₉H₃₅NO₂.HCl, calculated on the dried basis.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Identification
A. Dissolve a suitable quantity in acetone and evaporate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with dicyclomine hydrochloride RS or with the reference spectrum of dicyclomine hydrochloride.

B. To 3 ml of a 0.1 per cent w/v solution of sodium dodecyl sulphate, add 5 ml of chloroform and 0.05 ml of a 0.25 per cent w/v solution of methylene blue, mix gently and allow to separate; the chloroform layer is blue. Add 20 mg of the substance under examination dissolved in 2 ml of water, mix gently and allow to separate; the aqueous layer is blue and the chloroform layer is colourless.

C. Dissolve 10 mg in 5 ml of water and add 0.2 ml of 2 M nitric acid and 0.5 ml of silver nitrate solution; a white precipitate is produced.

Tests
Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 50 volumes of 1-propanol, 30 volumes of ethyl acetate, 15 volumes of water and 5 volumes of strong ammonia solution.

Test solution. Dissolve 0.5 g of the substance under examination in 10 ml of methanol.

Reference solution. Dilute 5 ml of the test solution to 50 ml with methanol and mix. To 2 ml of this solution add sufficient methanol to produce 100 ml.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with dilute potassium iodobismuthate solution. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.6 g, dissolve in 20 ml of anhydrous glacial acetic acid and add 10 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid, using crystal violet solution as indicator. Carry out a blank titration.
1 ml of 0.1 M perchloric acid is equivalent to 0.03460 g of C₁₉H₃₅NO₂.HCl.

**Dicyclomine Injection**

Dicyclomine Hydrochloride Injection

Dicyclomine Injection is a sterile, isotonic solution of Dicyclomine Hydrochloride in Water for Injections.

Dicyclomine Injection contains not less than 93.0 per cent and not more than 107.0 per cent of the stated amount of dicyclomine hydrochloride, C₁₉H₃₅NO₂,HCl.

**Identification**

A. To a volume containing 0.1 g of Dicyclomine Hydrochloride add 10 ml of water and 1 ml of hydrochloric acid, shake with 25 ml of ether and allow to separate. Extract the aqueous layer with 30 ml of chloroform, wash the extract with two quantities, each of 10 ml of water and filter the chloroform solution through anhydrous sodium sulphate. Evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with dicyclomine hydrochloride RS treated in the same manner.

B. In the Assay the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

**Tests**

**Bacterial endotoxins** (2.2.3). Not more than 17.2 Endotoxin Unit per mg of Dicyclomine Hydrochloride.

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** Determine by liquid chromatography (2.4.14).

*Solvent mixture.* 1 volume of 0.04 M phosphate buffer, pH 7.5 and 1 volume of acetonitrile.

*Test solution.* Dilute a volume containing about 20 mg of Dicyclomine Hydrochloride to 50.0 ml with the solvent mixture.

*Reference solution.* A 0.04 per cent w/v solution of dicyclomine hydrochloride RS in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane chemically bonded to porous silica (5 μm),
- mobile phase: a mixture of 70 volumes of 0.02 M phosphate buffer pH 7.5 prepared by dissolving 2.72 g of monobasic potassium phosphate in 450 ml of water, adjusting the pH to 7.5 ± 0.1 with 10 per cent w/v solution of sodium hydroxide, diluting to 500 ml with water and 30 volumes of acetonitrile,
- flow rate. 1 ml per minute,
- spectrophotometer set at 215 nm,
- a 20 μl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor for the analyte peak is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of C₁₉H₃₅NO₂,HCl in the injection.

**Storage.** Store protected from light, in single dose or multiple-dose containers.

**Dicyclomine Oral Solution**

Dicyclomine Hydrochloride Oral Solution; Dicycloverine Hydrochloride Oral Solution

Dicyclomine Oral Solution is a solution of Dicyclomine Hydrochloride in a suitable flavoured vehicle.

Dicyclomine Oral Solution contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dicyclomine hydrochloride, C₁₉H₃₅NO₂,HCl.

**Identification**

A. To a volume containing 0.1 g of Dicyclomine Hydrochloride add 10 ml of water and 1 ml of hydrochloric acid, shake with 25 ml of ether and allow to separate. Extract the aqueous layer with 30 ml of chloroform, wash the extract with two quantities, each of 10 ml of water and filter the chloroform solution through anhydrous sodium sulphate. Evaporate the filtrate to dryness, recrystallise the residue from hot acetone and dry at 105° for 30 minutes. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with dicyclomine hydrochloride or with the reference spectrum of dicyclomine hydrochloride.

B. Acidify the oral solution with 2 M nitric acid and add silver nitrate solution; a white precipitate is produced.

**Tests**

**Other tests.** Complies with the tests stated under Oral Liquids.

**Assay.** Weigh accurately a quantity containing about 5 mg of Dicyclomine Hydrochloride add 5 ml of sulphuric acid (10 per cent v/v) and 2 ml of 0.02 M potassium permanganate, mix, allow to stand, add 20 ml of water and 20 ml of chloroform to the decolorised solution and titrate with 0.001 M sodium...
dodecyl sulphate, using 1 ml of dimethyl yellow solution as indicator.

1 ml of 0.001 M sodium dodecyl sulphate is equivalent to 0.0003460 g of C₁₉H₃₅NO₂.HCl.

Determine the weight per ml of the oral solution (2.4.29), and calculate the content of C₁₉H₃₅NO₂.HCl, weight in volume.

Storage. Store protected from light.

**Dicyclomine Tablets**

Dicyclomine Hydrochloride Tablets; Dicycloverine Hydrochloride Tablets

Dicyclomine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of dicyclomine hydrochloride, C₁₉H₃₅NO₂.HCl.

**Identification**

A. Extract a quantity of the powdered tablets containing 0.2 g of Dicyclomine Hydrochloride with 20 ml of chloroform, filter, evaporate the filtrate to dryness, recrystallise the residue from hot acetone and dry at 105° for 4 hours. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with dicyclomine hydrochloride RS or with the reference spectrum of dicyclomine hydrochloride.

B. To 3 ml of a 0.1 per cent w/v solution of sodium dodecyl sulphate, add 5 ml of chloroform and 0.05 ml of a 0.25 per cent w/v solution of methylene blue, mix gently and allow to separate; the chloroform layer is blue. Add a quantity of the powdered tablets containing 20 mg of Dicyclomine Hydrochloride dispersed in 2 ml of water, mix gently and allow to separate; the aqueous layer is blue and the chloroform layer is colourless.

C. Shake a quantity of the powdered tablets containing 10 mg of Dicyclomine Hydrochloride with 5 ml of water and 0.2 ml of 2 M nitric acid, filter and add 0.5 ml of silver nitrate solution to the filtrate; a white precipitate is produced.

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 50 volumes of 1-propanol, 30 volumes of ethyl acetate, 15 volumes of water and 5 volumes of strong ammonia solution.

**Test solution.** Shake a quantity of the powdered tablets containing 0.2 g of Dicyclomine Hydrochloride with 8 ml of water and 2 ml of strong ammonia solution, extract with two quantities, each of 20 ml of chloroform, shake with anhydrous sodium sulphate, filter, evaporate the filtrate to dryness and dissolve the residue in 4 ml of chloroform.

**Reference solution.** Dilute 1 volume of the test solution to 500 volumes with chloroform.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with dilute potassium iodobismuthate solution. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 30 mg of Dicyclomine Hydrochloride, add 20 ml of water and shake. Add 10 ml of 1 M sulphuric acid, 1 ml of dimethyl yellow solution and 40 ml of chloroform, shake and titrate with 0.004 M sodium dodecyl sulphate, shaking vigorously and allowing the layers to separate after each addition, until a permanent orange-pink colour is produced in the chloroform layer.

1 ml of 0.004 M sodium dodecyl sulphate is equivalent to 0.001384 g of C₁₉H₃₅NO₂.HCl.

**Storage.** Store protected from light.

**Didanosine**

\[
\text{C}_{10}\text{H}_{12}\text{N}_{4}\text{O}_{3} \quad \text{Mol. Wt. 236.2}
\]

Didanosine is 2',3'-dideoxyinosine.

Didanosine contains not less than 98.0 per cent and not more than 102.0 per cent of C₁₀H₁₂N₄O₃, calculated on the dried basis.

**Description.** A white or almost white crystalline powder.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with didanosine RS or with the reference spectrum of didanosine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.
Tests

Specific optical rotation (2.4.22). –24.0° to –28.0°, determined in a 1.0 per cent w/v solution in water.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in 100 ml of the mobile phase.

Reference solution. A 0.001 per cent w/v solution of the substance under examination in the mobile phase.

Chromatographic system
– a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilica gel (5 µm),
– mobile phase: a filtered and degassed mixture of 6 volumes of acetonitrile and 94 volumes of water,
– flow rate. 1 ml per minute,
– spectrophotometer set at 254 nm,
– a 20 µl loop injector.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution, the area of any peak other than the principal peak is not greater than half of the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all such peaks is not greater than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more that 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14) as given under the test for Related substances using the following solutions.

Test solution. Dilute 5.0 ml of a 0.1 per cent w/v solution of the substance under examination in water to 100.0 ml with the mobile phase.

Reference solution. Dilute 5.0 ml of a 0.1 per cent w/v solution of didanosine RS in water to 100.0 ml with the mobile phase.

Inject the test solution. The test is not valid unless the column efficiency determined from the didanosine peak is not less than 5000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of C10H12N4O3.

Storage. Store protected from light.

Didanosine Capsules

Didanosine Capsules contain enteric-coated granules of Didanosine.

Didanosine Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of didanosine, C10H12N4O3.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

A. Apparatus No. 2

Medium. 1000 ml of 0.1 M hydrochloric acid.

Speed and time. 100 rpm and 120 minutes.

Determine by liquid chromatography (2.4.14).

Test solution. At the end of the test period dissolve all the granules from the basket in 750 ml of buffer solution pH 7.5 prepared by dissolving 1.41 g of disodium hydrogen orthophosphate anhydrous in 1000 ml of water, adjusting the pH to 7.5 with orthophosphoric acid and filtering, and dilute to 1000 ml with the buffer solution. Dilute suitably to get a solution containing about 0.005 per cent w/v of didanosine.

Reference solution. A 0.005 per cent w/v solution of didanosine RS in the buffer solution.

Chromatographic system
– a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded porous silica (5µm),
– mobile phase: a mixture of 950 volumes of buffer solution pH 7.5 and 50 volumes of acetonitrile,
– flow rate. 1.5 ml per minute.
– spectrophotometer set at 249 nm,
– a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates the tailing factor is not more than 1.5 and the relative standard deviation of replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the percentage of C10H12N4O3 released in the acid medium by subtracting the content of C10H12N4O3 determined in the Assay.

Not more than 10 per cent of the stated amount of C10H12N4O3 is dissolved in 120 minutes.

B. Apparatus No. 2

Storage.
Medium. 1000 ml of a buffer solution prepared by mixing 250 ml of 0.2 M tribasic sodium phosphate buffer and 750 ml of 0.1 M hydrochloric acid and adjusting the pH to 6.8 with 2 M hydrochloric acid or 2 M sodium hydroxide.

Speed and time. 100 rpm and 45 minutes.

Run for 120 minutes at 100 rpm using the medium given in method A. At the end of this period discard the medium from each vessel without losing any of the granules and fill the empty vessel with the dissolution medium preheated to 37º. After running the apparatus for 45 minutes, withdraw a suitable volume of the medium and dilute to get a concentration of about 0.005 per cent w/v of didanosine in the dissolution medium.

Determine by liquid chromatography (2.4.14).

Test solution. The solution obtained in the manner described above.

Reference solution. A 0.005 per cent w/v solution of didanosine RS in the dissolution medium.

Use the chromatographic system described in test A.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation of replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

D. Not less than 75 per cent of the stated amount of C₁₀H₁₂N₄O₃.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Weigh accurately a quantity of the contents of the capsules containing 50 mg of Didanosine, dissolve in 100.0 ml of the buffer solution pH 7.5 and filter. Dilute 5.0 ml of the solution to 50.0 ml with the buffer solution pH 7.5.

Reference solution. A 0.005 per cent w/v solution of didanosine RS in buffer solution pH 7.5.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5µm),
- mobile phase: a mixture of 950 volumes of buffer solution pH 7.5 and 50 volumes of acetonitrile,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 249 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 4500 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and the reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 4 times the area of the peak in the chromatogram obtained with the reference solution (b) (4.0 per cent) and the sum of all the secondary peaks is not more than 5.5 times the area of the peak in the chromatogram obtained with the reference solution (5.5 per cent).

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of the contents of the capsules containing 50 mg of Didanosine, dissolve in 100 ml of mobile phase and filter. Dilute 5.0 ml of the solution to 50.0 ml with mobile phase.

Reference solution. A 0.1 per cent w/v solution of didanosine RS in mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm packed with octadecylsilane bonded to porous silica (5µm),
- mobile phase: a mixture of 950 volumes of mobile phase and 50 volumes of acetonitrile,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 4500 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and the reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 4 times the area of the peak in the chromatogram obtained with the reference solution (b) (4.0 per cent) and the sum of all the secondary peaks is not more than 5.5 times the area of the peak in the chromatogram obtained with the reference solution (5.5 per cent).

D. Not less than 75 per cent of the stated amount of C₁₀H₁₂N₄O₃.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Weigh accurately a quantity of the contents of the capsules containing 100 mg of Didanosine, dissolve in 100 ml of mobile phase and filter.

Reference solution (a). A 0.1 per cent w/v solution of didanosine RS in mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm packed with octadecylsilane bonded to porous silica (5µm), (such as Lichrospher RP18e),
- mobile phase: a mixture of 95 volumes of a buffer solution prepared by dissolving 1.15 g of ammonium dihydrogen orthophosphate in 1000 ml of water, and 5 volumes of acetonitrile, adjust the pH to 6.8 with triethylamine and filter,
- flow rate. 1 ml per minute,
- spectrophotometer set at 249 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates the tailing factor is not more than 1.5 and the relative standard deviation of replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of C₁₀H₁₂N₄O₃ in the capsules.

Storage. Store protected from moisture, at a temperature not exceeding 30º.

Didanosine Tablets

Didanosine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of didanosine, C₁₀H₁₂N₄O₃. The tablets may contain permitted flavouring agents.

Identification
A. Shake a quantity of the powdered tablets containing 0.1 g of Didanosine with 80 ml of water, dilute to 100 ml with water and filter. Dilute 5 ml of the filtrate to 100 ml with water. When examined in the range 220 nm to 350 nm (2.4.7), the resulting solution shows an absorption maximum only at about 250 nm.
B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

**Tests**

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh accurately a quantity of the powdered tablets containing about 50 mg of Didanosine and transfer to a 50-ml volumetric flask. Add about 25 ml of buffer solution pH 7.0, and mix with the aid of ultrasound for 5 minutes, dilute to volume with the same solvent, mix and filter.

**Reference solution.** Weigh accurately about 50 mg didanosine RS and transfer to a 50-ml volumetric flask. Dissolve in about 25 ml of buffer solution pH 7.0 and dilute to volume with the same solvent. Dilute 5.0 ml of this solution to 50.0 ml with the same solvent. Dilute further 5.0 ml to 50.0 ml with the same solvent and filter through a membrane filter disc with an average pore diameter not greater than 0.45 μm.

**Chromatographic system**
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 μm) (such as Kromasil C18),
- mobile phase: filtered and degassed gradient mixtures of acetonitrile and buffer solution pH 7.0 prepared by dissolving 1.42 g of disodium hydrogen phosphate and 6.8 g of tetrabutylammonium hydrogen sulphate in 1000 ml of water, adjusting the pH to 7.0 ± 0.05 with sodium hydroxide solution,
- flow rate. 1.5 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 245 nm,
- a 5 μl loop injector.

<table>
<thead>
<tr>
<th>Time (in min.)</th>
<th>Buffer (pH 7.0)</th>
<th>Acetonitrile</th>
<th>Coment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 8</td>
<td>100 → 0</td>
<td>0</td>
<td>isocratic</td>
</tr>
<tr>
<td>20 – 25</td>
<td>70 → 30</td>
<td>30</td>
<td>linear gradient</td>
</tr>
<tr>
<td>26 – 35</td>
<td>100 → 0</td>
<td>0</td>
<td>re-equilibrium</td>
</tr>
</tbody>
</table>

Inject the reference solution. The test is not valid unless the column efficiency determined from the didanosine peak is not less than 3000 theoretical plates and the tailing factor is not more than 1.5.

Inject separately the buffer and test solution. Examine the chromatogram obtained with the buffer solution for any extraneous peaks and ignore the corresponding peaks observed in the chromatogram obtained with the test solution.

Any secondary peak observed in the chromatogram obtained with the test solution should not be more than 5.0 per cent and the sum of the areas of all the secondary peaks should not be more than 6.0 per cent when calculated by percentage area normalisation.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh accurately a quantity of the powdered tablets containing about 100 mg of Didanosine and transfer to a 100-ml volumetric flask. Add about 50 ml of buffer solution pH 7.0, mix with the aid of ultrasound for 10 minutes, dilute to volume with the same solvent, mix and filter through a membrane filter disc with an average pore diameter not greater than 0.45 μm.

**Reference solution.** A 0.1 per cent w/v solution of didanosine RS in buffer solution pH 7.0. Filter through a membrane filter disc with an average pore diameter not greater than 0.45 μm.

**Chromatographic system**
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 μm) (such as Kromasil C18),
- mobile phase: a filtered and degassed mixture of 5 volumes of acetonitrile and 95 volumes of a buffer solution prepared by dissolving 1.42 g of disodium hydrogen phosphate in 1000 ml of water, adjusting the pH to 7.5 ± 0.05 with dilute phosphoric acid,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 245 nm,
- a 5 μl loop injector.

Inject the reference solution and record the chromatogram for twice the retention time of didanosine. The test is not valid unless the column efficiency determined from the didanosine peak is not less than 4500 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the content of C10H12N4O3 in the tablets.

**Storage.** Store protected from light.

**Labelling.** The label states that the tablets should be chewed before swallowing.

**Dienoestrol**

Dienestrol

\[
\text{C}_{18}\text{H}_{20}\text{O}_{2} \quad \text{Mol. Wt.} \, 266.3
\]

Dienoestrol is \((E,E)-4,4'\text{[bis(ethylidene)ethylene]-diphenol.}\)
Dienoestrol contains not less than 98.5 per cent and not more than 101.5 per cent of \( \text{C}_{18}\text{H}_{18}\text{O}_2 \), calculated on the dried basis.

**Description.** A white or almost white, crystalline powder.

**Identification**

*Test A* may be omitted if tests *B*, *C*, and *D* are carried out. Tests *B* and *C* may be omitted if tests *A* and *D* are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with dienoestrol RS.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. Heat a mixture of about 1 mg in 5 ml of glacial acetic acid and 1 ml of a 1 per cent w/v solution of bromine in glacial acetic acid in a water-bath for 2 minutes. To 0.5 ml of the solution in a dry test tube add 0.5 ml of ethanol, mix and add 10 ml of water; a reddish-violet colour is produced. Add 5 ml of chloroform, shake vigorously and allow to separate; the chloroform layer is red and the aqueous layer is almost colourless.

D. Dissolve 0.5 mg in 0.2 ml of glacial acetic acid, add 1 ml of phosphoric acid and heat on a water-bath for 3 minutes; a reddish-violet colour is produced.

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 90 volumes of toluene and 10 volumes of diethylamine.

**Test solution (a).** Dissolve 0.5 g of the substance under examination in 5 ml of ethanol (95 per cent).

**Test solution (b).** Dilute 5 ml of test solution (a) to 100 ml with ethanol (95 per cent).

**Reference solution (a).** A 0.5 per cent w/v solution of dienoestrol RS in ethanol (95 per cent).

**Reference solution (b).** Dilute 5 ml of reference solution (a) to 50 ml with ethanol (95 per cent).

**Reference solution (c).** A solution containing 0.25 per cent w/v each of dienoestrol RS and stilbestrol RS in ethanol (95 per cent).

Apply to the plate 1 µl of each solution. After development, dry the plate in air, spray with ethanolic sulphuric acid (20 per cent v/v) and heat at 120° for 10 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (c) shows at least two clearly separated spots having approximately the same intensity.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 25 mg and dissolve in sufficient ethanol to produce 100.0 ml. To 5.0 ml of this solution add 10 ml of ethanol, dilute with 0.1 M sodium hydroxide to 250.0 ml and measure the absorbance of the resulting solution at the maximum at about 245 nm (2.4.7). Calculate the content of \( \text{C}_{18}\text{H}_{18}\text{O}_2 \) from the absorbance obtained by repeating the procedure using dienoestrol RS in place of the substance under examination.

**Storage.** Store protected from light.

**Dienoestrol Tablets**

Dienoestrol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dienoestrol, \( \text{C}_{18}\text{H}_{18}\text{O}_2 \).

**Identification**

A. Extract a quantity of the powdered tablets containing about 15 mg of Dienoestrol with ether and filter; evaporate the filtrate to dryness. Reserve a portion of the residue for test C. Heat a mixture of about 1 mg of the residue in 5 ml of glacial acetic acid and 1 ml of a 1 per cent w/v solution of bromine in glacial acetic acid in a water-bath for 2 minutes. To 0.5 ml of the solution in a dry test tube add 0.5 ml of ethanol, mix and add 10 ml of water; a reddish-violet colour is produced. Add 5 ml of chloroform, shake vigorously and allow to separate; the chloroform layer is red and the aqueous layer is almost colourless.

B. Dissolve 0.5 mg of the residue in 0.2 ml of glacial acetic acid, add 1 ml of phosphoric acid and heat on a water-bath for 3 minutes; a reddish-violet colour is produced.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 90 volumes of toluene and 10 volumes of diethylamine.

**Test solution.** Shake a quantity of the powdered tablets containing 2 mg of Dienoestrol with 4 ml of acetone, centrifuge and use the supernatant liquid.

**Reference solution (a).** A 0.05 per cent w/v solution of dienoestrol RS in acetone.
Reference solution (b). A solution containing 0.1 per cent w/v each of dienoestrol RS and stilbestrol RS in acetone.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with ethanolic sulphuric acid (20 per cent v/v) and heat at 120° for 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots having approximately the same intensity.

Tests

Uniformity of content. Comply with test stated under Tablets.

Powder one tablet and extract with successive quantities of ether until complete extraction is effected. Filter the ether solution and wash the filter with small quantities of ether. Evaporate the ether and add sufficient 0.1 M sodium hydroxide to produce a solution containing 0.0005 per cent w/v of Dienoestrol. Complete the test as described in the Assay beginning at the words “Measure the absorbance....”.

Calculate the content of C10H21N3O in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 10 mg of Dienoestrol and triturate with successive quantities of ether until complete extraction is effected. Filter the ether extracts and wash the filter with small quantities of ether. Evaporate the ether and add sufficient 0.1 M sodium hydroxide to produce a solution containing 0.0005 per cent w/v of Dienoestrol. Complete the test as described in the Assay beginning at the words “Measure the absorbance....”.

Calculate the content of C10H21N3O in the tablet.

Diethylcarbamazine Citrate is N,N-diethyl-4-methylpiperazine-1-carboxamide dihydrogen citrate.

Diethylcarbamazine Citrate contains not less than 98.0 per cent and not more than 101.0 per cent of C10H21NiO,C6H8O7, calculated on the dried basis.

Description. A white, crystalline powder; odourless; slightly hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with diethylcarbamazine citrate RS or with the reference spectrum of diethylcarbamazine citrate.

B. In the test for N,N’-Dimethylpiperazine and N-methylpiperazine, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution (a).

C. A 2 per cent w/v solution gives reaction A of citrates (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution is not more opalescent than opalescence standard OS2 (2.4.1), and not more intensely coloured than reference solution BYS6 (2.4.1).

N,N’-Dimethylpiperazine and N-methylpiperazine. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 65 volumes of methanol, 30 volumes of 2-butanone and 5 volumes of strong ammonia solution.

Test solution. Dissolve 0.5 g of the substance under examination in 10 ml of methanol.

Reference solution (a). A 5 per cent w/v solution of diethylcarbamazine citrate RS in methanol.

Reference solution (b). A 0.01 per cent w/v solution of N,N’-dimethylpiperazine in methanol.

Reference solution (c). A 0.01 per cent w/v solution of N-methylpiperazine in methanol.

Heavy metals (2.3.13). 1.0 g dissolved in 20 ml of water, 0.5 ml of 0.1 M hydrochloric acid and sufficient water to produce...
25 ml complies with the limit test for heavy metals, Method A (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven over phosphorus pentoxide at 60° at a pressure of 1.5 to 2.5 kPa for 4 hours.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh accurately about 25 mg of the substance under examination, dissolve in 20 ml of a 3.124 per cent w/v solution of potassium dihydrogen phosphate, dilute to 25.0 ml with the same solvent, mix well and filter. Dilute 5.0 ml of the filtrate to 50.0 ml with the potassium dihydrogen phosphate solution.

**Reference solution.** A 0.01 per cent w/v solution of diethylcarbamazine citrate RS in a 3.124 per cent w/v solution of potassium dihydrogen phosphate.

**Chromatographic system**
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilil silica gel (5 µm),
- mobile phase: a mixture of 100 volumes of methanol and 900 volumes of a 1 per cent solution of potassium dihydrogen phosphate,
- flow rate. 2.5 ml per minute,
- spectrophotometer set at 220 nm,
- a 20 µl loop injector.

Inject alternately suitable volumes of the test solution and reference solution. The test is not valid unless the relative standard deviation of the peak areas of diethylcarbamazine in replicate injections is not more than 2.0 per cent.

Calculate the content of C₁₀H₂₁N₃O₆C₆H₈O₇.

**Storage.** Store protected from moisture.

## Diethylcarbamazine Tablets

**Diethylcarbamazine Citrate Tablets**

Diethylcarbamazine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of diethylcarbamazine citrate, C₁₀H₂₁N₃O₆C₆H₈O₇.

**Identification**

To a quantity of the powdered tablets containing 0.15 g of Diethylcarbamazine Citrate add 15 ml of ethanol (95 per cent), shake for 5 minutes, filter and evaporate the filtrate to dryness. To the residue add 10 ml of 2 M sodium hydroxide and extract with three quantities, each of 10 ml, of chloroform. Dry the combined extracts over anhydrous sodium sulphate, filter and evaporate the chloroform. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with diethylcarbamazine citrate RS or with the reference spectrum of diethylcarbamazine citrate.

### Tests

**N,N'-Dimethylpiperazine and N-methylpiperazine.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 65 volumes of methanol, 30 volumes of 2-butanone and 5 volumes of strong ammonia solution.

**Test solution.** Dissolve 0.5 g of the substance under examination in 10 ml of methanol.

**Reference solution (a).** A 5 per cent w/v solution of diethylcarbamazine citrate RS in methanol.

**Reference solution (b).** A 0.01 per cent w/v solution of N,N'-dimethylpiperazine in methanol.

**Reference solution (c).** A 0.01 per cent w/v solution of N-methylpiperazine in methanol.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate at 105° and expose it to iodine vapour for 30 minutes. Any spots corresponding to N,N’-dimethylpiperazine and N-methylpiperazine in the chromatogram obtained with the test solution are not more intense than the spots in the chromatogram obtained with reference solutions (b) and (c) respectively.

### Dissolution (2.5.2).

**Apparatus.** No 1

Medium. 900 ml of water

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate. Dilute the filtrate, if necessary, with an equal volume of a 6.248 per cent w/v solution of potassium dihydrogen phosphate. Carry out the determination as described in the Assay. Calculate the content of C₁₀H₂₁N₃O₆C₆H₈O₇ using a solution of known concentration of diethylcarbamazine citrate RS in a 3.124 per cent w/v solution of potassium dihydrogen phosphate.

D. Not less than 75 per cent of the stated amount of C₁₀H₂₁N₃O₆C₆H₈O₇.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 25 mg of
Diethylcarbamazine Citrate, add 20 ml of a 3.124 per cent w/v solution of potassium dihydrogen phosphate and place in an ultrasonic bath for 5 minutes. Cool, dilute to 25.0 ml with the same solvent and filter. Dilute 5.0 ml of the filtrate to 50.0 ml with the same solvent.

Reference solution. A 0.01 per cent w/v solution of diethylcarbamazine citrate RS in a 3.124 per cent w/v solution of potassium dihydrogen phosphate.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 100 volumes of methanol and 900 volumes of a 1 per cent w/v solution of potassium dihydrogen phosphate,
- flow rate. 2.5 ml per minute,
- spectrophotometer set at 220 nm,
- a 20 µl loop injector.

Inject alternately suitable volumes of the test solution and reference solution. The test is not valid unless the relative standard deviation of the peak areas of diethylcarbamazine in replicate injections is not more than 2.0 per cent.

Calculate the content of $C_{10}H_{21}N_3O_2C_6H_8O_7$ in the tablets.

Storage. Store protected from moisture.

Diethyl Phenyl Acetamide

$C_{12}H_{17}NO$  Mol. Wt. 191.3

Diethyl Phenyl Acetamide is $N,N'$-diethylbenzenecetamide.

Diethyl Phenyl Acetamide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{12}H_{17}NO$, calculated on the anhydrous basis.

Description. A clear to faintly yellow liquid. It shall be free from suspended matter.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Boiling point (2.4.8), about 143º,

Relative density (2.4.29), about 1.010 at 30º,

Refractive index (2.4.27), 1.439 to 1.447 at 20º sodium D lines.

Water (2.3.43). Not more than 0.5 per cent.

Assay. Determine by gas chromatography (2.4.13).

Internal standard solution. A 3 per cent w/v solution of diethyl sebaeate in acetone.

Test solution. To 0.2 g of the substance under examination, add 10 ml of internal standard solution and dilute to the 100.0 ml with acetone.

Reference solution. To 50 mg of diethyl phenylacetamide RS, add 2.5 ml of internal standard solution and dilute to the 25.0 ml with acetone.

Chromatographic system
- a glass column 1.2 m x 2 mm packed with 10 per cent OV-101 on chromosorb WHP (100-120 mesh)
- temperature : column 150º, inlet port and detector at 300º,
- flow rate 3.0 ml per minute of the Nitrogen, 30 ml per minute of the Hydrogen, 210 ml per minute of the Air.

Inject 2 µl of the test solution and the reference solution. Calculate the content of $C_{12}H_{17}NO$. 

Storage. Store protected from light and moisture.

Diethyl Phthalate

$C_{12}H_{14}O_4$  Mol. Wt. 222.2

Diethyl phthalate is diethyl benzene-1,2-dicarboxylate.

Diethyl phthalate contains not less than 99.0 per cent and not more than 101.0 per cent of diethyl phthalate, $C_{12}H_{14}O_4$.

Description. A clear, oily liquid, colourless or very slightly yellow.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with diethyl phthalate RS.

B. Relative density (2.4.29). 1.117 to 1.121.

C. Determine by thin layer chromatography (2.4.17), coating the plate with silica gel GF 254.

Mobile phase. A mixture of 30 volumes of heptane and 70 volumes of ether.
**Test solution.** Dissolve 50 mg of the substance under examination in 10 ml of ether.

**Reference solution.** Dissolve 50 mg of diethyl phthalate RS in 10 ml of ether.

Apply to the plate 10 µl of each solution. After development dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution.

D. To about 0.1 ml, add 0.25 ml of sulphuric acid and 50 mg of resorcinol. Heat on a water-bath for 5 minutes. Allow to cool. Add 10 ml of water and 1 ml of strong sodium hydroxide solution. The solution becomes yellow or brownish-yellow and shows green fluorescence.

**Tests**

**Appearance.** The substance under examination is clear (2.4.1) and not more intensely coloured than reference solution YS6, (2.4.1).

**Acidity.** Dissolve 20.0 g in 50 ml of alcohol previously neutralised to phenolphthalein solution. Add 0.2 ml of phenolphthalein solution. Not more than 0.1 ml of 0.1 M sodium hydroxide is required to change the colour of the indicator to pink.

**Related substances.** Determine by gas chromatography (2.4.13).

**Internal standard solution.** Dissolve 60 mg of naphthalene in 20 ml of methylene chloride.

**Test solution (a).** Dissolve 1 g of the substance under examination in 20 ml of methylene chloride.

**Test solution (b).** Dissolve 1 g of the substance under examination in methylene chloride, add 2.0 ml of the internal standard solution and dilute to 20 ml with methylene chloride.

**Reference solution.** To 1 ml of test solution (a) add 10 ml of the internal standard solution and dilute to 100 ml with methylene chloride.

**Chromatographic system**
- a glass column 2.0 m x 2 mm, packed with silanised diatomaceous earth for gas chromatography (150 µm to 180 µm) impregnated with 3 per cent m/m of polymethylphenylsiloxane,
- temperature: column 150°, inlet port and detector at 225°,
- flow rate. 30 ml per minute of the carrier gas.

Inject 1 µl of the reference solution. The test is not valid unless the resolution between the peaks corresponding to naphthalene and diethyl phthalate is at least 10.

Inject 1 µl of test solution (a). In the chromatogram obtained, verify that there is no peak with the same retention time as the internal standard.

Inject separately 1 µl of test solution (b) and the reference solution. Continue the chromatography for three times the retention time of diethyl phthalate. From the chromatogram obtained with the reference solution, calculate the ratio (R) of the area of the peak due to diethyl phthalate to the area of the peak due to the internal standard. From the chromatogram obtained with test solution (b), calculate the ratio of the sum of the areas of any peaks, other than the principal peak and the peaks due to the internal standard and the solvent, to the area of the peak due to the internal standard; this ratio is not greater than R (1.0 per cent).

**Water (2.3.43).** Not more than 0.2 per cent, determined on 5.0 g.

**Sulphated ash (2.3.18).** Not more than 0.1 per cent, determined on 1.0 g.

**Assay.** Weigh accurately about 0.75 g, dissolve in 25.0 ml of 0.5 M alcoholic potassium hydroxide and add few glass beads. Boil on a water-bath under a reflux condenser for 1 hour. Add 1 ml of phenolphthalein solution and titrate immediately with 0.5 M hydrochloric acid. Carry out a blank titration.

1 ml of 0.5 M alcoholic potassium hydroxide is equivalent to 0.05556 g of C12H14O4.

**Storage.** Store protected from moisture.

**Diethyltoluamide**

Deet

\[
\text{C}_{12}\text{H}_{17}\text{NO} \quad \text{Mol. Wt. 191.3}
\]

Diethyltoluamide is \(N,N\)-diethyl-3-toluamide.

Diethyltoluamide contains not less than 95.0 per cent and not more than 103.0 per cent of \(C_7\text{H}_7\text{NO}\), calculated on the anhydrous basis.

**Description.** A colourless or faintly yellow liquid; odourless or almost odourless.

*CAUTION* — Diethyltoluamide is irritant to the eyes and mucous membranes.
Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with diethyltoluamide RS.

B. Heat 2 ml with 25 ml of a 50 per cent v/v solution of hydrochloric acid under a reflux condenser for 1 hour. Make the mixture alkaline with sodium hydroxide solution, cool and extract with three quantities, each of 30 ml, of ether. Reserve the aqueous layer. Evaporate the ether, dissolve the residue in 5 ml of dilute hydrochloric acid, cool to 5°, add 5 ml of sodium nitrite solution and allow to stand for 10 minutes at 5°. Add 10 ml of water and extract with two quantities, each of 20 ml, of ether. Evaporate the ether, add 1 g of phenol to the residue, cool and add 1 ml of sulphuric acid; an intense green colour is produced, which becomes red on pouring into water and green on making alkaline with dilute sodium hydroxide solution.

C. Acidify the aqueous layer reserved in test B with dilute hydrochloric acid, extract with two quantities, each of 20 ml, of ether and evaporate the ether from the combined extracts. The residue, after drying at 60°, melts at about 108° (2.4.21).

Tests

Weight per ml (2.4.29). 0.997 g to 1.000 g, determined at 20°.

Refractive index (2.4.27). 1.520 to 1.524.

Acidity. A solution of 10.0 g dissolved in 50 ml of ethanol (95 per cent) previously neutralised to phenolphthalein solution requires not more than 4.0 ml of 0.01 M sodium hydroxide to change the colour of the solution, using phenolphthalein solution as indicator.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

Assay. Weigh accurately about 0.3 g, add 7 ml of nitrogen-free sulphuric acid and carry out the determination of nitrogen (2.3.30), using 0.05 M sulphuric acid as the titrant.

1 ml of 0.05 M sulphuric acid is equivalent to 0.01913 g of C_{12}H_{17}NO.

Storage. Store protected from moisture in dry containers.

Digitoxin

Digitoxin is 3β-[(O-2,6-dideoxy-β-D-ribo-hexopyranosyl-(1→4)-O-2,6-dideoxy-β-D-ribo-hexopyranosyl-(1128W1A4)-2,6-dideoxy-β-D-ribo-hexopyranosyl]oxy]-14β-hydroxy-5β-card-20(22)-enolide.

Digitoxin contains not less than 95.0 per cent and not more than 103.0 per cent of C_{41}H_{64}O_{13}, calculated on the dried basis.

Description. A white or almost white powder; odourless.
Specific optical rotation (2.4.22). +16.0° to +18.5°, determined at 20° in a 2.5 per cent w/v solution in chloroform.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 90 volumes of chloroform, 40 volumes of cyclohexane and 15 volumes of methanol.

Solvent mixture. A mixture of equal volumes of chloroform and methanol.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml with solvent mixture.

Reference solution (a). A 1.0 per cent w/v solution of digitoxin RS in the same solvent mixture.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with the same solvent mixture.

Reference solution (c). A 0.02 per cent w/v solution of gitoxin RS in the same solvent mixture.

Reference solution (d). Dilute 5 ml of reference solution (b) to 10 ml with the same solvent mixture.

Reference solution (e). A solution containing 0.5 per cent w/v of digitoxin RS and 0.01 per cent w/v of gitoxin RS in the same solvent mixture.

Apply to the plate 5 µl of each solution and develop the chromatograms immediately after applying the solutions. After development, dry the plate in a current of cold air for 5 minutes. Repeat the development and again dry the plate in a current of cold air for 5 minutes. Spray with ethanolic sulphuric acid (10 per cent) and heat at 130° for 15 minutes. Examine the chromatograms in daylight. Any spot in the chromatogram obtained with the test solution corresponding to gitoxin is not more intense than the spot in the chromatogram obtained with reference solution (c). Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (e) shows clearly separated spots corresponds to digitoxin and gitoxin and the spot in the chromatogram obtained with reference solution (d) is clearly visible.

Sulphated ash (2.3.18). Not more than 0.1 per cent, determined on the residue obtained from the test for Loss on drying.

Loss on drying (2.4.19). Not more than 1.5 per cent, determined on 0.5 g by drying in an oven at 105° for 2 hours.

Assay. Weigh accurately about 40 mg, dissolve in sufficient ethanol (95 per cent) to produce 50.0 ml and dilute 5.0 ml of this solution to 100.0 ml with the same solvent. To 5.0 ml of this solution add 3.0 ml of alkaline picric acid solution, allow to stand in subdued light for 30 minutes and measure the absorbance of the resulting solution at the maximum at about 495 nm (2.4.7), using as the blank a mixture of 5.0 ml of ethanol (95 per cent) and 3.0 ml of alkaline picric acid solution. Calculate the content of C₄₁H₆₄O₁₃ from the absorbance obtained by repeating the operation using digitoxin RS in place of the substance under examination.

Storage. Store protected from moisture and light in a refrigerator (2° to 8°).

Digitoxin Tablets

Digitoxin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of digitoxin, C₄₁H₆₄O₁₃.

Identification

To a quantity of the powdered tablets containing 250 µg of Digitoxin add 1 ml of glacial acetic acid containing 0.01 per cent w/v of ferric chloride, shake for a few minutes, filter through sintered-glass and add cautiously 1 ml of sulphuric acid to the filtrate without mixing; a brown ring free from red colour is produced at the interface which gradually becomes blue and finally the upper layer acquires an indigo colour.

Tests

Dissolution (2.5.2).

Apparatus. No 2

Medium. 600 ml of freshly distilled water

Speed and time. 120 rpm and 60 minutes.

Place six tablets in each basket in the test

Withdraw a suitable volume of the medium and filter through a membrane filter disc having an average pore diameter not greater than 0.8 µm, rejecting the first 1 ml of the filtrate. Transfer 1.0 ml to a 10-ml volumetric flask, add 3.0 ml of a 0.1 per cent w/v solution of L-ascorbic acid in methanol and 0.2 ml of a 0.009M solution of hydrogen peroxide [prepared by accurately diluting hydrogen peroxide solution (100 vol) that has been standardised by titration with 0.02 M potassium permanganate], mix and dilute to volume with hydrochloric acid. After exactly 30 minutes measure the fluorescence of the solution (2.4.5), using an excitation wavelength of about 400 nm and an emission wavelength of about 570 nm and setting the spectrophotofluorimeter to zero with water and to 100 with a solution of suitable concentration of digitoxin RS prepared at the same time and treated in the same manner as the test solution.

D. Not less than 75 per cent of the stated amount of digitoxin, C₄₁H₆₄O₁₃.
Uniformity of content. Comply with test stated under Tablets. Test solution. For tablets containing 100 µg of Digitoxin, shake 1 tablet with 15 ml of methanol (50 per cent) for 30 minutes and dilute to 25.0 ml with the same solvent.

For tablets containing 200 µg of Digitoxin, shake 1 tablet with 30 ml of methanol (50 per cent) for 30 minutes and dilute to 50.0 ml with the same solvent.

Filter through a suitable membrane filter disc having an average pore diameter not greater than 0.8 µm, rejecting the first few ml of the filtrate. Transfer 1.0 ml to a 10-ml volumetric flask, add 3.0 ml of a 0.1 per cent w/v solution of L-absorbic acid in methanol and 0.2 ml of a 0.009M solution of hydrogen peroxide [prepared by accurately diluting hydrogen peroxide solution (100 vol) that has been standardised by titration with 0.02 M potassium permanganate], mix and dilute to volume with hydrochloric acid. After exactly 30 minutes measure the fluorescence of the solution (2.4.5), using an excitation wavelength of about 400 nm and an emission wavelength of about 570 nm and setting the spectrophotofluorimeter to zero with water. Calculate the content of digitoxin, C41H64O13, from the fluorescence obtained by carrying out the operation described above at the same time using a 0.0004 per cent w/v solution of digitoxin RS in methanol (50 per cent) and beginning at the words “Transfer 1.0 ml to a 10-ml volumetric flask....”.

Other tests. Comply with the tests stated under Tablets. Assay. Weigh and finely powder 20 tablets. Weigh accurately a quantity of the powder containing about 1.25 mg of Digitoxin, add 3.0 ml of water, swirl to disperse the powder and allow to stand for 10 minutes, swirling occasionally. Add 25.0 ml of glacial acetic acid, shake for 1 hour and filter, discarding the first few ml of the filtrate. To 4.0 ml of the filtrate add 1.0 ml of dimethyl sulphoxide, dilute to 25.0 ml with xanthydrol reagent, mix well and allow to stand in the dark for 4 ½ hours (solution A). At the same time prepare two further solutions in the same manner but using for solution B 4.0 ml of digitoxin standard solution and for solution C 4.0 ml of a mixture of 25 volumes of glacial acetic acid and 3 volumes of water and beginning at the words “add 1.0 ml of dimethyl sulphoxide....”. Measure the absorbances of solutions A and B at the maximum at about 550 nm (2.4.7), using solution C as the blank. Calculate the content of C41H64O13 from the absorbances obtained.

Storage. Store protected from light and moisture at a temperature not exceeding 30°.

Digoxin

C41H64O14 Mol. Wt. 780.9

Digoxin is 3β-[(O-2,6-dideoxy-β-D-ribo-hexopyranosyl- (1→4)-O-2,6-dideoxy-β-D-ribo-hexopyranosyl- (1128W1Ä4)-2, 6-dideoxy-β-D-ribo-hexopyranosyl)oxy]-12b,1428β-dihydroxy-5β-card-20(22)-enolide.

Digoxin contains not less than 95.0 per cent and not more than 103.0 per cent of C41H64O14, calculated on the dried basis.

Description. Colourless crystals or a white or almost white powder.

Identification. Colourless crystals or a white or almost white powder.

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with digoxin RS or with the reference spectrum of digoxin.

B. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

C. Dissolve about 1 mg in 2 ml of glacial acetic acid with the aid of gentle heat, cool and add 0.05 ml of ferric chloride test solution. Cautiously add 1 ml of sulphuric acid under the two liquids without mixing; a brown ring develops at the interface which gradually becomes blue and a green colour, finally passes to the upper layer.

D. Suspend about 0.5 mg in 0.2 ml of ethanol (60 per cent) and add 0.1 ml dinitrobenzoic acid solution and 0.1 ml of 2 M sodium hydroxide; a violet colour develops.

Tests

Appearance of solution. A 0.5 per cent w/v solution in a mixture of equal volumes of dichloromethane and methanol is clear (2.4.1), and colourless (2.4.1).
Specific optical rotation (2.4.22). +10.0° to +13.0°, determined in a 2.0 per cent w/v solution in anhydrous pyridine.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with kieselguhr G. Place the dry plate in a closed tank containing the necessary quantity of a mixture of 90 volumes of acetone and 10 volumes of formamide so that the plate dips about 5 mm into the liquid and allow the impregnating solvent to ascend at least 15 cm. Remove the plate from the tank, allow to stand for 30 minutes and then use immediately.

Mobile phase. A mixture of 50 volumes of 2-butanol, 50 volumes of xylene and 4 volumes of formamide.

Solvent mixture. A mixture of equal volumes of dichloromethane and methanol.

Test solution. Dissolve 0.1g of the substance under examination in 10 ml with solvent mixture.

Reference solution (a). A 1.0 per cent w/v solution of digoxin RS in the same solvent mixture.

Reference solution (b). Dilute 1 ml of reference solution (a) to 50 ml with the same solvent mixture.

Reference solution (c). A 0.01 per cent w/v solution of digoxin RS in the same solvent mixture.

Reference solution (d). Dilute 1 ml of reference solution (a) to 100 ml with the same solvent mixture.

Reference solution (e). A 0.02 per cent w/v of gitoxin RS in the same solvent mixture.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of cold air until only the lower edge is still moist. Repeat the development and dry the plate at 115° for 20 minutes. Allow to cool, spray with a mixture of 15 volumes of 25 per cent w/v solution of trichloroacetic acid in ethanol (95 per cent) and 1 volume of freshly prepared 3 per cent w/v solution of chloramine T and heat at 115° for 5 minutes. Examine in ultraviolet light at 365 nm. Any spots corresponding to digitoxin and gitoxin in the chromatogram obtained with the test solution are not more intense than the spots in the chromatograms obtained with reference solutions (d) and (e) respectively. Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (c).

Sulphated ash (2.3.18). Not more than 0.1 per cent, determined on the residue obtained from the test for Loss on drying.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 0.5 g by drying over phosphorus pentoxide at a pressure not exceeding 2.7 kPa.

Assay. Weigh accurately about 40 mg, dissolve in sufficient ethanol (95 per cent) to produce 50.0 ml and dilute 5.0 ml of this solution to 100.0 ml with the same solvent. To 5.0 ml of the resulting solution add 3.0 ml of alkaline picric acid solution, allow to stand in subdued light for 30 minutes and measure the absorbance of the solution at the maximum at about 495 nm (2.4.7), using as the blank a mixture of 5.0 ml of ethanol(95 per cent) and 3.0 ml of alkaline picric acid solution. Calculate the content of C_{41}H_{64}O_{14} from the absorbance obtained by repeating the operation using digoxin RS in place of the substance under examination.

Storage. Store protected from light and moisture at a temperature not exceeding 30°.

Digoxin Injection

Digoxin Injection is a sterile solution of Digoxin in Water for Injections and Ethanol or other suitable solvents.

Digoxin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of digoxin, C_{41}H_{64}O_{14}.

Identification

Evaporate 2 ml to dryness, dissolve the residue in 1 ml of glacial acetic acid containing 0.01 per cent w/v of ferric chloride and cautiously add 1 ml of sulphuric acid without mixing; a brown ring develops at the interface which gradually becomes blue and finally the upper layer acquires a blue colour.

Tests

pH (2.4.24). 6.7 to 7.3.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Transfer 20 ml, accurately measured, to a separating funnel containing 10 ml of water. Make alkaline with 5 M ammonia and extract with four quantities, each of 25 ml, of chloroform. Wash each extract with the same 10 ml of water. Evaporate the combined extracts to dryness on a water-bath, dissolve the residue in 5.0 ml of a mixture of 65 volumes of chloroform and 35 volumes of ethanol and add 20.0 ml of glacial acetic acid (solution A). To 5.0 ml of a 0.2 per cent w/v solution of digoxin RS in glacial acetic acid add 10.0 ml of a mixture of 65 volumes of chloroform and 35 volumes of methanol and sufficient glacial acetic acid to produce 50.0 ml (solution B). Dilute 5.0 ml of solution A to 25.0 ml with digoxin reagent, mix, allow to stand for 1 hour and measure the absorbance of the resulting solution at about 590 nm, using water as the blank (2.4.7). Calculate the content of
Digoxin Paediatric Solution

Paediatric Digoxin Elixir

Digoxin Paediatric Solution is a solution of Digoxin in a suitable flavoured vehicle.

Digoxin Paediatric Solution contains not less than 90.0 per cent and not more than 110.0 per cent w/v of the stated amount of digoxin, \( \text{C}_{41}\text{H}_{64}\text{O}_{14} \).

Identification

Digoxin Paediatric Solution should not be diluted before use and should be measured with a pipette.

Extract a quantity of the solution containing 250 µg of Digoxin with four quantities, each of 20 ml, of chloroform, washing each extract with the same 10 ml of water, evaporate the combined extracts to dryness and dissolve the residue in 1 ml of glacial acetic acid containing 0.01 per cent w/v of ferric chloride. Add cautiously 1 ml of sulphuric acid without mixing; a brown ring develops at the interface which gradually becomes blue and finally the upper layer acquires a blue colour.

Tests

pH (2.4.24). 6.8 to 7.2.

Other tests. Complies with the tests stated under Oral Liquids.

Assay. Extract an accurately measured volume containing about 5 mg of Digoxin with four quantities, each of 25 ml, of chloroform, washing each extract with the same 5 ml of water, and evaporate the combined extracts to dryness. To the residue add 3 ml of ethanol and carefully evaporate to dryness on a water-bath with the aid of a gentle current of air. Repeat the evaporation using a further 3 ml of ethanol and cool. Dissolve the residue in 5.0 ml of a mixture of 65 volumes of chloroform and 35 volumes of methanol, add 20.0 ml of glacial acetic acid and filter if necessary. Dilute 5.0 ml of the filtrate to 25.0 ml with digoxin reagent, allow to stand for 1 hour and measure the absorbance of the resulting solution at the maximum at about 590 nm (2.4.7). Calculate the content of \( \text{C}_{41}\text{H}_{64}\text{O}_{14} \) from the absorbance obtained by carrying out the operation described above at the same time but using a solution prepared by mixing 5.0 ml of a 0.2 per cent w/v solution of digoxin RS in glacial acetic acid with 10.0 ml of a mixture of 65 volumes of chloroform and 35 volumes of methanol and adding sufficient glacial acetic acid to produce 50.0 ml beginning at the words “Dilute 5.0 ml of the filtrate......” and using water as the blank.

Storage. Store protected from light in single dose containers.

Digoxin Tablets

Digoxin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of digoxin, \( \text{C}_{41}\text{H}_{64}\text{O}_{14} \).

Identification

To a quantity of the powdered tablets containing 250 µg of Digoxin add 1 ml of glacial acetic acid containing 0.01 per cent w/v of ferric chloride, shake for a few minutes, filter through sintered-glass and add cautiously 1 ml of sulphuric acid to the filtrate without mixing; a brown ring free from red colour is produced at the interface which gradually becomes blue and finally the upper layer acquires an indigo colour.

Tests

Dissolution (2.5.2).

Apparatus. No 2

Medium. 600 ml of freshly distilled water

Speed and time. 120 rpm and 60 minutes.

Place six tablets in each basket in a test

Withdraw a suitable volume of the medium and filter through a membrane filter disc having an average pore diameter not greater than 0.8 µm, rejecting the first 1 ml of the filtrate. Transfer 1.0 ml to a 10-ml volumetric flask, add 3.0 ml of a 0.1 per cent w/v solution of L-absorbic acid in methanol and 0.2 ml of a 0.009M solution of hydrogen peroxide (prepared by accurately diluting hydrogen peroxide solution (100 vol) that has been standardised by titration with 0.02 M potassium permanganate), mix and dilute to volume with hydrochloric acid. After exactly 2 hours measure the fluorescence of the solution (2.4.5), using an excitation wavelength of about 360 nm and an emission wavelength of about 490 nm and setting the spectrophotofluorimeter to zero with water and to 100 with a solution prepared at the same time as the test solution in the following manner. Dilute 2.5 ml of a 0.1 per cent w/v solution of digoxin RS in ethanol (80 per cent) to 100.0 ml with water, dilute the resulting solution further with water to produce a solution containing in 1 ml an amount of digoxin equal to one-hundredth of the strength of the tablets under examination, transfer 1.0 ml of the solution to a 10-ml volumetric flask and carry out the operation described above, beginning at the words “add 3.0 ml...”.
D. Not less than 75 per cent of the stated amount of digoxin, C_{41}H_{64}O_{14}.

**Uniformity of content.** Comply with the test stated under Tablets.

**Test solution.** For tablets containing 250 µg of Digoxin, place 1 tablet with 10 ml of water at 37°, agitate to disintegrate, add 56 ml of ethanol (95 per cent), shake for 60 minutes and add sufficient ethanol (80 per cent) to produce 100.0 ml.

For tablets containing 125 µg and 62.5 µg of Digoxin, repeat the above procedure by using proportionately smaller quantities of water, ethanol (95 per cent) and ethanol (80 per cent).

Filter through a suitable membrane filter disc having an average pore diameter not greater than 0.8 µm, rejecting the first few ml of the filtrate. Transfer 1.0 ml to a 10-ml volumetric flask, add 3.0 ml of a 0.1 per cent w/v solution of L-ascorbic acid in methanol and 0.2 ml of a 0.009 M solution of hydrogen peroxide (prepared by accurately diluting hydrogen peroxide solution (100 vol) that has been standardised by titration with 0.02 M potassium permanganate), mix and dilute to volume with hydrochloric acid. After exactly 2 hours measure the fluorescence of the solution (2.4.5), using an excitation wavelength of about 360 nm and an emission wavelength of about 490 nm and setting the spectrophotofluorimeter to zero with water. Calculate the content of digoxin, C_{41}H_{64}O_{14}, from the fluorescence obtained by carrying out the operation described above at the same time using a 0.00025 per cent hydrogen peroxide solution in ethanol (80 per cent) and beginning at the words “Transfer 1.0 ml to a 10-ml volumetric flask...”

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and finely powder 20 tablets. Weigh accurately a quantity of the powder containing about 1.25 mg of Digoxin, add 3.0 ml of water, swirl to disperse the powder and allow to stand for 10 minutes, swirling occasionally. Add 25.0 ml of glacial acetic acid, shake for 1 hour and filter, discarding the first few ml of the filtrate. To 4.0 ml of the filtrate add 1.0 ml of dimethyl sulphoxide, dilute to 25.0 ml with xanthydrol reagent, mix well and allow to stand in the dark for 4 1/2 hours (solution A). At the same time prepare two further solutions in the same manner but using for solution B 4.0 ml of digoxin standard solution and for solution C 4.0 ml of a mixture of 25 volumes of glacial acetic acid and 3 volumes of water and beginning at the words “add 1.0 ml of dimethyl sulfoxide...”. Measure the absorbances of solutions A and B at the maximum at about 545 nm (2.4.7), using solution C as the blank. Calculate the content of C_{41}H_{64}O_{14} from the absorbances obtained.

**Storage.** Store protected from light.

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**Diiodohydroxyquinoline**

**Iodoquinol**

\[ \text{C}_{9} \text{H}_{10} \text{I}_{2} \text{NO} \quad \text{Mol. Wt. 396.9} \]

Diiodohydroxyquinoline is 5,7-diiodoquinolin-8-ol.

Diiodohydroxyquinoline contains not less than 97.0 per cent and not more than 100.5 per cent of C_{9}H_{10}I_{2}NO, calculated on the dried basis.

**Description.** A light yellowish to yellowish-brown, microcrystalline powder; odourless or with a faint odour.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with diiodohydroxyquinoline RS or with the reference spectrum of diiodohydroxyquinoline.

B. Dissolve 10 mg in 100 ml of dioxan and dilute 5 ml to 100 ml with ethanol. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 258 nm; absorbance at about 258 nm, about 0.53.

C. Heat a few crystals with about 1 ml of sulphuric acid; violet vapours of iodine are evolved.

**Tests**

**Acidity or alkalinity.** Shake 0.5 g with 10 ml of water previously neutralised to phenolphthalein solution. The solution is colourless and not more than 0.1 ml of 0.1 M sodium hydroxide is required to change the colour of the solution to pink.

**Free iodine and iodide.** Shake 1.0 g with 20 ml of water for 30 seconds, allow to stand for 5 minutes and filter. To 10 ml of the filtrate add 1 ml of 1 M sulphuric acid and 2 ml of chloroform and shake; the chloroform layer does not become violet. To the mixture add 5 ml of 1 M sulphuric acid and 1 ml of potassium dichromate solution and shake for 15 seconds; the colour of the chloroform layer does not become more intense than that produced by diluting 2 ml of a 0.016 per cent w/v solution of potassium iodide to 10 ml with water, adding 6 ml of 1 M sulphuric acid, 1 ml of potassium dichromate solution and 2 ml of chloroform and shaking for 15 seconds.

**Related substances.** Determine by gas chromatography (2.4.13).
**Diiodohydroxyquinoline Tablets**

Iodoquinol Tablets

Diiodohydroxyquinoline Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of diiodohydroxyquinoline, C$_9$H$_5$I$_2$NO.

**Identification**

A. Triturate a quantity of the powdered tablets containing about 50 mg of Diiodohydroxyquinoline with 10 ml of carbon disulphide, filter and evaporate the solvent. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with diiodohydroxyquinoline RS or with the reference spectrum of diiodohydroxyquinoline.

B. Shake a quantity of the powdered tablets containing about 10 mg of Diiodohydroxyquinoline with 100 ml of dioxan, filter and dilute 5 ml of the filtrate to 100 ml with ethanol. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 258 nm; absorbance at about 258 nm, about 0.53 (2.4.7).

**Tests**

**Soluble iodides.** Digest a quantity of the powdered tablets containing 0.1 g of Diiodohydroxyquinoline with 5 ml of water for 10 minutes, cool and filter. To the filtrate add 1 ml of 3 M hydrochloric acid, 0.1 ml of ferric chloride test solution and 2 ml of chloroform, shake gently and allow to separate; any violet colour in the chloroform is not more intense than that in a blank to which 1 ml of a 0.02 per cent w/v solution of potassium iodide has been added.

**Disintegration** (2.5.1). 30 minutes.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 12 mg of Diiodohydroxyquinoline and determine by the oxygen-flask method (2.3.34), using a mixture of 10 ml of water and 2 ml of 1 M sodium hydroxide as the absorbing liquid. When the process is complete, add to the flask an excess (5 ml to 10 ml) of acetic bromine solution and allow to stand for 2 minutes. Remove the excess of bromine by the addition of formic acid (about 0.5 ml to 1 ml). Rinse the sides of the flask with water and sweep out any bromine vapour above the liquid with a current of air. Add 1 g of potassium iodide and titrate with 0.02 M sodium thiosulphate using starch solution, added towards the end of the titration, as the indicator.

1 ml of 0.02 M sodium thiosulphate is equivalent to 0.0006616 g of C$_9$H$_5$I$_2$NO.

**Storage.** Store protected from light.

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**Test solution.** Add 0.5 ml of N,O-bis (trimethylsilyl)acetamide to 0.5 ml of a solution in pyridine containing 0.4 per cent w/v of each of 5-chloro-8-hydroxyquinoline, 5,7-dichloro-8-hydroxyquinoline and 5-chloro-7-ido-8-hydroxyquinoline and 0.04 per cent w/v of the substance under examination, mix, allow to stand for 15 minutes and add 5 ml of a 0.05 per cent w/v solution of dibutylphthalate (internal standard) in hexane.

**Reference solution (a).** Add 0.5 ml of N,O-bis (trimethylsilyl)acetamide to a mixture of 0.1 g of the substance under examination and 0.5 ml of pyridine, mix, allow to stand for 15 minutes and add 5 ml of hexane.

**Reference solution (b).** Treat a mixture of 0.1 g of the substance under examination and 0.5 ml of pyridine as described for the test solution.

**Chromatographic system**

- a glass column 1.5 m x 4 mm, packed with silanised diatomaceous support (100 to 120 mesh) coated with 3 per cent w/w of methyl silicone gum,
- temperature: column, 190°, inlet port and detector, 240°,
- flame ionisation detector,
- nitrogen as carrier gas.

In the chromatogram obtained with the test solution the peaks following the solvent peak, in order of emergence, are due to (a) 5-chloro-8-hydroxyquinoline, (b) 5,7-dichloro-8-hydroxyquinoline, (c) the internal standard, (d) 5-chloro-7-ido-8-hydroxyquinoline and (e) diiodohydroxyquinoline. In the chromatogram obtained with reference solution (b) calculate the content of 5-chloro-8-hydroxyquinoline, 5,7-dichloro-8-hydroxyquinoline and 5-chloro-7-ido-8-hydroxyquinoline by reference to the corresponding peaks in the chromatogram obtained with the test solution. The total content of the named impurities and any other impurities does not exceed 4.0 per cent w/w.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over phosphorus pentoxide at a pressure not exceeding 0.7 kPa for 4 hours.

**Assay.** Weigh accurately about 0.3 g and dissolve in 50 ml of anhydrous pyridine. Titrate with 0.1 M tetrabutylammonium hydroxide, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M tetrabutylammonium hydroxide is equivalent to 0.03969 g of C$_9$H$_5$I$_2$NO.

**Storage.** Store protected from light.
Diloxanide Furoate

![Chemical Structure](image)

C₁₄H₁₁Cl₂NO₄  Mol. Wt. 328.2

Diloxanide Furoate is 4-\((N\)-methyl-2,2-dichloroacetamido)phenyl 2-furoate. Diloxanide Furoate contains not less than 98.0 per cent and not more than 102.0 per cent of C₁₄H₁₁Cl₂NO₄, calculated on the dried basis.

**Description.** A white or almost white, crystalline powder; odourless or almost odourless

**Identification.**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with diloxanide furoate RS or with the reference spectrum of diloxanide furoate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in ethanol (95 per cent) shows an absorption maximum only at about 258 nm; absorbance at about 258 nm, about 0.70.

C. On 20 mg determine by the oxygen-flask method (2.3.34), using 10 ml of 1 M sodium hydroxide as the absorbing liquid. When the process is complete, acidify the liquid with nitric acid and add silver nitrate solution; a white precipitate is produced.

**Tests**

**Free acidity.** Shake 3.0 g with 50 ml of water, filter and wash the residue with three quantities, each of 20 ml of water. Titrate the combined filtrate and washings with 0.1 M sodium hydroxide using phenolphthalein solution as indicator; not more than 1.3 ml is required.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel HF254.

**Mobile phase.** A mixture of 96 volumes of dichloromethane and 4 volumes of methanol.

**Test solution.** Dissolve 0.5 g of the substance under examination in 5 ml of chloroform.

**Reference solution.** Dilute 1 ml of the test solution to 100 ml with chloroform and mix. Dilute 5 ml of the resulting solution to 20 ml with chloroform.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Sulphated ash (2.3.18).** Not more than 0.1 per cent.

**Loss on drying (2.3.19).** Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.3 g and dissolve in 50 ml of anhydrous pyridine. Titrate with 0.1 M tetrabutylammonium hydroxide, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M tetrabutylammonium hydroxide is equivalent to 0.03282 g of C₁₄H₁₁Cl₂NO₄.

**Storage.** Store protected from light.

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Diloxanide Tablets

Diloxanide Furoate Tablets

Diloxanide Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of diloxanide furoate, C₁₄H₁₁Cl₂NO₄.

**Identification.**

A. Extract a quantity of the powdered tablets containing 0.2 g of Diloxanide Furoate with 20 ml of chloroform, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with diloxanide furoate RS or with the reference spectrum of diloxanide furoate.

B. On 20 mg of the residue obtained in test A determine by the oxygen-flask method (2.3.34), using 10 ml of 1 M sodium hydroxide as the absorbing liquid. When the process is complete, acidify the liquid with nitric acid and add silver nitrate solution; a white precipitate is produced.

C. The residue obtained in test A melts at 114° to 116° (2.4.21).

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel HF254.

**Mobile phase.** A mixture of 96 volumes of dichloromethane and 4 volumes of methanol.

**Test solution.** Dissolve 0.5 g of Diloxanide Furoate with 5 ml of chloroform, centrifuge and use the supernatant liquid.

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Reference solution. Dilute 1 ml of the test solution to 100 ml with chloroform and mix. Dilute 5 ml of the resulting solution to 20 ml with chloroform.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 40 mg of Diloxanide Furate, shake with 150 ml of ethanol (95 per cent) for 30 minutes, add sufficient ethanol (95 per cent) to produce 200.0 ml, mix and filter. Dilute 10.0 ml of the filtrate to 250.0 ml with ethanol (95 per cent) and measure the absorbance of the resulting solution at the maximum at about 258 nm (2.4.7). Calculate the content of C₁₄H₁₁Cl₂NO₄ taking 705 as the specific absorbance at 258 nm.

Storage. Store protected from light.

Diltiazem Hydrochloride

C₂₂H₂₆N₂O₄S.HCl  Mol. Wt. 451.0

Diltiazem Hydrochloride is s-2,3,4,5-tetrahydro-5-(2-dimethylaminoethyl)-2-(4-methoxyphenyl)-4-oxobenzo[b]thiazepin-3-yl acetate monohydrochloride.

Diltiazem Hydrochloride contains not less than 98.5 per cent and not more than 101.5 per cent of C₂₂H₂₆N₂O₄S.HCl, calculated on the dried basis.

Description. A white, crystalline powder or small crystals.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with diltiazem hydrochloride RS.

B. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to diltiazem hydrochloride in the chromatogram obtained with the reference solution.

C. A 5 per cent w/v solution gives the reactions of chlorides (2.3.1).

Tests

Specific optical rotation (2.4.22). +110° to +116°, determined in a 1.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.12g of the substance under examination in 100 ml of methanol.

Reference solution. A solution containing 0.12 per cent w/v each of diltiazem hydrochloride RS and desacetyl diltiazem hydrochloride RS in methanol.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 50 volumes of a buffer solution containing 0.116 per cent w/v of d-10-camphorsulphonic acid in 0.1 M sodium acetate, with the pH adjusted to 6.2 by the addition of 0.1 M sodium hydroxide, 25 volumes of acetonitrile and 25 volumes of methanol, filtered and degassed,
- flow rate. 1.6 ml per minute,
- spectrophotometer set at 240 nm,
- a 10 µl loop injector.

Inject the reference solution and measure the peak responses of all the peaks. The relative retention times for desacetyl diltiazem and diltiazem are about 0.65 and 1.0 respectively. The resolution between desacetyl diltiazem and diltiazem is not less than 3, and the theoretical plates for the diltiazem peak is not less than 1200. The relative standard deviation of the peak response for replicate injections due to diltiazem hydrochloride and desacetyl diltiazem is not more than 2.0 per cent.

Inject the test solution and the reference solution and measure the responses of all the peaks.

Calculate the percentage content of desacetyl diltiazem hydrochloride in the substance under examination by comparing the area of the peaks due to desacetyl diltiazem and diltiazem in the chromatograms of the test solution and the reference solution and from the content of desacetyl diltiazem hydrochloride in the reference solution. Similarly, calculate the percentage content of each impurity peak other than the peaks due to diltiazem and desacetyl diltiazem with that of the peak due to desacetyl diltiazem in the chromatogram obtained...
with the test solution and from the content of desacetyl diltiazem hydrochloride in the reference solution. Desacetyl diltiazem hydrochloride content is not more than 0.5 per cent w/v, the total impurities including desacetyl diltiazem hydrochloride content is not more than 1 per cent w/v with no individual impurity more than 0.5 per cent w/v.

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals. Method A (20 ppm).

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

**Assay.** Determine by liquid chromatography (2.4.14) as given under the test for Related substances using the following solutions.

**Test solution.** Dissolve 0.12 g of the substance under examination in 100 ml of methanol.

**Reference solution (a).** A solution containing 0.0012 per cent w/v each of diltiazem hydrochloride RS and desacetyl diltiazem hydrochloride RS in methanol.

**Reference solution (b).** Dissolve 60 mg of diltiazem hydrochloride RS in 50 ml of methanol.

Inject reference solution (a) and check the system suitability parameters like the relative retention times, the resolution and the column efficiency in terms of theoretical plates.

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and reference solution (b).

Calculate the content of C$_{22}$H$_{26}$N$_2$O$_4$S,HCl.

**Storage.** Store protected from light.

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**Diltiazem Tablets**

Diltiazem Hydrochloride Tablets

Diltiazem Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of diltiazem hydrochloride, C$_{22}$H$_{26}$N$_2$O$_4$S, HCl. The tablets may be coated.

**Identification**

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to diltiazem hydrochloride in the chromatogram obtained with the reference solution.

**Tests**

**Other tests.** Comply with the tests stated under Tablets.

**Dissolution (2.5.2).**

**Apparatus.** No 1

**Medium.** 900 ml of freshly distilled water

**Speed and time.** 100 rpm and 30 minutes and 3 hours

Withdraw a suitable volume of the medium after 30 minutes and 3 hours. Filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate. Dilute a suitable volume of the filtrate with the same solvent and measure the absorbance of the resulting solution at the maximum at about 240 nm (2.4.7). Calculate the content of C$_{22}$H$_{26}$N$_2$O$_4$S,HCl from the absorbance obtained from a solution of known concentration of diltiazem hydrochloride RS.

Use the following acceptance criteria for the 30-minute time interval. At $S_1$: no unit is more than $D$; at $S_2$, the average value is equal to or less than $D$, and no unit is greater than $D + 10$ per cent; at $S_3$, the average value is equal to or less than $D$, not more than 2 units are more than $D + 10$ per cent and no unit is more than $D + 25$ per cent. Use the acceptance criteria in Acceptance Table 1(2.5.2) for the 3-hour time interval.

D. Not less than 60 per cent of the stated amount of C$_{22}$H$_{26}$N$_2$O$_4$S,HCl is dissolved in 30 minutes and not less than 80 per cent is dissolved in 3 hours.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.6 g of Diltiazem Hydrochloride, add 200 ml of methanol, mix with the aid of ultrasound for 1 hour, cool and dilute to 500.0 ml with methanol; centrifuge 25 ml at 3500 rpm for 15 minutes and use the clear, supernatant liquid.

**Reference solution (a).** A solution containing 0.0012 per cent w/v each of diltiazem hydrochloride RS and desacetyl diltiazem hydrochloride RS in methanol.

**Reference solution (b).** Dissolve 60 mg of diltiazem hydrochloride RS in 50 ml of methanol.

**Chromatographic system**

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 50 volumes of a buffer solution containing 0.116 per cent w/v of d-10-camphorsulphonic acid in 0.1 M sodium acetate, with the pH adjusted to 6.2 by the addition of 0.1 M sodium hydroxide, 25 volumes of acetonitrile and 25 volumes of methanol, filtered and degassed,
- flow rate. 1.6 ml per minute,
- spectrophotometer set at 240 nm,
- a 10 µl loop injector.

Inject reference solution (a) and measure the peak responses of all the peaks. The relative retention times for desacetyl...
diltiazem and diltiazem are about 0.65 and 1.0 respectively. The resolution between desacetyl diltiazem and diltiazem is not less than 3, and the theoretical plates for the diltiazem peak is not less than 1200.

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and reference solution (b). Calculate the content of C\(_{22}\)H\(_{26}\)N\(_2\)O\(_4\)S.HCl in the tablets.

**Storage.** Store protected from light.

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**Dimercaprol**

B.A.L.

![Dimercaprol molecule](image)

C\(_{10}\)H\(_8\)O\(_2\)S \quad \text{Mol. Wt. 124.2}

Dimercaprol is (RS)-2,3-dimercaptopropanol.

Dimercaprol contains not less than 98.5 per cent w/w and not more than 101.5 per cent w/w of C\(_{10}\)H\(_8\)O\(_2\)S.

**Description.** A clear, colourless or slightly yellow liquid; odour, strong, characteristic and alliaceous.

**Identification**

A. Dissolve 0.1 ml in 4 ml of water and to 2 ml of the solution add lead acetate solution; a yellow precipitate is obtained.

B. To 2 ml of the solution prepared for test A add 1 ml of 0.05 M iodine; the colour of iodine is immediately discharged.

C. In a ground-glass-stoppered tube suspend 0.6 g of sodium bismuthate, previously heated to 200° for 2 hours, in a mixture of 6 ml of water and 2.8 ml of a 10 per cent w/w solution of phosphoric acid. Add 0.2 ml of the substance under examination, mix and allow to stand for 10 minutes shaking frequently. To 1 ml of the supernatant liquid add 5 ml of a 0.4 per cent w/v solution of chromotropic acid sodium salt in sulphuric acid, mix and heat for 15 minutes in a water-bath; a violet-red colour is produced.

**Tests**

**Appearance of solution.** The substance under examination is clear (2.4.1), and not more intensely coloured than reference solution BS6 or BYS6 (2.4.1).

**pH** (2.4.24). 5.0 to 6.5, determined in a saturated solution.

**Refractive index** (2.4.27). 1.568 to 1.574, determined at 20°.

**Weight per ml** (2.4.29). 1.238 g to 1.240 g.

**Iron** (2.3.14). Ignite 2.0 g with 1 g of anhydrous sodium carbonate, cool, dissolve the residue in 15 ml of dilute hydrochloric acid and dilute to 45 ml with water; the resulting solution complies with the limit test for iron (20 ppm).

**Halides.** To 2.0 g add 25 ml of 0.5 M ethanolic potassium hydroxide and heat under a reflux condenser for 2 hours. Remove the ethanol by evaporation in a current of warm air, add 20 ml of water and cool. Add a mixture of 10 ml of strong hydrogen peroxide solution and 40 ml of water. Boil gently for 10 minutes; cool and filter rapidly. Add 10 ml of dilute nitric acid and 5 ml of 0.1 M silver nitrate and titrate with 0.1 M ammonium thiocyanate using ferric ammonium sulphate solution as indicator. Repeat the operation without the substance under examination. The difference in the volumes of 0.1 M ammonium thiocyanate used in the two titrations is not more than 1.0 ml.

**Assay.** Weigh accurately about 0.1 g, dissolve in 40 ml of methanol and add 20 ml of 0.1 M hydrochloric acid and 50.0 ml of 0.05 M iodine. Allow to stand for 10 minutes and titrate with 0.1 M sodium thiosulphate. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of iodine required. 1 ml of 0.05 M iodine is equivalent to 0.00621 g of C\(_{10}\)H\(_8\)O\(_2\)S.

**Storage.** Store protected from light in well-filled containers in a refrigerator (2° to 8°).

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**Dimercaprol Injection**

B.A.L. Injection

Dimercaprol injection is a sterile solution of Dimercaprol in a mixture of Benzyl Benzoate and Arachis Oil.

Dimercaprol Injection contains not less than 90.0 per cent and more than 110.0 per cent of the stated amount of dimercaprol, C\(_{10}\)H\(_8\)O\(_2\)S.

**Description.** A clear, yellow, viscous solution, having a pungent, disagreeable odour.

**Tests**

**Acidity.** Shake with an equal volume of water for 2 minutes and set aside for separation; pH of the aqueous layer after filtration through a neutral filter is 4.5 to 6.5 (2.4.24).

**Refractive index** (2.4.27). 1.481 to 1.486, determined at 20°.

**Weight per ml** (2.4.29). About 0.95 g.

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).
Activated Dimethicone

Simethicone; Activated Polydimethylsiloxane

\[
\text{H}_3\text{C}\left[\begin{array}{c}
\text{Si}-\text{O}\
\text{Si}\text{-(CH}_3\text{)}_2\text{Si}-\
\text{Si}\text{-(CH}_3\text{)}_2\text{SiO}_2
\end{array}\right]_n \text{CH}_3 + \text{SiO}_2
\]

\[(\text{CH}_3)_3\text{Si}[\text{OSi(CH}_3\text{)}_2\text{]CH}_3+\text{SiO}_2\]

Activated Dimethicone is a mixture of fully methylated linear siloxane polymers containing repeating \((\text{CH}_3)_3\text{SiO-}\) units stabilised with trimethylsiloxy, \((\text{CH}_3)_3\text{SiO-}\), end-blocking units and finely divided silicon dioxide.

Activated Dimethicone contains not less than 90.0 per cent and not more than 99.0 per cent of polydimethylsiloxane, \([\text{-(CH}_3\text{)}_3\text{SiO-}]_n\), and not less than 4.0 per cent and not more than 7.0 per cent of silicon dioxide, \(\text{SiO}_2\).

Description. A translucent, grey viscous liquid; almost odourless.

Identification

A. To 50 mg add 25 ml of carbon tetrachloride and swirl to disperse. Add 50 ml of dilute hydrochloric acid and shake for 5 minutes. Transfer to a separating funnel and remove about 5 ml of the lower layer to a stoppered tube containing 0.5 g of anhydrous sodium sulphate. Shake vigorously and centrifuge the mixture until a clear supernatant liquid is obtained. The resulting liquid complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with dimethicone RS. Ignore the region of the spectrum from 850 to 750 cm\(^{-1}\) since slight differences may be observed depending on the degree of polymerisation.

B. Heat 0.5 g in a test-tube over a small flame until white fumes are evolved. Invert the test-tube over a second tube containing 1 ml of a 0.1 per cent w/v solution of chromotropic acid sodium salt in sulphuric acid so that the fumes reach the solution. Shake the second tube for about 10 seconds and heat on a water-bath for 5 minutes; the solution is violet.

C. To 50 mg in a platinum crucible add 0.15 ml of sulphuric acid and ignite until a white residue is obtained; the residue gives the reaction of silicates (2.3.1).

Tests

Acidity. To 2.0 g add 25 ml of a mixture of equal volumes of ethanol and ether previously neutralised to 0.2 ml of bromothymol blue solution and shake; not more than 0.15 ml of 0.01 M sodium hydroxide is required to change the colour of the solution to blue.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Defoaming activity. Weigh accurately about 0.2 g, transfer to a 100-ml bottle, add 50 ml of 2-methylpropanol and shake vigorously, warming, if necessary, to effect solution. Add dropwise, 0.5 ml of this solution to a clean, unused, cylindrical 250-ml glass jar, fitted with a 50-mm cap, containing 100 ml of a 1.0 per cent w/v solution of octoxinol. Cap the jar and clamp it in an upright position in a wrist-action shaker capable of moving the jar through a radius of 13.3 ± 0.4 cm (measured from the centre of the shaft to the centre of the jar) and an arc of 10 degrees at a frequency of 300 ± 30 strokes per minute. Shake for 10 seconds and record the time required in seconds for the foam to collapse. The time for foam collapse is determined at the instant the first portion of foam-free liquid surface appears, measured from the end of the shaking period. The defoaming activity time is not more than 15 seconds.

Assay. For polydimethylsiloxane - Weigh accurately about 50 mg, transfer to a narrow-mouthed glass bottle and add 25 ml of carbon tetrachloride. Swirl to disperse, add 50 ml of dilute hydrochloric acid, close the bottle securely with a cap having an inert liner and shake for exactly 5 minutes. Transfer the mixture to a 125-ml separating funnel and remove about 5 ml of the lower layer to a stoppered test-tube containing 0.5 g of anhydrous sodium sulphate. Close the test-tube, agitate vigorously and centrifuge the mixture until a clear supernatant liquid is obtained. Prepare a blank by mixing 10 ml of carbon tetrachloride with 0.5 g of anhydrous sodium sulphate and centrifuging to obtain a clear supernatant liquid. Determine the absorbance of a 0.5-mm layer of the solution at the maximum at about 7.9 µm in a suitable infra-red spectrophotometer (2.4.6), using the blank to set the instrument. Calculate the content of \([\text{-(CH}_3\text{)}_3\text{SiO-}]_n\), from the absorbance obtained by repeating the Assay on a 0.2 per cent w/v solution of dimethicone RS in place of the substance under examination.

For silicon dioxide - Mix thoroughly and weigh accurately about 1.0 g; transfer to a tared, sintered-glass filtering crucible.
(porosity No. 4) and pass through the filter, with suction, 200 ml of carbon tetrachloride, added with stirring in small portions, followed by similar washing of the material on the filter with 200 ml of n-hexane, and discard the filtrates. Place the filtering crucible in a muffle furnace at room temperature, raise the temperature of the furnace to 550°. Heat at 550° ± 25° for 2 hours. Cool the filtering crucible with its contents in a desiccator, weigh and calculate the content of silicon dioxide, SiO₂, in the sample taken.

Diphenhydramine Hydrochloride

Diphenhydramine Hydrochloride is 2-benzhydryloxyethylidimethylamine hydrochloride.

Diphenhydramine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of C₁₇H₂₁NO.HCl, calculated on the dried basis.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with diphenhydramine hydrochloride RS or with the reference spectrum of diphenhydramine hydrochloride. Examine the substances as discs prepared using potassium chloride IR.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.025 per cent w/v solution in ethanol (95 per cent) shows absorption maxima at about 253 nm, 258 nm and 264 nm; absorbance at about 253 nm, about 0.31, at about 258 nm, about 0.38 and at about 264 nm, about 0.3.

C. To 0.05 ml of a 5 per cent w/v solution add 2 ml of sulphuric acid; an intense yellow colour develops which changes to red on the addition of 0.5 ml of nitric acid. Add 15 ml of water, cool, add 5 ml of chloroform and shake; an intense violet colour develops in the chloroform layer.

D. Gives the reactions of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in carbon dioxide-free water, and a 5-fold dilution thereof, are both clear (2.4.1). The 5.0 per cent solution is not more intensely coloured than reference solution BYS6 (2.4.1).

pH (2.4.24). 4.0 to 6.0, determined in a 5.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel H.

Mobile phase. A mixture of 80 volumes of chloroform, 20 volumes of methanol and 1 volume of diethylamine.

Test solution. Dissolve 0.2 g in 10 ml of methanol immediately before use.

Reference solution. Dilute 1 ml of the test solution to 100 ml with methanol immediately before use.

Apply to the plate 5 μl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air for 5 minutes, spray with sulphuric acid and heat at 120° for 10 minutes until spots appear. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.5 g, dissolve in 20 ml of anhydrous glacial acetic acid and add 10 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid, using crystal violet solution as indicator. Carry out a blank titration. 1 ml of 0.1 M perchloric acid is equivalent to 0.02918 g of C₁₇H₂₁NO.HCl.

Storage. Store protected from light.

Diphenhydramine Capsules

Diphenhydramine Capsules contain not less than 93.0 per cent and not more than 107.0 per cent of the stated amount of diphenhydramine hydrochloride, C₁₇H₂₁NO.HCl.

Identification

Extract a quantity of the contents of the capsules containing 0.1 g of Diphenhydramine Hydrochloride with two quantities, each of 15 ml, of chloroform. Evaporate the combined extracts to dryness on a water-bath and dry the residue at 80° for 1 hour. The residue melts at about 168° (2.4.21), and complies with the following tests.
A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with diphenhydramine hydrochloride RS or with the reference spectrum of diphenhydramine hydrochloride. Examine the substances as discs prepared using potassium chloride IR.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.025 per cent w/v solution in ethanol (95 per cent) shows absorption maxima at about 253 nm, 258 nm and 264 nm; absorbance at about 253 nm, about 0.31, at about 258 nm, about 0.38 and at about 264 nm, about 0.3.

C. To 0.05 ml of a 5 per cent w/v solution add 2 ml of sulphuric acid; an intense yellow colour develops which changes to red on the addition of 0.5 ml of nitric acid. Add 15 ml of water, cool, add 5 ml of chloroform and shake; an intense violet colour develops in the chloroform layer.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel H.

Mobile phase. A mixture of 80 volumes of chloroform, 20 volumes of methanol and 1 volume of diethylamine.

Test solution. Shake a quantity of the contents of the capsules containing 100 mg of Diphenhydramine Hydrochloride with three quantities, each of 10 ml, of chloroform, filter and evaporate the combined filtrate almost to dryness; dissolve the residue in 5 ml of chloroform.

Reference solution. Dilute 1 ml of the test solution to 100 ml with chloroform.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air for 5 minutes, spray with sulphuric acid and heat at 120° for 10 minutes until spots appear. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Other tests. Comply with the tests stated under Capsules.

Assay. Weigh accurately a quantity of the mixed contents of 20 capsules and transfer to a 100-ml volumetric flask, add sufficient water to produce 100.0 ml, shake well to dissolve and filter. To an accurately measured volume of the filtrate containing 0.3 g of Diphenhydramine Hydrochloride add 5 g of sodium chloride and 5 ml of sodium hydroxide solution and extract with successive quantities, each of 20 ml, of ether until complete extraction is effected. Wash the combined extracts with two quantities, each of 5 ml, of water, extract the combined washings with two quantities, each of 10 ml, of ether, add the ether to the combined ether extracts and evaporate to about 10 ml. Add 25.0 ml of 0.1 M hydrochloric acid, warm gently to complete the removal of ether, cool and titrate the excess of acid with 0.1 M sodium hydroxide using methyl red solution as indicator.

1 ml of 0.1 M hydrochloric acid is equivalent to 0.02918 g of C_{17}H_{21}NO,HCl.

Storage. Store protected from moisture.

Diphenoxylate Hydrochloride

C_{30}H_{32}N_{2}O_{2},HCl

Mol. Wt. 489.1

Diphenoxylate Hydrochloride is ethyl 1-(3-cyano-3,3-diphenylpropyl)-4-phenylpiperidine-4-carboxylate hydrochloride.

Diphenoxylate Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of C_{30}H_{32}N_{2}O_{2},HCl, calculated on the dried basis.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with diphenoxylate hydrochloride RS or with the reference spectrum of diphenoxylate hydrochloride.

B. Dissolve about 30 mg in 5 ml of methanol, add 0.25 ml of nitric acid and 0.4 ml of silver nitrate solution. Shake and allow to stand; a curdled precipitate is formed. Centrifuge and rinse the precipitate with three quantities, each of 2 ml, of methanol. Carry out this operation rapidly in subdued light. Suspend the precipitate in 2 ml of water and add 1.5 ml of 10 M ammonia; the precipitate dissolves easily.

C. Gives reaction A of chlorides (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution in dichloromethane is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1). Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with a suitable octadecylsilyl silica gel (5 µm) containing a fluorescent indicator with a maximum intensity at 254 nm.
Mob *ile phase. A mixture of 60 volumes of *dioxan, 30 volumes of a 5.9 per cent w/v solution of sodium chloride and 10 volumes of methanol.

**Test solution.** Dissolve 1 g of the substance under examination in a mixture of 1 volume of methanol and 2 volumes of dichloromethane and dilute to 20 ml with the same solvent mixture.

Reference solution (a). Dilute 0.5 ml of the test solution to 100 ml with the same solvent mixture.

Reference solution (b). Dissolve 0.5 g of the substance under examination in 25 ml of a 1.5 per cent w/v solution of potassium hydroxide in methanol and add 1 ml of water. Heat on a water-bath under a reflux condenser for 4 hours. Cool and add 25 ml of 0.5 M hydrochloric acid and shake with 100 ml of dichloromethane. Evaporate the organic layer to dryness on a water-bath. Dissolve the residue in 10 ml of a mixture of 1 volume of methanol and 2 volumes of dichloromethane, add 10 ml of test solution and dilute to 25 ml with a mixture of 1 volume of methanol and 2 volumes of dichloromethane.

Apply to the plate 1 µl of each solution. Allow the mobile phase to rise 7 cm in an unsaturated tank. Dry the plate in an oven at 160° for 15 minutes and place the plate while hot in a closed tank containing 20 ml of fuming nitric acid for 30 minutes. Remove the plate and heat it again at 160° for 15 minutes. Allow to cool and examine immediately in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated principal spots.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.4 g, dissolve in 40 ml of ethanol (95 per cent) and add 5 ml of 0.01 M hydrochloric acid. Titrate with 0.1 M ethanolic sodium hydroxide, determining the end-point potentiometrically (2.4.25). Read the volume added between the two points of inflection.

1 ml of 0.1 M ethanolic sodium hydroxide is equivalent to 0.04891 g of C$_{30}$H$_{32}$N$_2$O$_2$HCl.

**Storage.** Store protected from light.

**Disodium Edetate**

![Disodium Edetate structure](image_url)

Disodium Edetate is disodium ethylenediaminetetraacetate dihydrate.

Disodium Edetate contains not less than 98.5 per cent and not more than 101.0 per cent of C$_{10}$H$_{14}$N$_2$Na$_2$O$_8$2H$_2$O.

**Description.** A white, crystalline powder; odourless.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with disodium edetate RS.

B. Dissolve 2 g in 25 ml of water, add 6 ml of lead nitrate solution, shake and add 3 ml of potassium iodide solution; no yellow precipitate is produced. Make alkaline to red litmus paper with 2 M ammonia and add 5 ml of ammonium oxalate solution; no precipitate is produced.

C. Dissolve 0.5 g in 10 ml of water, add 0.5 ml of a 10 per cent w/v solution of calcium chloride, make alkaline to red litmus paper with 2 M ammonia and add 3 ml of ammonium oxalate solution; no precipitate is produced.

D. Gives the reactions of sodium salts (2.3.1).

**Tests**

**Appearance of solution.** A 5.0 per cent w/v solution in carbon dioxide-free water is clear (2.4.1), and colourless (2.4.1).

**pH** (2.4.24). 4.0 to 5.5, determined in a 5.0 per cent w/v solution.

**Heavy Metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

**Iron** (2.3.14). 20 ml of a 2.5 per cent w/v solution complies with the limit test for iron (80 ppm). Add 0.25 g of calcium chloride to each solution before adding mercaptoacetic acid.

**Assay.** Weigh accurately about 0.5 g, dissolve in sufficient water to produce 300 ml and add 2 g of hexamine and 2 ml of 2 M hydrochloric acid. Titrate with 0.1 M lead nitrate using about 50 mg of xylénol orange triturate as indicator.

1 ml of 0.1 M lead nitrate is equivalent to 0.03722 g of C$_{10}$H$_{14}$N$_2$Na$_2$O$_8$2H$_2$O.

**Disodium Edetate Injection**

Disodium Edetate Injection is a sterile solution of Disodium Edetate in Water for Injections, containing varying amounts of the disodium and trisodium salts as a result of pH adjustment.

Disodium Edetate Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of disodium edetate, C$_{10}$H$_{14}$N$_2$Na$_2$O$_8$. 
Identification
To a volume containing about 3 g of Disodium Edetate add 3 M hydrochloric acid to adjust the pH to 5.0 and evaporate to dryness on a steam-bath to dryness. The residue so obtained complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with disodium edetate RS.

B. Dissolve 2 g in 25 ml of water, add 6 ml of lead nitrate solution, shake and add 3 ml of potassium iodide solution; no yellow precipitate is produced. Make alkaline to red litmus paper with 2 M ammonia and add 5 ml of ammonium oxalate solution; no precipitate is produced.

C. Dissolve 0.5 g in 10 ml of water, add 0.5 ml of a 10 per cent w/v solution of calcium chloride, make alkaline to red litmus paper with 2 M ammonia and add 3 ml of ammonium oxalate solution; no precipitate is produced.

Tests
pH (2.4.24). 6.5 to 7.5.

Bacterial endotoxins (2.2.3). Not more than 0.2 Endotoxin Unit per mg of disodium edetate.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Dilute an accurately measured volume containing about 0.6 g of Disodium Edetate with water to produce 100 ml, mix and add 2 g of hexamine and 2 ml of 2 M hydrochloric acid. Titrate with 0.1 M lead nitrate using about 50 mg of xylenol orange triturate as indicator.

1 ml of 0.1 M lead nitrate is equivalent to 0.03722 g of C10H14N2Na2O8,2H2O.

Storage. Store in single dose containers.

Labelling. The label states the strength in terms of anhydrous disodium edetate contained in a suitable dose-volume.

Disulfiram

\[
\begin{align*}
&\text{C}_{10}\text{H}_{18}\text{N}_{2}\text{S}_{4} \\
&\text{Mol. Wt. 296.5}
\end{align*}
\]

Disulfiram is tetraethylthiuram disulphide.

Disulfiram contains not less than 98.5 per cent and not more than 101.0 per cent of C_{10}H_{18}N_{2}S_{4}, calculated on the dried basis.

Description. A white or almost white, crystalline powder.

Identification
Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with disulfiram RS or with the reference spectrum of disulfiram.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Dissolve about 10 mg in 10 ml of methanol and add 2 ml of a 0.05 per cent w/v solution of cupric chloride in methanol; a yellow colour is produced which changes to greenish-yellow.

Tests
Diethyldithiocarbamate. Dissolve 0.2 g in 10 ml of ether, add 5 ml of phosphate buffer pH 8.0, shake vigorously, reject the ether layer and wash the aqueous layer with 10 ml of ether. To the aqueous layer add 0.2 ml of a 0.4 per cent w/v solution of cupric sulphate and 5 ml of carbon tetrachloride and shake well. Any yellow colour in the carbon tetrachloride layer is not more intense than that of a standard prepared at the same time and in the same manner using 0.2 ml of a freshly prepared 0.015 per cent w/v solution of sodium diethyldithiocarbamate in place of the substance under examination (150 ppm).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 70 volumes of n-hexane and 30 volumes of butyl acetate.

Test solution (a). Dissolve 0.2 g of the substance under examination in 10 ml of ethyl acetate.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with ethyl acetate.

Reference solution (a). Dissolve 0.2 g of disulfiram RS in ethyl acetate.

Reference solution (b). A 0.2 per cent w/v solution of disulfiram RS with ethyl acetate.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method C (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.
Loss on drying. Not more than 0.5 per cent, determined on 1.0 g by drying in an oven over phosphorus pentoxide at 50° at a pressure of 1.5 to 2.5 kPa.

Assay. Weigh accurately about 0.45 g and dissolve in 80 ml of acetone. Add 20 ml of a 2 per cent w/v solution of potassium nitrate. Titrate with 0.1 M silver nitrate determining the end-point potentiometrically, using a silver indicator electrode and a silver-silver chloride double-junction electrode saturated with potassium nitrate.

1 ml of 0.1 M silver nitrate is equivalent to 0.05930 g of C_{10}H_{20}N_{2}S_{4}.

Storage. Store protected from light.

Disulfiram Tablets

Disulfiram Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of disulfiram, C_{10}H_{20}N_{2}S_{4}.

Identification

A. Extract a quantity of the powdered tablets containing 0.2 g of Disulfiram by boiling with 5 ml of carbon tetrachloride, filter and evaporate the filtrate to dryness. The residue, after drying at 40° at a pressure not exceeding 0.7 kPa, complies with the following test.

Determine by infrared absorption spectrophotometry. Compare the spectrum with that obtained with disulfiram RS or with the reference spectrum of disulfiram.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with the reference solution.

C. Extract a quantity of the powdered tablets containing 0.3 g of Disulfiram with ethanol (95 per cent), filter and evaporate the filtrate to dryness. Dissolve the residue in 5 ml of ethanol (95 per cent), add 1 ml of potassium cyanide solution; a yellow colour is produced which changes to green and then darkens to bluish-green.

Tests

Diethyldithiocarbamate. Shake a quantity of the powdered tablets containing 0.1 g of Disulfiram with 10 ml of chloroform and filter. To the filtrate add 10 ml of 0.1 M sodium hydroxide, shake, reject the chloroform layer and wash the aqueous layer with three quantities, each of 10 ml, of chloroform. To the aqueous layer add 0.25 ml of a 0.4 per cent w/v solution of cupric sulphate and 2 ml of carbon tetrachloride, shake and allow to separate. The lower layer is not more intensely coloured than reference solution BYS4 (2.4.1).

Related substances. Determine by thin-layer chromatography, coating the plate with silica gel GF254.

Mobile phase. A mixture of 70 volumes of n-hexane and 30 volumes of butyl acetate.

Test solution (a). Extract a quantity of the powdered tablets containing 0.5 g of Disulfiram with 20 ml of ethyl acetate and filter.

Test solution (b). Dilute 1 ml of test solution (a) to 100 ml with ethyl acetate.

Reference solution. A 0.025 per cent w/v solution of disulfiram RS in ethyl acetate.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with the reference solution.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.4 g of Disulfiram and shake with 75 ml of methanol for 30 minutes. Add sufficient methanol to produce 100.0 ml, mix, filter and dilute 5.0 ml of the filtrate to 100.0 ml with methanol. To 5.0 ml add sufficient of a 0.1 per cent w/v solution of cupric chloride in methanol to produce 25.0 ml, mix and allow to stand for 1 hour. Measure the absorbance of the resulting solution at the maximum at about 400 nm (2.4.7), using as the blank a solution prepared by diluting 5.0 ml of methanol to 25.0 ml with the cupric chloride solution. Calculate the content of C_{10}H_{20}N_{2}S_{4} from the absorbance obtained by repeating the operation using 5.0 ml of 0.02 per cent w/v solution of disulfiram RS in methanol beginning at the words “add sufficient of a 0.1 per cent w/v solution of cupric chloride...”.

Storage. Store protected from light.

Dithranol

Anthralin; Dioxyanthranol

Dithranol is 1,8-dihydroxyanthrone.

Dithranol contains not less than 98.5 per cent and not more than 101.0 per cent of C_{14}H_{10}O_{3}, calculated on the dried basis.
**Description.** A yellow or orange-yellow, microcrystalline powder; odourless or almost odourless.

**Identification**
A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dithranol RS* or with the reference spectrum of dithranol.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in chloroform shows absorption maxima at about 255 nm, 287 nm and 354 nm; absorbances at the maxima, about 0.55, 0.5 and 0.45 respectively.

C. Melts at about 178° (2.4.21).

**Tests**

**Dihydroxyanthracecne.** Dissolve 0.1 g in 5 ml of hot benzene; a clear yellow or orange solution is produced.

**Dihydroxyanthraquinone.** Dissolve 1 mg in a few drops of sulphuric acid; an orange solution with no trace of violet colour is produced.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.2 g and dissolve in 50 ml of anhydrous pyridine. Titrate with 0.1 M tetrabutylammonium hydroxide, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M tetrabutylammonium hydroxide is equivalent to 0.02262 g of C_{14}H_{10}O_{3}.

**Storage.** Store protected from light.

---

**Dithranol Ointment**

Dithranol Ointment contains Dithranol, in fine powder, in a suitable base.

Dithranol Ointment contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dithranol, C_{14}H_{10}O_{3}.

**Identification**
A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Heat a quantity containing 0.5 mg of Dithranol with 5 ml of 1 M sodium hydroxide on a water-bath with constant stirring; a pink colour is produced in the aqueous layer.

**Tests**

**Dihydroxyanthracecne.** Dissolve a quantity containing 0.1 g of Dithranol in 5 ml of hot benzene; a yellow or orange solution is produced.

**Dihydroxyanthraquinone.** Dissolve a quantity containing 1 mg of Dithranol in a few drops of sulphuric acid; an orange solution with no trace of violet colour is produced.

**Other tests.** Complies with the tests stated under Ointments.

**Assay.** Determine by liquid chromatography (2.4.14)

**Test solution.** Weigh accurately a quantity of the ointment containing about 5 mg of Dithranol, disperse in 20 ml of dichloromethane, add 1.0 ml of glacial acetic acid, dilute to 100.0 ml with hexane and filter.

**Reference solution.** Add 1.0 ml of glacial acetic acid to 20.0 ml of a 0.025 per cent w/v solution of *dithranol RS* in dichloromethane and add sufficient hexane to produce 100.0 ml.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm, packed with porous silica particles (5 µm),
- mobile phase: a mixture of 82 volumes of hexane, 5 volumes of dichloromethane and 1 volume of glacial acetic acid,
- flow rate. 2 ml per minute,
- spectrophotometer set at 354 nm,
- a 20 µl loop injector.

Inject the test solution and reference solution. Calculate the content of C_{14}H_{10}O_{3} in the ointment.

**Storage.** Store protected from light.

---

**Docusate Sodium**

Dioctyl Sodium Sulphosuccinate

\[
\text{Na}^+ \quad \begin{array}{c} \text{H}_3\text{C} \\ \text{H}_{3}\text{C} \\ \text{O} \end{array} \quad \begin{array}{c} \text{O} \quad \text{SO}_3 \quad \text{Na} \\ \text{CH}_3 \\ \text{CH}_3 \end{array} 
\]

C_{39}H_{75}NaO_{8}S  
Mol. Wt. 444.6

Docusate Sodium is sodium 1,4-bis[(2-ethylhexyl)oxy]-1,4-dioxobutane-2-sulphonate.

Docusate Sodium contains not less than 98.0 per cent and not more than 101.0 per cent of C_{39}H_{75}NaO_{8}S, calculated on the anhydrous basis.

**Description.** White or almost white, waxy masses or flakes, hygroscopic.
Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with docusate sodium RS.

B. Ignite 0.75 g in the presence of dilute sulphuric acid, until an almost white residue is obtained. Cool and add 5 ml of water. Filter. 2 ml of the filtrate gives reaction (a) of sodium salts (2.3.1).

Tests

Alkalinity. Dissolve 1.0 g in 100 ml of a mixture of equal volumes of methanol and water, previously neutralised to methyl red solution. Add 0.1 ml of methyl red solution. Not more than 0.2 ml of 0.1 M hydrochloric acid is required to change the colour of the indicator to red.

Related non-ionic substances. Determine by gas chromatography (2.3.13).

Internal standard solution. Dissolve 10 mg of methyl behenate in 50 ml of hexane.

Test solution (a). Dissolve 0.1 g of the substance under examination in 2.0 ml of the internal standard solution and dilute to 5.0 ml with hexane. Pass the solution, at a rate of about 1.5 ml per minute, through a column 10 mm in internal diameter, packed with 5 g of basic aluminium oxide and previously washed with 25 ml of hexane. Elute with 5 ml of hexane and discard the eluate. Elute with 20 ml of a mixture of equal volumes of ether and hexane. Evaporate the eluate to dryness and dissolve the residue in 2.0 ml of hexane.

Test solution (b). Prepare as described for test solution (a) but dissolving 0.1 g of the substance under examination in 5.0 ml of hexane and using a new column.

Reference solution. Dilute 2.0 ml of the internal standard solution to 5.0 ml with hexane.

Chromatographic system
- a glass column 2 m x 2 mm, packed with silanised diatomaceous earth for gas chromatography (150 µm to 180 µm) impregnated with 3 per cent m/m of polymethylphenylsiloxane,
- temperature : column 230°, inlet port and detector at 280°,
- flow rate: 30 ml per minute of the nitrogen carrier gas.

Inject 1 ml of the test solution (a), (b) and the reference solution. There is no peak with the same retention time as the internal standard in the chromatogram obtained with test solution (b). The area of any impurity peak is not more than the area of the peak due to the internal standard (0.4 per cent).

Chlorides (2.3.12). Dissolve 5.0 g in 50 ml of alcohol (50 per cent v/v) and add 0.1 ml of potassium dichromate solution. Not more than 0.5 ml of 0.1 M silver nitrate is required to change the colour of the indicator from yellow to orange (350 ppm).

Sodium sulphate. Not more than 2 per cent.

Dissolve 0.25 g in 40 ml of a mixture of 20 volumes of water and 80 volumes of 2-propanol. Adjust to pH between 2.5 and 4.0 using perchloric acid solution. Add 0.4 ml of naphtharson solution and 0.1 ml of 0.0125 per cent w/v solution of methylene blue. Not more than 1.5 ml of 0.025 M barium perchlorate is required to change the colour of the indicator from yellowish-green to yellowish-pink.

Heavy metals (2.3.13). Dissolve 4.0 g in 20 ml of alcohol (80 per cent v/v). 12 ml of the solution complies with the limit test for heavy metals, Method B (10 ppm).

Water (2.3.43). Not more than 3.0 per cent, determined on 0.25 g.

Assay. Weigh accurately about 1.0 g, dissolve in 25.0 ml of 0.5 M alcoholic potassium hydroxide and heat on a water-bath under reflux for 45 minutes. Cool, add 0.25 ml of phenolphthalein solution and titrate with 0.5 M hydrochloric acid until the red colour disappears. Carry out a blank titration.

1 ml of 0.5 M hydrochloric acid is equivalent to 0.1112 g of C_{22}H_{24}ClN_{5}O_{2}, calculated on the dried basis.

Storage. Store protected from moisture.

Domperidone Maleate

C_{22}H_{24}ClN_{5}O_{2}C_{4}H_{4}O_{4}, Mol. Wt. 542.0

Domperidone Maleate is 5-chloro-1-[1-[3-(2,3-dihydro-2-oxo-1H-benzimidazol-1-yl)propyl]-4-piperidinyl]-1,3-dihydro-2H-benzimidazol-2-one maleate.

Domperidone Maleate contains not less than 99.0 per cent and not more than 101.0 per cent of domperidone maleate, C_{22}H_{24}ClN_{5}O_{2}C_{4}H_{4}O_{4}, calculated on the dried basis.

Description. A white or almost white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with domperidone.
maleate RS or with the reference spectrum of domperidone maleate. If the spectra obtained show differences, dissolve the substance under examination and the reference substance separately in the minimum volume of 2-propanol, evaporate to dryness on a water-bath and record new spectra using the residues.

**Tests**

**Appearance of solution.** Dissolve 0.2 g in dimethylformamide and dilute to 20.0 ml with the same solvent. The solution is clear (2.4.1) and not more intensely coloured than reference solution Y6 (2.4.1).

**Related substances.** Determine by liquid chromatography (2.4.14).

*NOTE - Prepare the solutions immediately before use.*

**Test solution.** Dissolve 0.1 g of the substance under examination in dimethylformamide and dilute to 10 ml with the same solvent.

**Reference solution (a).** Dissolve 10 mg of domperidone maleate RS and 15 mg of droperidol RS in dimethylformamide and dilute to 100 ml with the same solvent.

**Reference solution (b).** Dilute 1 ml of the test solution to 100 ml with dimethylformamide. Dilute 5 ml of the solution to 20 ml with dimethylformamide.

**Chromatographic system**
- a stainless steel column 10 cm x 4.6 mm, packed with base-deactivated octadecylsilyl silica gel (3 µm),
- mobile phase: a mixture of 3 volumes of methanol and 7 volumes of a 0.5 per cent w/v solution of ammonium acetate,
- flow rate. 1.5 ml per minute,
- a linear gradient programme with the mobile phase changing to methanol over 10 minutes, followed by elution with methanol for 2 minutes,
- spectrophotometer set at 280 nm,
- a 10 µl loop injector.

Equilibrate the column for at least 30 minutes with methanol and then equilibrate with the initial mobile phase.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is at least 50 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are: domperidone maleate, about 6.5 minutes and droperidol, about 7 minutes. The test is not valid unless the resolution between the peaks due to domperidone maleate and droperidol is at least 2.0. If necessary adjust the concentration of methanol in the mobile phase or adjust the time programme for the linear gradient.

Inject dimethylformamide as a blank, the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent); the sum of the areas of all peaks, other than the principal peak, is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak in the chromatogram obtained with the blank run, any peak due to maleic acid at the beginning of the chromatogram and any peak with an area less than 0.2 times that of the principal peak in the chromatogram obtained with reference solution (b).

**Heavy metals** (2.3.13.). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm). Prepare the standard using 2 ml of lead standard solution (10 ppm Pb).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 100º to 105º.

**Assay.** Dissolve 0.4 g in 50 ml of anhydrous acetic acid. Titrate with 0.1 M perchloric acid using 0.2 ml of naphtholbenzein solution as indicator, until the colour changes from orange-yellow to green.

1 ml of 0.1 M perchloric acid is equivalent to 0.0542 g of C₂₆H₂₈ClN₅O₆.

**Storage.** Store protected from light.

**Domperidone Tablets**

Domperidone Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of domperidone, C₂₂H₂₄ClN₅O₂.

**Identification**

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel F254 or using a precoated plate (such as Merck silica gel 60 F254 plates).

**Mobile phase.** A mixture of 5 volumes of a solution prepared by dissolving 1.36 g of sodium acetate in 50 ml of water, adjusting the pH to 4.7 with dilute acetic acid and adding sufficient water to produce 100 ml, 18 volumes of methanol, 23 volumes of dichloromethane and 54 volumes of ethyl acetate.

**Test solution.** Shake a quantity of the powdered tablets containing 10 mg of domperidone with 10 ml of a mixture of
equal volumes of dichloromethane and methanol and filter through a glass microfibre filter (such as Whatman GF/C).

**Reference solution.** A 0.127 per cent w/v solution of domperidone maleate RS in a mixture of equal volumes of dichloromethane and methanol.

Apply to the plate 10 µl of each solution. After development, allow the plate to dry in air and examine in ultraviolet light at 254 nm. Spray the plate with potassium iodobismuthate solution and examine again. With each method of visualisation, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

**Tests**

**Dissolution** (2.5.2).

Apparatus No.1

- Medium. 900 ml of 0.1 M hydrochloric acid
- Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, diluted with the dissolution medium if necessary, at 286 nm (2.4.7), using the dissolution medium as the blank. Calculate the content of C₂₂H₂₄ClN₅O₂ in the medium if necessary, at 286 nm (2.4.7), using the dissolution recorder. The reference solution (a) is at least 50 per cent of the full scale of the recorder.

Equilibrate the column for at least 30 minutes with methanol and equilibrate with the initial mobile phase for at least 5 minutes. Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (a) is at least 50 per cent of the full scale of the recorder.

Inject a mixture of equal volumes of 0.01 M hydrochloric acid and methanol as a blank, the test solution and reference solutions (a) and (b). The test is not valid unless, in the chromatogram obtained with reference solution (b), the resolution between the two principal peaks is at least 2. If necessary adjust the concentration of methanol in the mobile phase or adjust the time programme for the linear gradient.

In the chromatogram obtained with the test solution the area of any secondary peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent) and the sum of the areas of any secondary peaks is not greater than twice the area of the principal peak in the chromatogram obtained with the reference solution (a) (0.5 per cent). Ignore any peak in the chromatogram obtained with the blank solution and any peak with an area less than 0.2 times the area of the peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14) as described under Related substances using the following solutions.

**Test solution.** Add sufficient methanol to 10 whole tablets to produce a solution containing 0.02 per cent w/v of domperidone, mix with the aid of ultrasound for 20 minutes and filter through a glass microfibre filter (such as Whatman GF/C). To 50.0 ml of the filtrate add 1 ml of 0.1 M hydrochloric acid and sufficient water to produce 100.0 ml.

**Reference solution.** A solution containing 0.0127 per cent w/v of domperidone maleate RS in a mixture of equal volumes of 0.002 M hydrochloric acid and methanol.

<table>
<thead>
<tr>
<th>Time (in min.)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
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<tbody>
<tr>
<td>0</td>
<td>30</td>
<td>70</td>
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<tr>
<td>10</td>
<td>100</td>
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</table>

A solution containing 0.015 per cent w/v of droperidol RS in a mixture of equal volumes of 0.01 M hydrochloric acid and methanol.

- Chromatographic system
  - A stainless steel column 10 cm x 4.6 mm, packed with base-deactivated, end-capped octadecylsilyl silica gel (3 µm) (such as Hypersil BDS).
Calculate the content of C₂₂H₂₄ClN₅O₂ in the tablets.

**Storage.** Store protected from moisture.

**Labelling.** The label states the strength in terms of the equivalent amount of domperidone.

### Donepezil Hydrochloride

![Chemical Structure](DONEPEZIL HYDROCHLORIDE)

\[
\text{C}_{24}\text{H}_{29}\text{NO}_3\cdot\text{HCl} \quad \text{Mol. Wt. 415.5}
\]

Donepezil Hydrochloride is \((\pm)-2-[(1\text{-benzyl}-4\text{-piperidyl})\text{methyl}]-5,6\text{-dimethoxy}-1\text{-indanone hydrochloride.}

Donepezil Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of C₂₄H₂₉NO₃·HCl, calculated on the anhydrous basis.

**Description.** A white to off-white powder.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with donepezil hydrochloride RS or with the reference spectrum of donepezil hydrochloride.

B. When examined in the range 200 nm to 400 nm (2.4.7), a 0.001 per cent w/v solution in methanol shows absorption maximum at about 230 nm, 268 nm and 313 nm.

**Tests**

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 50 mg of the substance under examination in 50 ml of mobile phase.

**Reference solution (a).** A 0.1 per cent w/v solution of donepezil hydrochloride RS in mobile phase.

**Reference solution (b).** Dilute 1 ml of reference solution (a) to 100 ml with mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 600 volumes of water, 400 volumes of methanol and 1 volume of triethylamine, adjust the pH to 3.0 with orthophosphoric acid and filter,
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- a 20 µl loop injector.

Inject reference solution (a). Test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

**Heavy metals** (2.3.13). The residue obtained from residue on ignition complies with limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.19). Not more than 0.2 per cent.

**Water** (2.3.43). Not more than 7.0 per cent, determined on 1.0 g.

**Assay.** Weigh accurately about 0.4 g, dissolve in a mixture of 40 ml of glacial acetic acid and 10 ml of 5 per cent of mercuric acetate in glacial acetic acid. Titrate with 0.1 M perchloric acid. Determine the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.04155 g of C₂₄H₂₉NO₃·HCl.

**Storage.** Store protected from moisture.

### Donepezil Tablets

Donepezil Hydrochloride Tablets

Donepezil Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of donepezil hydrochloride, C₂₄H₂₉NO₃·HCl.

**Identification**

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

**Tests**

**Dissolution** (2.5.2).

Apparatus No. 1
Medium. 900 ml of 0.1 M hydrochloric acid.
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with
the medium if necessary, at the maximum at about 230 nm (2.4.7). Calculate the content of C₂₄H₂₉NO₃.HCl in the medium from the absorbance obtained from a solution of known concentration of donepezil hydrochloride RS in the same medium.

D. Not less than 75 per cent of the stated amount of C₂₄H₂₉NO₃.HCl.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Solvent mixture.** 25 volumes of acetonitrile and 75 volumes of water.

**Test solution.** Weigh accurately a quantity of powdered tablets containing about 50 mg of Donepezil Hydrochloride, add 25 ml of solvent mixture, sonicate for 15 minutes and make up the volume to 50 ml with solvent mixture and filter.

**Reference solution (a).** A 0.1 per cent w/v solution of donepezil hydrochloride RS in solvent mixture.

**Reference solution (b).** Dilute 1 ml of reference solution (a) to 100 ml with solvent mixture.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm packed octadecylsilane bonded to porous silica (5 µm),
- column temperature 50º,
- mobile phase: A. a buffer solution pH 6.5 prepared by adding 1 ml of orthophosphoric acid in 1000 ml of water, adjust the pH to 6.5 with triethylamine and filter,
- B. acetonitrile,
- a linear gradient programme using the conditions given below,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 286 nm,
- a 20 µl loop injector.

<table>
<thead>
<tr>
<th>Time (mins.)</th>
<th>Mobile phase A (per cent w/v)</th>
<th>Mobile phase B (per cent w/v)</th>
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<tr>
<td>50</td>
<td>75</td>
<td>25</td>
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</tbody>
</table>

Inject reference solution (b). Test is not valid unless the column efficiency is not less than 20000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 1.5 times the area of the peak in the chromatogram obtained with reference solution (b) (1.5 per cent) and the sum of areas of all the secondary peaks is not more than 3 times the area of the peak in the chromatogram obtained with the reference solution (b) (3.0 per cent).

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Transfer intact tablets and sonicate to disperse the tablets completely, add mobile phase and sonicate again for 30 minutes and cool and make up the volume with mobile phase. Allow the excipients to settle down completely and dilute finally with mobile phase to obtain a solution of final concentration of 0.01 per cent w/v and filter.

**Reference solution.** A 0.01 per cent w/v solution of donepezil hydrochloride RS in mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 40º,
- mobile phase: a mixture of 60 volumes of buffer pH 2.2 prepared by dissolving about 6.8 g of potassium dihydrogen phosphate in 1000 ml of water. Add 5 ml of triethylamine and adjust the pH to 2.2 with orthophosphoric acid, filter and 40 volumes of methanol,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 268 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 7000 theoretical plates. The tailing factor is not more than 1.5. The relative standard deviation of replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution

Calculate the content of C₂₄H₂₉NO₃.HCl

**Storage.** Store protected from moisture, at a temperature not exceeding 25º.

**Dothiepin Hydrochloride**

Dosulepin Hydrochloride

\[
\text{C}_{19}H_{21}NS\cdot\text{HCl}
\]

Mol. Wt. 331.9

Dothiepin Hydrochloride is 3-(6H-dibenzo[\textit{b,e}]thiepin-11-ylidene)propyldimethylamine hydrochloride, consisting predominantly of the E-isomer.
Dothiepin Hydrochloride contains not less than 98.0 per cent and not more than 101.0 per cent of \( \text{C}_{19}\text{H}_{21}\text{NS},\text{HCl} \), calculated on the dried basis.

**Description.** A white to faintly yellow, crystalline powder; odourless or almost odourless.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with dothiepin hydrochloride RS or with the reference spectrum of dothiepin hydrochloride.

B. Dissolve 1 mg in 5 ml of sulphuric acid; a dark red colour is produced.

C. On 20 mg determine by the oxygen-flask method (2.3.34), using a mixture of 15 ml of water and 1 ml of hydrogen peroxide solution (20 volume) as the absorbing liquid. The solution gives the reactions of sulphates (2.3.1).

D. Gives reaction A of chlorides (2.3.1).

**Tests**

**pH** (2.4.24). 3.0 to 4.5, determined in a 5.0 per cent w/v solution.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel HF254.

**Mobile phase.** A mixture of 90 volumes of 1,2-dichloroethane, 10 volumes of 2-propanol and 1 volume of strong ammonia solution.

Prepare the following solutions freshly before use.

**Test solution (a).** Dissolve 1.0 g of the substance under examination in 10 ml of chloroform.

**Test solution (b).** Dissolve 0.4 g of the substance under examination in 10 ml of chloroform.

**Reference solution.** A solution containing 0.02 per cent w/v each of 11-(3-dimethylamino-propylidene)-6H-dibenzo[b,e]thiepin-5-oxide RS and 6H-dibenzo[b,e]thiepin-11-one RS in chloroform.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the reference solution the spot with the lower \( R_f \) value is more intense than any corresponding spot in the chromatogram obtained with test solution (b). In the chromatogram obtained with test solution (a) any secondary spot other than any spot corresponding to the spot with the lower \( R_f \) value in the chromatogram obtained with the reference solution is not more intense than the proximate spot in the chromatogram obtained with the reference solution.

**Z-Isomer.** Determine by gas chromatography (2.4.13).

**Test solution.** Dissolve 0.5 g of the substance under examination in 100 ml of methanol.

**Reference solution.** A 0.5 per cent w/v solution of dothiepin hydrochloride RS in methanol.

Chromatographic system

- a glass column 1.8 m x 3 mm, packed with acid-washed, silanised diatomaceous support (100 to 120 mesh) coated with 3 per cent w/w of cyanopropylmethyl phenyl methyl silicone fluid (such as OV-225),
- temperature:
  - column.200°,
  - inlet port. 260°,
- flame ionisation detector,
- nitrogen as carrier gas.

In the chromatogram obtained with the reference solution a peak due to Z-dothiepin is present with a retention time of approximately 0.83 relative to the retention time of the principal peak which is due to \( E \)-dothiepin. In the chromatogram obtained with the test solution the area of any peak corresponding to Z-dothiepin is not greater than 7.5 per cent of the sum of the areas of the peaks due to Z-dothiepin and \( E \)-dothiepin.

**Heavy metals** (2.3.13). Dissolve the residue obtained in the test for Sulphated ash in 0.5 ml of hydrochloric acid, evaporate to dryness, dissolve the residue in 2 ml of water, neutralise to phenolphthalein solution with dilute sodium hydroxide and dilute to 15 ml with water, 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent, determined on 1.5 g.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.6 g, dissolve in 100 ml of acetone and add 15 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid, using 3 ml of a saturated solution of methyl orange in acetone as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.03319 g of \( \text{C}_{19}\text{H}_{21}\text{NS},\text{HCl} \).

**Storage.** Store protected from light.

**Dothiepin Capsules**

Dothiepin Hydrochloride Capsules; Dosulepin Capsules; Dosulepin Hydrochloride Capsules;

Dothiepin Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of dothiepin hydrochloride, \( \text{C}_{19}\text{H}_{21}\text{NS},\text{HCl} \).
Identification

Extract a quantity of the contents of the capsules containing 0.1 g of Dothiepin Hydrochloride with 20 ml of ethanol, filter and remove the ethanol from the filtrate by evaporation. The residue complies with the following tests.

A. Dissolve 1 mg in 5 ml of sulphuric acid; a dark red colour is produced.

B. On 20 mg determine by the oxygen-flask method (2.3.34), using a mixture of 15 ml of water and 1 ml of hydrogen peroxide solution (20 volume) as the absorbing liquid. The solution gives the reactions of sulphates (2.3.1).

C. Gives reaction A of chlorides (2.3.1).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel HF254.

Mobile phase. A mixture of 90 volumes of 1,2-dichloroethane, 10 volumes of 2-propanol and 1 volume of strong ammonia solution.

Test solution (a). Extract a quantity of the contents of the capsules containing 0.25 g of Dothiepin Hydrochloride by shaking for 2 minutes with 5 ml of chloroform, centrifuge and use the supernatant liquid.

Test solution (b). Dilute 2 ml of test solution (a) to 5 ml with chloroform.

Reference solution. A freshly prepared solution containing 0.02 per cent w/v each of 11-(3-dimethylamino-propyldiene)-6H-dibenzo[b,e]thiepin-5-oxide RS and 6H-dibenzo[b,e]thiepin-11-one RS in chloroform.

Apply to the plate 5 µl of the reference solution and 10 µl of the test solutions. After development, dry the plate in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the reference solution the spot with the lower Rf value is more intense than any corresponding spot in the chromatogram obtained with test solution (b). In the chromatogram obtained with test solution (a) any secondary spot other than any spot corresponding to the spot with the lower Rf value in the chromatogram obtained with the reference solution is not more intense than the proximate spot in the chromatogram obtained with the reference solution.

Z-Isomer. Determine by gas chromatography (2.4.13).

Test solution. Use the supernatant liquid obtained by extracting a quantity of the mixed contents of 20 capsules containing 25 mg of Dothiepin Hydrochloride with 5 ml of methanol and centrifuging.

Reference solution. A 0.5 per cent w/v solution of dothiepin hydrochloride RS in methanol.

Doxepin Hydrochloride

\[
\text{C}_{19}\text{H}_{21}\text{NO}\cdot\text{HCl}
\]

Mol. Wt. 315.8

Doxepin Hydrochloride is 3-(6H-dibenzo[b,e]oxepin-11-ylidene)propylidimethylamine hydrochloride. It consists of a mixture of \( Z \) and \( E \) isomers.

Doxepin Hydrochloride contains not less than 98.0 per cent and not more than 101.0 per cent of \( \text{C}_{19}\text{H}_{21}\text{NO}\cdot\text{HCl} \), calculated on the dried basis.
Description. A white, crystalline powder; odour, slight and amine-like.

Identification
A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with doxepin hydrochloride RS or with the reference spectrum of doxepin hydrochloride.
B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.004 per cent w/v solution in 0.01 M methanolic hydrochloric acid shows an absorption maximum only at about 297 nm; absorbance at about 297 nm, about 0.60.
C. Dissolve 5 mg in 2 ml of nitric acid; a red colour is produced.
D. Gives reaction A of chlorides (2.3.1).

Tests
Z-isomer. 13.0 per cent to 18.5 per cent, determined by the following method.
Determine by gas chromatography (2.4.13).
Test solution. Dissolve 0.5 g of the substance under examination in 100 ml of methanol.
Reference solution. A 0.5 per cent w/v solution of doxepin hydrochloride RS in methanol.
Chromatographic system
- a glass column 1.5 m x 4 mm, packed with acid-washed, silanised diatomaceous support (100 to 120 mesh) coated with 3 per cent w/w of cyanopropylmethyl phenyl methyl silicone fluid (such as OV-225),
- temperature: column 200°, inlet port 260°,
- flame ionisation detector,
- nitrogen as carrier gas.

In the chromatogram obtained with the reference solution a peak due to Z-doxepin immediately precedes and is adequately separated from the principal peak which is due to E-doxepin. Measure the areas or heights of the peaks due to Z-doxepin and E-doxepin in the chromatograms obtained with the test solution and the reference solution. Calculate the content of the Z-isomer in the substance under examination.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.6 g, dissolve in 100 ml of acetone and add 15 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid, using 3 ml of a saturated solution of methyl orange in acetone as indicator. Carry out a blank titration.
1 ml of 0.1 M perchloric acid is equivalent to 0.03158 g of C_{19}H_{21}NO.HCl.

Storage. Store protected from light.

Doxepin Capsules
Doxepin Hydrochloride Capsules
Doxepin Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of doxepin, C_{19}H_{21}NO.

Identification
Wash a quantity of the contents of the capsules containing 0.1 g of doxepin with 3 quantities, each of 5 ml, of light petroleum (40° to 60°). Dry the residue in air and extract with 3 quantities, each of 10 ml, of chloroform, evaporate the combined extracts to dryness and dry the residue at 105°. The dried residue complies with the following tests.
A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with doxepin hydrochloride RS or with the reference spectrum of doxepin hydrochloride.
B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.004 per cent w/v solution in 0.01 M methanolic hydrochloric acid shows an absorption maximum only at about 297 nm; absorbance at about 297 nm, about 0.60.
C. Dissolve 5 mg in 2 ml of nitric acid; a red colour is produced.
D. Gives reaction A of chlorides (2.3.1).

Tests
Z-isomer. 13.0 per cent to 18.5 per cent, determined by the following method.
Determine by gas chromatography (2.4.13).
Test solution. Use the supernatant liquid obtained by extracting a quantity of the mixed contents of 20 capsules containing 25 mg of doxepin with 5 ml of methanol and centrifuging.
Reference solution. A 0.5 per cent w/v solution of doxepin hydrochloride RS in methanol.
Chromatographic system
- a glass column 1.5 m x 4 mm, packed with acid-washed, silanised diatomaceous support (100 to 120 mesh) coated with 3 per cent w/w of cyanopropylmethyl phenyl methyl silicone fluid (such as OV-225),
In the chromatogram obtained with the reference solution a peak due to Z-doxepin immediately precedes and is adequately separated from the principal peak which is due to E-doxepin. Measure the areas or heights of the peaks due to Z-doxepin and E-doxepin in the chromatograms obtained with the test solution and the reference solution. Calculate the content of the Z-isomer in the capsules.

Other tests. Comply with the tests stated under Capsules.

Assay. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 30 mg of doxepin, add 50 ml of 0.1 M methanolic hydrochloric acid, shake for 30 minutes and add sufficient 0.01 M methanolic hydrochloric acid to produce 100.0 ml. Centrifuge 40 ml of this solution and dilute 10.0 ml of the clear supernatant liquid to 100.0 ml with 0.01 M methanolic hydrochloric acid. Measure the absorbance of the resulting solution at the maximum at about 297 nm (2.4.7). Calculate the content of C₁₉H₂₁NO taking 150 as the specific absorbance at 297 nm.

Storage. Store protected from light and moisture.

Labelling. The label states the strength in terms of the equivalent amount of doxepin.

Doxorubicin Hydrochloride

\[
\begin{align*}
C_{27}H_{29}NO_{11} \cdot HCl
\end{align*}
\]

Mol. Wt. 580.0

Doxorubicin Hydrochloride is (8S,10S)-10-[[3-amino-2,3,6-trideoxy-\(\alpha\)-L-lyxo-hexopyranosyl]oxy]-6,8,11-trihydroxy-8-hydroxyacetyl-1-methoxy-7,8,9,10-tetrahydronaphthacene-5,12-dione hydrochloride, a substance produced by the growth of certain strains of \(\text{Streptomyces coeruleorubidus}\) or \(\text{S. peucetius}\) or obtained by any other means.

Doxorubicin Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of C₁₉H₂₁NO₁₁·HCl, calculated on the anhydrous and solvent-free basis.

Description. An orange-red, crystalline powder; hygroscopic. CAUTION - Doxorubicin Hydrochloride is poisonous. It must be handled with care avoiding contact with skin and inhalation of airborne particles.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with doxorubicin hydrochloride RS.

B. When examined in the range 220 nm to 550 nm (2.4.7), a 0.001 per cent w/v solution in ethanol (95 per cent) exhibits maxima at about 234 nm, 252 nm, 288 nm, 475 nm, 495 nm and 530 nm.

C. In the test for Related substances, the principal peak in the chromatogram obtained with test solution (b) corresponds to the peak in the chromatogram obtained with reference solution (c).

D. Dissolve 10 mg in 0.5 ml of nitric acid, add 0.5 ml of water and heat over a flame for 2 minutes. Allow to cool and add 0.5 ml of silver nitrate solution; a white precipitate is produced.

Tests

pH (2.4.24). 4.0 to 5.5, determined in a 0.5 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution (a). Dissolve 50 mg of the substance under examination in sufficient of the mobile phase to produce 50 ml.

Test solution (b). Dilute 10 ml of test solution (a) to 100 ml with the mobile phase.

Reference solution (a). Dissolve 10 mg of doxorubicin hydrochloride RS and 10 mg of epirubicin hydrochloride RS in the mobile phase and dilute to 50 ml with the mobile phase. Dilute 10 ml of this solution to 100 ml with the mobile phase.

Reference solution (b). Dilute 5 ml of reference solution (a) to 20 ml with the mobile phase.

Reference solution (c). Dissolve 50 mg of doxorubicin hydrochloride RS in the mobile phase and dilute to 50 ml with the mobile phase. Dilute 10 ml of this solution to 100 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of equal volumes of acetonitrile and a solution containing 2.88 g per litre of sodium acetate buffer (pH 6.5) as eluent.
dodecyl sulphate and 2.25 g per litre of phosphoric acid,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject test solution (a) and reference solutions (a) and (b). Continue the chromatography for 3.5 times the retention time of doxorubicin of about 8 minutes. The test is not valid unless in the chromatogram obtained with reference solution (a) the resolution factor between the peaks due to doxorubicin and epirubicin is at least 2.0.

In the chromatogram obtained with the test solution the area of any peak other than the principal peak is not more than the area of the peak corresponding to doxorubicin in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.1 times the area of the peak corresponding to doxorubicin in the chromatogram obtained with reference solution (b) (0.5 per cent).

Acetone and ethanol. Not more than 2.0 per cent w/w together of which not more than 0.5 per cent w/w is acetone, determined by gas chromatography (2.4.13), injecting 1 µl of each of two solutions

Test solution. A 5.0 per cent w/v solution of the substance under examination and 0.1 per cent w/v of dioxon in water.

Reference solution. 0.05 per cent w/v of acetone, 0.05 per cent w/v of ethanol and 0.1 per cent w/v of the internal standard.

Chromatographic system
- a glass column 2 m x 3 mm, packed with acid-washed diatomaceous support (180 to 250 mesh) impregnated with 10 per cent w/w of polyethylene glycol 20,000 (such as Carbowax 20M or Chromosorb E/AW),
- temperature: column, 70°, inlet port and detector. 125°,
- flow rate. 30 ml per minute of the carrier gas.

Water (2.3.43). Not more than 4.0 per cent, determined on 0.1 g.

Assay. Determine by liquid chromatography (2.4.14) by the procedure described under the test for Related substances. Inject test solution (b) and reference solution (c). Calculate the content of C$_{27}$H$_{29}$NO$_{11}$HCl.

Doxorubicin Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture. If the material is sterile, it should be stored in sterile, tamper-evident containers and sealed so as to exclude micro-organisms.

Labelling. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

Doxorubicin Injection

Doxorubicin Hydrochloride Injection

Doxorubicin Injection is a sterile solution of Doxorubicin Hydrochloride in Water for Injections made isotonic with Sodium Chloride, Dextrose or other suitable added substances. It is either supplied as preformed solution or it is prepared by dissolving the contents of a sealed container containing Doxorubicin Hydrochloride with or without auxiliary substances in the requisite amount of Water for Injections or Sodium Chloride Injection as directed on the label.

Doxorubicin Injection contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of doxorubicin hydrochloride, C$_{27}$H$_{29}$NO$_{11}$HCl.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 2.5 to 4.5 for the preformed solution and 4.5 to 6.5, determined in the injection prepared in accordance with the directions on the label.

Bacterial endotoxins (2.2.3). Not more than 2.2 Endotoxin Units per mg of doxorubicin hydrochloride, determined in a solution prepared by diluting the injection, if necessary, with water BET to obtain a concentration of 2.0 mg of doxorubicin hydrochloride per ml.

Sterility. Complies with the test for sterility, Method A, (2.2.11), using the entire contents of all the containers collected aseptically.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. A solution containing 0.05 per cent w/v of doxorubicin hydrochloride prepared by diluting an accurately measured volume of the injection containing not less than
2 mg of Doxorubicin Hydrochloride with the mobile phase or by dissolving the contents of the sealed container in sufficient mobile phase to give a solution of the same strength.

**Reference solution (a).** A 0.05 per cent w/v solution of doxorubicin hydrochloride RS in the mobile phase.

**Reference solution (b).** A solution containing 0.002 per cent w/v each of doxorubicin hydrochloride RS and epirubicin hydrochloride RS in the mobile phase.

**Chromatographic system**
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilil silica gel (5 µm),
- mobile phase: a mixture of 50 volumes of a solution containing 0.288 per cent w/v of sodium dodecyl sulphate and 0.23 per cent w/v of phosphoric acid, 45 volumes of acetonitrile and 5 volumes of methanol,
- flow rate. 0.8 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject reference solution (b) six times. The assay is not valid unless the resolution factor between the peaks due to doxorubicin and epirubicin is not less than 2.0 and the relative standard deviation for the area of the peak due to doxorubicin is not more than 1.0 per cent. If these requirements are not met, adjust the operating conditions.

Calculate the content of C_{27}H_{29}NO_{11},HCl in the injection.

**Storage.** Store the sealed container at a temperature not exceeding 30°. Store the preformed solution protected from light in a refrigerator. Use the solution prepared in the liquid stated on the label immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the instructions of the manufacturer.

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**Doxycycline Hydrochloride**

**Doxycycline Hyclate**

![Doxycycline Hydrochloride structure](image)

C_{22}H_{24}N_{2}O_{8},HCl,½C_{2}H_{5}OH,½H_{2}O  \text{Mol. Wt. 513.0}

Doxycycline Hydrochloride is (4S,4aR,5S,5aR,6R,12aS)-4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide hydrochloride hemiethanolate hemihydrate, an antimicrobial substance obtained from oxytetracycline or methacycline or by any other means.

Doxycycline Hydrochloride has a potency not less than 880 µg and not more than 940 µg of C_{22}H_{24}N_{2}O_{8} per mg, calculated on the anhydrous and ethanol-free basis.

**Description.** A yellow, crystalline powder; odour, slightly ethanolic; hygroscopic.

**Identification**

*Test A* may be omitted if tests *B, C and D* are carried out. Tests *B and C* may be omitted if tests *A and D* are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with doxycycline hydrochloride RS or with the reference spectrum of doxycycline hydrochloride.

B. Determine by thin-layer chromatography (2.4.7), coating the plate with silica gel *H*.

**Mobile phase.** A mixture of 59 volumes of dichloromethane, 35 volumes of methanol and 6 volumes of water.

**Test solution.** Dissolve 50 mg of the substance under examination in 100 ml of methanol.

**Reference solution (a).** A 0.05 per cent w/v solution of doxycycline hydrochloride RS in methanol.

**Reference solution (b).** A solution containing 0.05 per cent w/v each of doxycycline hydrochloride RS and tetracycline hydrochloride RS in methanol.

Spray the plate evenly with a 10 per cent w/v solution of disodium edetate the pH of which has been adjusted to 9.0 with 10 M sodium hydroxide. Allow the plate to dry in a horizontal position for at least 1 hour. Immediately before use dry it at 110° for 1 hour. Apply to the plate 1 µl of each solution. After development, dry the plate in a current of air and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. To about 2 mg add 5 ml of sulphuric acid; a yellow colour is produced.

D. A 5 per cent w/v solution gives the reactions of chlorides (2.3.1).

**Tests**

**pH (2.4.24).** 2.0 to 3.0, determined in a 1.0 per cent w/v solution.

**Specific optical rotation (2.4.22).** -105° to -120°, determined within 5 minutes of preparing, in a 1.0 per cent w/v solution in
a mixture of 1 volume of 1 M hydrochloric acid and 99 volumes of methanol.

**Light absorption** (2.4.7). Absorbance of a 0.001 per cent w/v solution in a mixture of 1 volume of 1 M hydrochloric acid and 99 volumes of methanol, measured within 1 hour of preparing the solution, at the maximum at about 349 nm, 0.300 to 0.335.

**Light-absorbing impurities.** Dissolve 0.1 g in sufficient of a mixture of 1 volume of 1 M hydrochloric acid and 99 volumes of methanol to produce 10 ml. Absorbance of the solution at about 490 nm, when measured within 1 hour of preparing the solution, not more than 0.07 (2.4.7).

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 80 mg of the substance under examination in 100 ml of 0.01 M hydrochloric acid.

**Reference solution (a).** A 0.08 per cent w/v solution of 6-epidoxycycline hydrochloride RS in 0.01 M hydrochloric acid.

**Reference solution (b).** A 0.08 per cent w/v solution of 6-epidoxycycline hydrochloride RS in 0.01 M hydrochloric acid.

**Reference solution (c).** A 0.08 per cent w/v solution of methacycline hydrochloride RS in 0.01 M hydrochloric acid.

**Reference solution (d).** A solution containing 0.0016 per cent of 6-epidoxycycline in the chromatogram obtained with reference solution (d); the area of any peak appearing between the solvent peak and the peak corresponding to methacycline or 6-epidoxycycline is not greater than 25 per cent of that of the peak corresponding to 6-epidoxycycline in the chromatogram obtained with reference solution (d).

**Ethanol.** 4.3 to 6.0 per cent w/w of C₂H₆O, determine by gas chromatography (2.4.13).

**Test solution (a).** A 1 per cent w/v solution of the substance under examination in a 0.05 per cent v/v solution of 1-propanol (internal standard) in water (solution A).

**Test solution (b).** A 1 per cent w/v solution of the substance under examination in water.

**Reference solution.** A 0.05 per cent v/v solution of ethanol in solution A.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm, packed with styrene-divinylbenzene co-polymer (8 to 10 µm),
- mobile phase: a solution prepared by adding 60 g of 2-methyl-2-propanol to a volumetric flask with the aid of 200 ml of water, adding 400 ml of phosphate buffer pH 8.0, 50 ml of a 1 per cent w/v solution of tetrabutyl ammonium hydrogen sulphate previously adjusted to pH 8.0 with 2 M sodium hydroxide and 10 ml of a 4 per cent w/v solution of disodium edetate previously adjusted to pH 8.0 with 2 M sodium hydroxide and diluting to 1 litre with water,
- column temperature: 60°
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Using reference solution (e) adjust the attenuation to obtain peaks with a height corresponding to at least 50 per cent of full-scale deflection on the chart paper. The test is not valid unless (a) the resolution factor between the first peak (methacycline) and the second peak (6-epidoxycycline) is at least 1.25, (b) the resolution factor between the second peak and the third peak (doxycycline) is at least 2.0 (adjust the content of 2-methyl-2-propanol in the mobile phase if necessary) and (c) the symmetry factor for the third peak is at most 1.25. Inject reference solution (a) six times. The test is not valid unless the relative standard deviation of the area of the peak due to doxycycline is not more than 1.0 per cent.

Inject the test solution and reference solution (d). In the chromatogram obtained with the test solution the area of any peak corresponding to methacycline or 6-epidoxycycline is not greater than the area of the corresponding peak in the chromatogram obtained with reference solution (d); the area of any peak appearing between the solvent peak and the peak corresponding to methacycline and the area of any peak appearing on the tail of the main peak is not greater than 25 per cent of that of the peak corresponding to 6-epidoxycycline in the chromatogram obtained with reference solution (d).

**Heavy metals** (2.3.13). 0.4 g complies with the limit test for heavy metals, Method B (50 ppm).

**Sulphated ash** (2.3.18). Not more than 0.4 per cent.

**Water** (2.3.43). 1.4 to 2.8 per cent, determined on 1.2 g.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh accurately 80 mg of the substance under examination and dissolve in 100 ml of 0.01 M hydrochloric acid.

**Reference solution.** A 0.08 per cent w/v solution of doxycycline hydrochloride RS in 0.01 M hydrochloric acid.
Chromatographic system
– a stainless steel column 25 cm x 4.6 mm, packed with styrene-divinylbenzene co-polymer (8 to 10 µm),
– column temperature: 60°
– mobile phase: a solution prepared by adding 60 g of 2-methyl-2-propanol to a volumetric flask with the aid of 200 ml of water, adding 400 ml of phosphate buffer pH 8.0, 50 ml of a 1 per cent w/v solution of tetrabutyl ammonium hydrogen sulphate previously adjusted to pH 8.0 with 2 M sodium hydroxide and 10 ml of a 4 per cent w/v solution of disodium edetate previously adjusted to pH 8.0 with 2 M sodium hydroxide and diluting to 1 litre with water,
– flow rate. 1 ml per minute,
– spectrophotometer set at 254 nm,
– a 20 µl loop injector.

Inject the reference solution six times. The test is not valid unless the relative standard deviation of the area of the peak due to doxycycline is not more than 1.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the content of C22H24N2O8.

Doxycycline Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 1.14 Endotoxin Units per mg.

Doxycycline Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light and moisture at a temperature not exceeding 30°. If the substance is intended for use in the manufacture of parenteral preparations, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

Labelling. The label states, where applicable, that the material is sterile.

Identification
A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel H.

Mobile phase. A mixture of 59 volumes of dichloromethane, 35 volumes of methanol and 6 volumes of water.

Test solution. Shake a quantity of the contents of the capsules containing 50 mg of anhydrous doxycycline with 100 ml of methanol for 1 to 2 minutes, centrifuge and use the supernatant liquid. Prepare freshly.

Reference solution (a). A 0.05 per cent w/v solution of doxycycline hydrochloride RS in methanol.

Reference solution (b). A solution containing 0.05 per cent w/v each of doxycycline hydrochloride RS and tetracycline hydrochloride RS in methanol.

Spray the plate evenly with a 10 per cent w/v solution of disodium edetate the pH of which has been adjusted to 9.0 with 10 M sodium hydroxide. Allow the plate to dry in a horizontal position for at least 1 hour. Immediately before use dry it at 110° for 1 hour. Apply to the plate 1 µl of each solution. After development, dry the plate in a current of air and examine it in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

B. To 0.5 mg of the contents of the capsules add 2 ml of sulphuric acid; a yellow colour is produced.

C. A 5 per cent w/v solution of the contents of the capsules gives the reactions of chlorides (2.3.1).

Tests
Light-absorbing impurities. Dissolve the contents of 5 capsules as completely as possible in sufficient of a mixture of 1 volume of 1 M hydrochloric acid and 99 volumes of methanol to produce a solution containing the equivalent of 1.0 per cent w/v of anhydrous doxycycline and filter. Absorbance of the filtrate at about 490 nm, not greater than 0.2 (2.4.7), calculated with reference to the dried contents of the capsules.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the contents of the capsules containing 7 mg of anhydrous doxycycline in 10 ml of 0.01 M hydrochloric acid, filter and use the filtrate.

Reference solution (a). A 0.08 per cent w/v solution of doxycycline hydrochloride RS in 0.01 M hydrochloric acid.

Reference solution (b). A 0.08 per cent w/v solution of 6-epidoxycycline hydrochloride RS in 0.01 M hydrochloric acid.
Reference solution (c). A 0.08 per cent w/v solution of methacycline hydrochloride RS in 0.01 M hydrochloric acid.

Reference solution (d). A solution containing 0.0016 per cent w/v each of 6-epidoxyccline hydrochloride RS and methacycline hydrochloride RS in 0.01 M hydrochloric acid.

Reference solution (e). Dilute a mixture of 4 volumes of reference solution (a), 1.5 volumes of reference solution (a) and 1 volume of reference solution (c) to 25 volumes with 0.01 M hydrochloric acid.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with styrene-divinylbenzene co-polymer (8 to 10 µm),
- column temperature 60°,
- mobile phase: a solution prepared by adding 60 g of 2-methyl-2-propanol to a volumetric flask with the aid of 200 ml of water, adding 400 ml of phosphate buffer pH 8.0, 50 ml of a 1 per cent w/v solution of tetrabutyl ammonium hydrogen sulphate previously adjusted to pH 8.0 with 2 M sodium hydroxide and 10 ml of a 4 per cent w/v solution of disodium edetate previously adjusted to pH 8.0 with 2 M sodium hydroxide and diluting to 1000 ml with water,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Using reference solution (e) adjust the attenuation to obtain peaks with a height corresponding to at least 50 per cent of full-scale deflection of the recorder. The test is not valid unless (a) the resolution factor between the first peak (methacycline) and the second peak (6-epidoxycycline) is at least 1.25, (b) the resolution factor between the second peak and the third peak (doxycycline) is at least 2.0 (adjust the content of 2-methylpropan-2-ol in the mobile phase if necessary).

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution (d) and record the chromatograms. In the chromatogram obtained with the test solution the area of any peak corresponding to methacycline or 6-epidoxycycline is not greater than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (2 per cent, with reference to doxycycline hydrochloride), the area of any peak appearing between the solvent peak and the peak corresponding to methacycline and the area of any peak appearing on the tail of the main peak is not greater than 25 per cent of that of the peak corresponding to 6-epidoxycycline in the chromatogram obtained with reference solution (d) (0.5 per cent, with reference to doxycycline hydrochloride).

Loss on drying (2.4.19). Not more than 8.5 per cent, determined on 0.5 g of the contents of the capsules by drying in an oven at 105° for 2 hours.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve the mixed contents of 20 capsules containing about 17.5 mg of anhydrous doxycycline in sufficient 0.01 M hydrochloric acid to produce 25.0 ml and dilute 4.0 ml of this solution to 25.0 ml with the same solvent.

Reference solution. A 0.0128 per cent w/v solution of doxycycline hydrochloride RS in 0.01 M hydrochloric acid.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with styrene-divinylbenzene co-polymer (8 to 10 µm),
- column temperature 60°,
- mobile phase: a solution prepared by adding 60 g of 2-methyl-2-propanol to a volumetric flask with the aid of 200 ml of water, adding 400 ml of phosphate buffer pH 8.0, 50 ml of a 1 per cent w/v solution of tetrabutyl ammonium hydrogen sulphate previously adjusted to pH 8.0 with 2 M sodium hydroxide and 10 ml of a 4 per cent w/v solution of disodium edetate previously adjusted to pH 8.0 with 2 M sodium hydroxide and diluting to 1 litre with water,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject the reference solution six times. The test is not valid unless the relative standard deviation of the area of the peak due to doxycycline is not more than 1.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the content of C21H28O2 in the capsules.

Storage. Store protected from light and moisture at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of doxycycline.

Dydrogesterone

Dydrogesterone is \( \text{C}_{21}\text{H}_{28}\text{O}_{2} \) Mol. Wt. 312.5

Dydrogesterone is \( 9\beta,10\alpha \)-pregna-4,6,20-diene-3,20-dione.
Dydrogesterone contains not less than 97.0 per cent and not more than 103.0 per cent of C21H28O2, calculated on the dried basis.

**Description.** A white or almost white, crystalline powder; odourless or almost odourless.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with dydrogesterone RS or with the reference spectrum of dydrogesterone.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

**Tests**

**Specific optical rotation** (2.4.22). –446° to –464°, determined in a 1.0 per cent w/v solution in dioxan.

**Light absorption** (2.4.7). When examined in the range 230 nm to 360 nm, the final solution obtained in the Assay shows an absorption maximum only at about 286 nm. The ratio of the absorbance at about 240 nm to that at about 286 nm is not more than 0.12.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 20 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

**Reference solution (a).** Dissolve 20 mg of dydrogesterone RS in the mobile phase and dilute to 100 ml with the mobile phase.

**Reference solution (b).** Dilute 1 ml of the test solution to 500 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilyl silica gel (3 µm),
- column temperature 40º,
- mobile phase: a mixture of 53 volumes of water with 26 volumes of ethanol (95 per cent) and 21 volumes of acetonitrile,
- flow rate, 1 ml per minute,
- spectrophotometer set at 280 nm and 385 nm,
- a 10 µl loop injector.

The relative retention time with respect to dydrogesterone (retention time, about 10.5 minutes): impurity A (6-dehydroyprogesterone), about 1.16, impurity B (17α-dydrogesterone) about 1.32 and impurity C (Δ 8-14 triene dydrogesterone), about 0.97.

The response factors relative to that of dydrogestrone: impurity A (6-dehydroyprogesterone) 1.0, impurity B (17α-
dydrogesterone), 1.0 and impurity C (Δ 8-14 triene dydrogesterone), 0.899.

The test is not valid unless the column efficiency is not less than 10000 theoretical plates in the chromatogram obtained with the test solution.

For impurity A and impurity B, spectrophotometer set at 280 nm.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution the area of any peak corresponding to impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the area of any peak corresponding to impurity B is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent).

For impurity C, spectrophotometer set at 385 nm:

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution the area of any peak corresponding to impurity C, using the response factor, is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent).

The area of any other individual impurity is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

The sum of the areas of all the impurities is not greater than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent, determined on 2.0 g.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105º for 3 hours.

**Assay.** Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject alternately the test solution and reference solution (a).

Calculate the content of C21H28O2.

**Storage.** Store protected from light and moisture.

### Dydrogesterone Tablets

Dydrogesterone Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of dydrogesterone, C21H28O2.

**Identification**

Extract a quantity of the powdered tablets containing 60 mg of Dydrogesterone with 20 ml of methanol, filter and evaporate
the filtrate to dryness. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with dydrogesterone RS or with the reference spectrum of dydrogesterone.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

**Tests**

**Related substances.** Determine by liquid chromatography (2.4.14).

*Test solution.* Determine the average weight of 20 tablets. Do not powder the tablets. Accurately weigh 20 tablets. Add 25 ml of water and mix with aid of ultrasound until complete disintegration has occurred. Add 200 ml of acetonitrile and mix with aid of ultrasound for 15 minutes, dilute to 500.0 ml with water and mix. Centrifuge a part of this solution in a closed tube for about 5 minutes. Dilute a suitable volume of the supernatant liquid with the mobile phase to obtain a solution containing about 0.1 mg of dydrogesterone per ml and mix. Filter a part of this solution, discarding the first few ml of the filtrate.

*Reference solution (a).* Weigh accurately about 10 mg of dydrogesterone RS, add 40 ml of acetonitrile and mix with aid of ultrasound and dilute to 100.0 with water.

*Reference solution (b).* Dilute 1 ml of the test solution to 200.0 ml with the mobile phase.

**Chromatographic system**

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilil silica gel (3 μm),
- column temperature 40º,
- mobile phase: a mixture of 600 volumes of water and 425 volumes of acetonitrile,
- flow rate. 1 ml per minute,
- spectrophotometer set at 310 nm,
- a 20 μl loop injector.

The relative retention time with respect to dydrogesterone (retention time, about 10.5 minutes); impurity A (6-dehydroprogesterone), about 1.16 and impurity B (17α-dydrogesterone), about 1.32.

The response factors relative to dydrogestrone are 0.74 for impurity A and 1.0 for impurity B.

The test is not valid unless the column efficiency is not less than 10000 theoretical plates in the chromatogram obtained with test solution.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution the area of any peaks corresponding to 6-dehydroprogesterone and 17α-dydrogesterone are not more than the area of the principal peak obtained with reference solution (b) (0.5 per cent each) and the sum of all impurities found is not more than twice the area of the principal peak obtained with reference solution (b) (1.0 per cent).

**Uniformity of content.** Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14) as given under Related substances using the following test solution.

*Test solution.* To one tablet add 5 ml of water and mix with the aid of ultrasound. Add 40 ml of acetonitrile and mix with the aid of ultrasound for 15 minutes. Add about 50 ml of water and swirl for 15 minutes. Dilute with sufficient water to produce 100.0 ml.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject alternately the test solution and reference solution (a). Calculate the content of C_{21}H_{28}O_{2} in the tablets.

**Storage.** Store protected from light.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Form</th>
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<tbody>
<tr>
<td>Econazole Nitrate</td>
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<tr>
<td>Econazole Cream</td>
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<tr>
<td>Econazole Pessaries</td>
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<tr>
<td>Efavirenz</td>
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<tr>
<td>Efavirenz Capsules</td>
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<tr>
<td>Efavirenz Tablets</td>
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<td>Emetine Hydrochloride</td>
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<td>Emetine Injection</td>
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<tr>
<td>Emtricitabine</td>
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<td>Emtricitabine Capsules</td>
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<tr>
<td>Emulsifying Wax</td>
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<tr>
<td>Enalapril Maleate</td>
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<td>Enalapril Maleate Tablets</td>
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<tr>
<td>Ephedrine</td>
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<td>Ephedrine Hydrochloride</td>
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<td>Ephedrine Oral Solution</td>
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<td>Ergotamine Tartrate</td>
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<td>Ergotamine Injection</td>
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<td>Erythromycin</td>
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<td>Erythromycin Stearate</td>
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<td>Erythromycin Stearate Tablets</td>
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</table>
Ethacrynic Acid
Ethacrynic Acid Tablets
Ethambutol Hydrochloride
Ethambutol Hydrochloride Tablets
Ethambutol And Isoniazid Tablets
Ethanol
Ethanol (95 Per Cent)
Anaesthetic Ether
Ethinyloestradiol
Ethinyloestradiol Tablets
Ethionamide
Ethionamide Tablets
Ethopropazine Hydrochloride
Ethopropazine Tablets
Ethosuximide
Ethosuximide Capsules
Ethosuximide Syrup
Ethylcellulose
Ethyl Chloride
Ethyl Oleate
Ethylene diamine Hydrate
Ethylstrenol
Ethylstrenol Tablets
Etoposide
Etoposide Capsules
Etoposide Injection
Etoposide Concentrate
Econazole Nitrate

\[
\begin{align*}
\text{C}_{18}\text{H}_{15}\text{Cl}_{3}\text{N}_{2}\text{O},\text{HNO}_3 \\
\text{Mol. Wt. 444.7}
\end{align*}
\]

Econazole Nitrate is (RS)-1-[2-(4-chlorophenylmethoxy)-2-(2,4-dichlorophenyl)ethyl]-1H-imidazole nitrate.

Econazole Nitrate contains not less than 98.5 per cent and not more than 101.5 per cent of C\(_{18}\)H\(_{15}\)Cl\(_{3}\)N\(_2\)O.HNO\(_3\), calculated on the dried basis.

**Description.** A white or almost white, crystalline powder.

**Identification**

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with econazole nitrate RS or with the reference spectrum of econazole nitrate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.04 per cent w/v solution in a mixture of 1 volume of 0.1 M hydrochloric acid and 9 volumes of 2-propanol shows absorption maxima at about 265 nm, 271 nm and 280 nm; the ratio of the absorbance at the maximum at about 271 nm to that at the maximum at about 280 nm is 1.55 to 1.70.

C. In the test for Related substances examine the chromatograms obtained in ultraviolet light before spraying. The principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

D. Gives reaction A of nitrates (2.3.1).

**Tests**

**Appearance of solution.** A 1.0 per cent w/v solution in methanol is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 60 volumes of dioxan, 40 volumes of toluene and 1 volume of strong ammonia solution.

**Sulphated ash (2.3.18).** Not more than 0.1 per cent.

**Loss on drying (2.4.19).** Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

**Assay.** Weigh accurately about 0.4 g and dissolve in 50 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.04447 g of C\(_{18}\)H\(_{15}\)Cl\(_{3}\)N\(_2\)O.HNO\(_3\).

**Storage.** Store protected from light.

Econazole Cream

Econazole Nitrate Cream

Econazole Cream contains Econazole Nitrate in a suitable basis.

Econazole Cream contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of econazole nitrate, C\(_{18}\)H\(_{15}\)Cl\(_{3}\)N\(_2\)O.HNO\(_3\).

**Identification**

A. Mix a quantity of the cream containing 40 mg of Econazole Nitrate with 20 ml of a mixture of 1 volume of 1 M sulphuric acid and 4 volumes of methanol and shake with two quantities, each of 50 ml, of carbon tetrachloride, discarding the organic
layers. Make the aqueous phase alkaline with 2 M ammonia and extract with two quantities, each of 40 ml, of chloroform. Combine the chloroform extracts, shake with 5 g of anhydrous sodium sulphate, filter and dilute the filtrate to 100 ml with chloroform. Evaporate 50 ml to dryness and dissolve the residue in 50 ml of a mixture of 1 volume of 0.1 M hydrochloric acid and 9 volumes of 2-propanol.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 265 nm, 271 nm and 280 nm. The ratio of the absorbance at about 271 nm to that at about 280 nm is 1.55 to 1.70.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to econazole in the chromatogram obtained with the reference solution (a).

**Tests**

**Other tests.** Complies with the tests stated under Creams.

**Assay.** Determine by gas chromatography (2.4.13).

**Test solution.** Mix a quantity of the cream containing about 40 mg of Econazole Nitrate with 20 ml of a mixture of 1 volume of 0.5 M sulphuric acid and 4 volumes of methanol and shake with two quantities, each of 50 ml, of carbon tetrachloride. Wash each organic layer in turn with the same 10-ml quantity of a mixture of 1 volume of 0.5 M sulphuric acid and 4 volumes of methanol. Combine the aqueous phase and the washings, make alkaline with 2 M ammonia and extract with two quantities, each of 50 ml, of chloroform. To the combined extracts add 10.0 ml of a 0.3 per cent w/v solution of 1,2,3,4-tetraphenylcyclopenta-1,3-diene (internal standard) in chloroform and 5 g of anhydrous sodium sulphate, shake, filter, evaporate the filtrate to a low volume and add sufficient chloroform to produce 10.0 ml.

**Reference solution (a).** Shake 40 mg of econazole nitrate RS with 10.0 ml of a 0.3 per cent w/v solution of the internal standard in chloroform and 0.2 ml of strong ammonia solution, add 1 g of anhydrous sodium sulphate, shake again and filter.

**Reference solution (b).** Prepare in the same manner as reference solution (a) but omit the addition of the internal standard solution.

Chromatographic system

- a glass column 1.5 m x 2 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) (such as OV-17),
- temperature: column, 270°;
  inlet port and detector, 300°,
- flow rate: 30 ml per minute of the carrier gas.

Calculate the content of C_{18}H_{15}Cl_{3}N_{2}O,HNO_{3} in the cream.

**Storage.** Store protected from light at a temperature not exceeding 30°. If it is packed in aluminium tubes the inner surfaces of the tubes should be coated with a suitable lacquer.

**Econazole Pessaries**

Econazole Nitrates Pessaries; Econazole Vaginal Tablets

Econazole Pessaries contain Econazole Nitrate in a suitable basis.

Econazole Pessaries contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of econazole nitrate, C_{18}H_{15}Cl_{3}N_{2}O,HNO_{3}.

**Identification**

A. Mix a quantity of the crushed pessaries containing 40 mg of Econazole Nitrate with 20 ml of a mixture of 1 volume of 0.5 M sulphuric acid and 4 volumes of methanol and shake with two quantities, each of 50 ml, of carbon tetrachloride, discarding the organic layers. Make the aqueous phase alkaline with 2 M ammonia and extract with two quantities, each of 40 ml, of chloroform. Combine the chloroform extracts, shake with 5 g of anhydrous sodium sulphate, filter and dilute the filtrate to 100 ml with chloroform. Evaporate 50 ml to dryness and dissolve the residue in 50 ml of a mixture of 1 volume of 0.1 M hydrochloric acid and 9 volumes of 2-propanol.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 265 nm, 271 nm and 280 nm. The ratio of the absorbance at about 271 nm to that at about 280 nm is 1.55 to 1.70.

B. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (b).

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), using a precoated silica gel plate (such as Merck silica gel 60 plates).

**Mobile phase.** A mixture of 70 volumes of chloroform, 20 volumes of methanol and 10 volumes of an 85 per cent w/v solution of formic acid.

**Test solution.** Mix a quantity of the crushed pessaries containing 40 mg of Econazole Nitrate with 40 ml of methanol and heat under a reflux condenser for 15 minutes. Allow to cool, filter, wash the filter paper with methanol and evaporate the filtrate and washings to a volume of about 5 ml. Filter through a filter paper (such as Whatman No. 50 paper), wash
the paper with methanol, evaporate the filtrate and washings to dryness and dissolve the residue in 2 ml of methanol.

Reference solution (a). Dilute 0.5 ml of the test solution to 100 ml with methanol.

Reference solution (b). A 2.0 per cent w/v solution of econazole nitrate RS in methanol.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and expose to iodine vapour for 1 hour. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a). Ignore any spot with an Rf value higher than 0.9.

Other tests. Complies with the tests stated under Pessaries.

Assay. Dissolve 5 pessaries in 250.0 ml of anhydrous glacial acetic acid with the aid of gentle heat and allow to cool. Titrate 100.0 ml of the solution with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.04447 g of C18H15Cl3N2O,HNO3.

Storage. Store protected from light.

Efavirenz

\[
\text{C}_{14}\text{H}_{9}\text{ClF}_{3}\text{NO}_{2} \quad \text{Mol. Wt. 315.7}
\]

Efavirenz is (4S)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.

Efavirenz contains not less than 98.0 per cent and not more than 102.0 per cent of C14H9ClF3NO2, calculated on the dried basis.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with efavirenz RS or with the reference spectrum of efavirenz.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). –90.0° to –100.0°, determined in a 0.5 per cent w/v solution in methanol.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of methanol.

Reference solution. Dilute 1 ml of the test solution to 10 ml with methanol.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated octadecylsilyl silica gel (5 µm),
- mobile phase: a filtered and degassed mixture of 50 volumes of acetonitrile and 50 volumes of a 0.86 per cent w/v solution of ammonium dihydrogen phosphate, the pH of which is adjusted to 3.0 ± 0.05 with phosphoric acid,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the column efficiency determined from the efavirenz peak is not less than 6000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution. Determine the amount of related substances by area normalisation method. The content of any individual impurity is not more than 0.5 per cent and the sum of all the impurities is not more than 1.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying at 105° in an oven for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.12 g of the substance under examination in 100 ml of methanol.

Reference solution. A 0.12 per cent w/v solution of efavirenz RS in methanol.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a filtered and degassed mixture of 50 volumes of acetonitrile and 50 volumes of a 0.86 per
cent w/v solution of ammonium dihydrogen phosphate, the pH of which is adjusted to 3.0 ± 0.05 with phosphoric acid,
– flow rate. 1.5 ml per minute,
– spectrophotometer set at 254 nm,
– a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the column efficiency determined from the efavirenz peak is not less than 6000 theoretical plates and the tailing factor is not more than 2.0.

Inject alternately the test solution and the reference solution.
Calculate the content of C₁₄H₉ClF₃NO₂.

Storage. Store protected from light.

Efavirenz Capsules

Efavirenz Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of efavirenz, C₁₄H₉ClF₃NO₂.

Identification

A. When examined in the range 220 nm to 350 nm (2.4.7), the test solution in the Assay shows an absorption maximum at about 252 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Mix well the contents of 20 capsules and shake a quantity of the mixed contents containing about 5 mg of Efavirenz with sufficient methanol to obtain a solution containing 1 mg per ml of Efavirenz.

Reference solution. A 0.1 per cent w/v solution of efavirenz RS in methanol.

Chromatographic system
– a stainless steel column 25 cm x 4.6 mm, packed with base deactivated octadecylsilyl silica gel (5 µm),
– mobile phase: a filtered and degassed mixture of 50 volumes of acetonitrile and 50 volumes of a 0.86 per cent w/v solution of ammonium dihydrogen phosphate, the pH of which is adjusted to 3.0 ± 0.05 with phosphoric acid,
– flow rate. 1.5 ml per minute,
– spectrophotometer set at 252 nm,
– a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the column efficiency determined from the efavirenz peak is not less than 6000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution. Determine the amount of related substances by the area normalisation method. The content of any individual impurity is not more than 1.0 per cent and the sum of all impurities is not more than 2.0 per cent.

Dissolution (2.5.2).

Apparatus. No. 1
Medium. 900 ml of a 1 per cent w/v solution of sodium lauryl sulphate
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate and dilute a suitable volume of the filtrate if necessary, with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 252 nm (2.4.7). Calculate the content of efavirenz, C₁₄H₉ClF₃NO₂ in the medium from the absorbance obtained from a solution of known concentration of efavirenz RS in the same solvent.

D. Not less than 70 per cent of the stated amount of C₁₄H₉ClF₃NO₂.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Mix well the contents of 20 capsules and shake a quantity of the mixed contents containing about 60 mg of Efavirenz with sufficient methanol to obtain a solution containing 6 mg of Efavirenz per ml. Disperse the mixture with the aid of ultrasound for 20 minutes, filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate, and dilute 10.0 ml of the filtrate to 50.0 ml with methanol.

Reference solution. A 0.12 per cent w/v solution of efavirenz RS in methanol.

Chromatographic system
– a stainless steel column 25 cm x 4.6 mm, packed with base deactivated octadecylsilyl silica gel (5 µm),
– mobile phase: a filtered and degassed mixture of 50 volumes of acetonitrile and 50 volumes of a 0.86 per cent w/v solution of ammonium dihydrogen phosphate, the pH of which is adjusted to 3.0 ± 0.05 with phosphoric acid,
– flow rate. 1.5 ml per minute,
– spectrophotometer set at 252 nm,
– a 20 µl loop injector.
Inject the reference solution. The test is not valid unless the column efficiency determined from the efavirenz peak is not less than 6000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the content of C14H9ClF3NO2.

Efavirenz Tablets

Efavirenz Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of efavirenz, C14H9ClF3NO2.

Identification

A. When examined in the range 220 nm to 350 nm (2.4.7), the test solution in the Assay shows an absorption maximum at about 252 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the powdered tablets with a suitable quantity of methanol to obtain a mixture containing 0.1 per cent w/v of Efavirenz and filter through a membrane filter disc with an average pore diameter not exceeding 1.0 µm, rejecting the first few ml of the filtrate.

Reference solution. A 0.1 per cent w/v solution of efavirenz RS in methanol.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated octadecylsilyl silica gel (5 µm),
- mobile phase: a filtered and degassed mixture of 50 volumes of acetonitrile and 50 volumes of a 0.86 per cent w/v solution of ammonium dihydrogen phosphate, the pH of which is adjusted to 3.0 ± 0.05 with phosphoric acid,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 252 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the column efficiency determined from the efavirenz peak is not less than 6000 theoretical plates and the tailing factor is not more than 2.0.

Dissolution (2.5.2).

Apparatus. No. 1

Medium. 900 ml of a 1 per cent w/v solution of sodium lauryl sulphate

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, reject the first few ml of the filtrate and dilute a suitable volume of the filtrate if necessary, with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 252 nm (2.4.7). Calculate the content of efavirenz, C14H9ClF3NO2 in the medium from the absorbance obtained from a solution of known concentration of efavirenz RS in the same solvent.

D. Not less than 70 per cent of the stated amount of C14H9ClF3NO2.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablets containing about 100 mg of Efavirenz and shake with sufficient methanol to obtain a mixture containing 6 mg of Efavirenz per ml. Disperse the mixture with the aid of ultrasound for 20 minutes, filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate, and dilute 10.0 ml of the filtrate to 50.0 ml with methanol.

Reference solution. A 0.12 per cent w/v solution of efavirenz RS in methanol.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated octadecylsilyl silica gel (5 µm),
- mobile phase: a filtered and degassed mixture of 50 volumes of acetonitrile and 50 volumes of a 0.86 per cent w/v solution of ammonium dihydrogen phosphate, the pH of which is adjusted to 3.0 ± 0.05 with phosphoric acid,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 252 nm,
- a 20 µl loop injector.

Inject the test solution. Determine the amount of related substances by the area normalisation method. The content of any individual impurity is not more than 1.0 per cent and the sum of all impurities is not more than 2.0 per cent.
Inject alternately the test solution and the reference solution. Calculate the content of C₁₄H₉ClF₃NO₂.

Emetine Hydrochloride

\[
\begin{align*}
\text{H}_3\text{CO} & \quad \text{H}_3\text{CO} \\
\text{H} & \quad \text{N} \\
\text{CH}_3 & \quad \text{CH}_3 \\
\text{OCH}_3 & \quad \text{OCH}_3 \\
\text{H} & \quad \text{H} \\
,2\text{HCl},7\text{H}_2\text{O} &
\end{align*}
\]

C₂₉H₄₀N₂O₄·2HCl,7H₂O  Mol. Wt. 679.7

Emetine Hydrochloride is 6',7',10,11-tetramethoxyemetan dihydrochloride heptahydrate.

Emetine Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of C₂₉H₄₀N₂O₄·2HCl, calculated on the dried basis.

Description. A white or very slightly yellowish, crystalline powder; odourless. Develops a faint yellow tint on exposure to light.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with emetine hydrochloride RS or with the reference spectrum of emetine hydrochloride.

B. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution (a).

C. Sprinkle 5 mg on the surface of 1 ml of a 5 per cent w/v solution of ammonium molybdate in sulphuric acid; a bright green colour develops.

D. Gives reaction A of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution is clear (2.4.1) and not more intensely coloured than reference solution YS5 or BYS5 (2.4.1).

pH (2.4.24). 4.0 to 6.0, determined in a 2.0 per cent w/v solution.

Specific optical rotation (2.4.22). +16.0° to +19.0°, determined in a 5.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 200 volumes of chloroform, 40 volumes of 2-methoxyethanol, 10 volumes of methanol and 1 volume of diethylamine.

Prepare the following solutions freshly:

Solvent mixture. A 1 per cent v/v solution of 2 M ammonia in methanol.

Test solution. Dissolve 50 mg of the substance under examination in 100 ml with solvent mixture.

Reference solution (a). A 0.05 per cent w/v solution of emetine hydrochloride RS in the same solvent.

Reference solution (b). A 0.001 per cent w/v solution of isoemetine hydrobromide RS in the same solvent.

Reference solution (c). A 0.001 per cent w/v solution of cephaeline hydrochloride RS in the same solvent.

Reference solution (d). A 0.0005 per cent w/v solution of emetine hydrochloride RS in the same solvent.

Reference solution (e). Mix 1 ml each of reference solutions (a), (b) and (c). Prepare immediately before use.

Apply to the plate 10 µl of each of the test solution and reference solutions (a) to (d) and 30 µl of reference solution (e). After development, dry the plate in air until the odour of solvent is no longer detectable, spray with a 0.5 per cent w/v solution of iodine in chloroform, heat at 60° for 15 minutes and examine in ultraviolet light at 365 nm. Any spots corresponding to isoemetine and cephaeline in the chromatogram obtained with the test solution are not more intense than the spots in the chromatograms obtained with reference solution (b) and (c) respectively. Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (d). The test is not valid unless the chromatogram obtained with reference solution (e) shows three clearly separated spots.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). 15.0 to 19.0 per cent, determined on 0.5 g by drying in an oven at 105° for 3 hours.

Assay. Weigh accurately about 0.4 g, dissolve in 40 ml of anhydrous glacial acetic acid and add 7 ml of mercury acetate solution. Titrate with 0.1 M perchloric acid, using crystal violet solution as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02768 g of C₂₉H₄₀N₂O₄·2HCl.
Storage. Store protected from light.

Emetine Injection

Emetine Hydrochloride Injection

Emetine Injection is a sterile solution of Emetine Hydrochloride in Water for Injections.

Emetine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of emetine hydrochloride, C₂₉H₄₀N₂O₄·2HCl·7H₂O.

Identification

A. To a volume of the injection containing 0.2 g of Emetine Hydrochloride add an excess of 5 M sodium hydroxide and extract with ether. Evaporate the ether, dissolve the residue in a few ml of ethanol (95 per cent), add 0.25 ml of carbon disulphide, boil and scratch the sides of the container with a glass rod; a crystalline precipitate separates which melts at about 206° (2.4.21).

B. Evaporate 1 ml on a water-bath to dryness. The residue complies with the following tests.

Sprinkle 5 mg on the surface of 1 ml of a 5 per cent w/v solution of ammonium molybdate in sulphuric acid; a bright green colour develops.

C. Gives reaction A of chlorides (2.3.1).

Tests

pH (2.4.24). 2.7 to 4.0.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Dilute a volume containing about 0.2 g of Emetine Hydrochloride to 20 ml with water, add 10 ml of 5 M sodium hydroxide and shake with successive quantities, each of 50 ml, of ether until complete extraction of the alkaloid is effected. Wash the combined ether extracts with successive quantities, each of 10 ml, of water until the washings, after extraction with a further 50 ml of ether, are neutral to litmus paper. Mix the ether solutions, add 20 ml of water and 10.0 ml of 0.1 M hydrochloric acid, shake, allow to separate and collect the aqueous layer, shake the ether solution with two further quantities, each of 20 ml, of water, mix the aqueous solutions and titrate with 0.1 M sodium hydroxide using methyl red solution as indicator.

1 ml of 0.1 M hydrochloric acid is equivalent to 0.03398 g of C₂₉H₄₀N₂O₄·2HCl·7H₂O.

Storage. Store protected from light.

Emtricitabine

C₈H₁₀FN₃O₃S

Mol. Wt. 247.3

Emtricitabine is 4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2-(1H)-pyrimidone.

Emtricitabine contain not less than 98.0 per cent and not more than 102.0 per cent of C₈H₁₀FN₃O₃S, calculated on the dried basis.

Description. A white to off-white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with emtricitabine RS or with the reference spectrum of emtricitabine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). -125.0° to -150.0°, determined in a 0.5 per cent w/v solution in methanol.

Enantiomeric purity. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 50 ml of the mobile phase.

Reference solution. Dissolve 25 mg of racemic emtricitabine RS in 25 ml of the mobile phase.

Chromatographic system

– a stainless steel column 25 cm x 4.6 mm, packed with a chiral stationary phase (5 µm) (such as Chirobiotic V),
– mobile phase: a mixture of 1000 volumes of methanol, 2 volumes of diethyl amine and 1 volume of glacial acetic acid,
– flow rate. 0.5 ml per minute,
– spectrophotometer set at 277 nm,
– a 10 µl loop injector.

Inject the reference solution. The elution order is, the 5-fluoro-1-(2R,5S)-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine isomer followed by the other isomer. The resolution between the two isomers should not be less than 2.0.
Inject the test solution and measure the areas of the two isomers.

Calculate the content of the 5-fluoro-1-(2R,5S)-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine isomer by area normalization, not less than 99.0 per cent.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 25 mg of the substance under examination in 25 ml of the mobile phase.

**Reference solution (a).** A 0.1 per cent w/v solution of emtricitabine RS in the mobile phase.

**Reference solution (b).** Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm, packed with pentafluorophenyl bonded to silica (5 µm) (such as F-5 Supelco discovery),
- mobile phase: a mixture of 99 volumes of 0.025 M ammonium acetate solution with the pH adjusted to 5.0 and 1 volume of methanol, 
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 277 nm,
- a 20 µl loop injector.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0, the column efficiency is not less than 2000 theoretical plates.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak due to the reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak due to the reference solution (b) (2.0 per cent).

**Heavy metals** (2.3.13). 1 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1 g by drying in an oven at 105º.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 25 mg of the substance under examination in 25.0 ml of the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

**Reference solution.** A 0.1 per cent w/v solution of emtricitabine RS in the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm, packed with pentafluorophenyl bonded to silica (5 µm) (such as F-5 Supelco discovery),
- mobile phase: a mixture of 95 volumes of 0.025 M ammonium acetate solution adjusted the pH to 5.0 and 5 volumes of methanol,
- flow rate. 1 ml per minute,
- spectrophotometer set at 277 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 2000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of C₈H₁₀FN₃O₃S.

**Storage.** Store protected from light and moisture.

**Emtricitabine Capsules**

Emtricitabine Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of emtricitabine, C₈H₁₀FN₃O₃S.

**Identification**

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Disperse the quantity of contents of the capsules containing 1 mg of Emtricitabine in 100 ml with methanol and filter.

When examined in the range 200 nm to 400 nm (2.4.7), the filtrate shows absorption maxima at the same wavelengths as 0.001 per cent w/v solution of emtricitabine RS in methanol.

**Tests**

**Dissolution** (2.5.2).

Apparatus. No 1

Medium. 900 ml of 0.1 M hydrochloric acid.

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not more than 0.5 µm, rejecting the first 2 ml of the filtrate.

Determine by liquid chromatography (2.4.14).

**Test solution.** Use the filtrate.

**Reference solution.** Dissolve 27.5 mg of emtricitabine RS in 15 ml of methanol, dilute to 25 ml with the mobile phase. Dilute 2 ml of the solution to 10 ml with the dissolution medium.

Use the chromatographic system described under Assay.

Inject the test solution and the reference solution.
D. Not less than 75 per cent of the stated amount of C₈H₁₀FN₃O₃S.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh a quantity of the contents of the capsules containing 100 mg of Emtricitabine, disperse in 100 ml of the mobile phase and filter.

**Reference solution (a).** A 0.1 per cent w/v solution of emtricitabine RS in the mobile phase.

**Reference solution (b).** Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm, packed with pentafluorophenyl bonded to silica (5 µm) (such as F-5 Supelco discovery),
- mobile phase: a mixture of 99 volumes of a buffer solution prepared by dissolving 1.9 g of ammonium acetate in 1000 ml of water and adjusting the pH to 5.0 with glacial acetic acid, and 1 volume of methanol,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 277 nm,
- a 10 µl loop injector.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 2000 theoretical plates.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak due to the reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 3 times the area of the peak due to the reference solution (b) (3.0 per cent).

**Other tests.** Comply with the tests stated under Capsules.

**Water** (2.3.43). Not more than 5.0 per cent, determined on 0.5 g.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh accurately a quantity of the mixed contents of 20 capsules containing about 100 mg of Emtricitabine, disperse in 100.0 ml of methanol and filter. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

**Reference solution.** A 0.1 percent w/v solution of emtricitabine RS in methanol. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Intersil ODS 3V),
- mobile phase: a mixture of 80 volumes of a buffer solution prepared by dissolving 1.9 g of ammonium acetate in 1000 ml of water and adjusting the pH to 3.8 with glacial acetic acid, and 20 volumes of methanol,
- flow rate. 1 ml per minute,
- spectrophotometer set at 277 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 2000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of C₈H₁₀FN₃O₃S in the capsules.

**Storage.** Store protected from moisture.

**Emulsifying Wax**

**Anionic Emulsifying Wax**

Emulsifying Wax is a waxy solid containing 90 parts of Cetostearyl Alcohol, 10 parts of Sodium Lauryl Sulphate or sodium salts of similar sulphated higher primary aliphatic alcohols, and 4 parts of Purified Water.

**Description.** An almost white or pale yellow, waxy solid or flakes; odour, faint and characteristic. It becomes soft on warming.

**Identification**

The residue obtained in the test for Unsaponifiable matter melts at about 52° (2.4.21).

**Tests**

**Acidity.** Weigh accurately about 20.0 g, add a mixture of 40 ml of ether and 75 ml of ethanol (95 per cent), previously neutralised to phenolphthalein solution, and warm gently until solution is effected. Titrade with 0.1 M sodium hydroxide using phenolphthalein solution as indicator until a pink colour which persists for at least 15 seconds is obtained. Not more than 1.0 ml of 0.1 M sodium hydroxide is required.

**Alkalinity.** 25 ml of a 20 per cent w/v dispersion in warm ethanol (95 per cent), previously neutralised to phenolphthalein solution and cooled, exhibits no colour on the addition of 0.5 ml of phenolphthalein solution.

**Saponification value** (2.3.37). Not more than 2.0, determined on 20.0 g.

**Unsaponifiable matter** (2.3.39). Not less than 86.0 per cent, calculated on the anhydrous basis, determined on 5 g and omitting the titration of the residue.

**Iodine value** (2.3.28). Not more than 3.0, determined by the iodine monochloride method.
**Alcohols.** To 3.5 g of the residue obtained in the test for Unsaponifiable matter add 12 g of stearic anhydride and 10 ml of xylene and heat gently under a reflux condenser for 30 minutes. Cool, add a mixture of 40 ml of pyridine and 4 ml of water; reflux for a further 30 minutes and titrate the hot solution with 1 M sodium hydroxide using phenolphthalein solution as indicator. Repeat the operation omitting the residue. The difference between the titrations is not less than 12.8 ml and not more than 14.2 ml.

**Sodium alkyl sulphates.** Not less than 8.7 per cent, calculated as C_{12}H_{25}O_4SNa, on the anhydrous basis, determined by the following method. Weigh accurately about 0.25 g, dissolve as completely as possible in 15 ml of chloroform, add 30 ml of water, 10 ml of 1 M sulphuric acid and 1 ml of dimethyl yellow-oracet blue B solution and titrate with 0.004 M benzethonium chloride, shaking vigorously and allowing the layers to separate after each addition, until the chloroform layer acquires a permanent clear green colour.

1 ml of 0.004 M benzethonium chloride is equivalent to 0.001154 g of C_{12}H_{25}O_4SNa.

**Water (2.3.43).** Not more than 4.0 per cent, determined on 0.6 g.

**Enalapril Maleate**

![Enalapril Maleate structure](image)

C_{20}H_{28}N_2O_5,C_4H_4O_4, Mol. Wt. 492.5


Enalapril Maleate contains not less 98.0 per cent and not more than 102.0 per cent of C_{20}H_{28}N_2O_5,C_4H_4O_4, calculated on the dried basis.

**Description.** An off-white, crystalline powder.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with enalapril maleate RS or with the reference spectrum of enalapril maleate.

B. Melts at about 144° (2.4.21).

**Tests**

**Specific optical rotation** (2.4.22). –41.0° to –43.5°, determined in a 1.0 per cent w/v solution in methanol.

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 2 hours.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh accurately about 30 mg of the substance under examination and dissolve in 100.0 ml of the mobile phase.

**Reference solution.** A freshly prepared 0.03 per cent w/v solution of enalapril maleate RS in the mobile phase.

**Chromatographic system**

- a stainless steel column 25 cm x 4.6 mm, packed with a rigid spherical styrene divinylbenzene copolymer (5 to 10 µm),
- mobile phase: a mixture of 4 volumes of mixed phosphate buffer pH 6.8 and 1 volume of acetonitrile,
- column temperature 70°,
- flow rate, 1 ml per minute,
- spectrophotometer set at 210 nm,
- a 20 µl loop injector.

Inject the references solution. The test is not valid unless the column efficiency is not less than 300 theoretical plates and the relative standard deviation for the area of the peak due to enalapril maleate is not more than 1.0 per cent.

Inject the test solution and reference solution.

Calculate the content of C_{20}H_{28}N_2O_5,C_4H_4O_4.

**Enalapril Maleate Tablets**

Enalapril Maleate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of enalapril maleate, C_{20}H_{28}N_2O_5,C_4H_4O_4.

**Identification**

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

**Tests**

**Uniformity of content** (For tablets containing 10 mg or less). Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

**Test solution.** Finely crush one tablet, transfer to a 50-ml volumetric flask, add about 30 ml of mixed phosphate buffer pH 2.0, disperse with the aid of ultrasound for 15 minutes,
shake for another 30 minutes, dilute to volume with the buffer solution, mix and filter. Dilute a portion of the filtrate with the buffer solution to obtain a solution containing 0.01 per cent w/v of Enalapril Maleate.

**Reference solution.** A 0.01 per cent w/v solution of enalapril maleate RS in the same buffer solution.

**Chromatographic system**
- a stainless steel column 20 cm x 4.6 mm, packed with octylsilyl silica gel (3 to 10 µm),
- mobile phase: a filtered and degassed mixture of 75 volumes of phosphate buffer pH 2.0 and 25 volumes of acetonitrile,
- column temperature 50°,
- flow rate 2 ml per minute,
- spectrophotometer set at 215 nm,
- a 50 µl loop injector.

Inject alternately the test solution and the reference solution. Calculate the content of C_{20}H_{28}N_{2}O_{5},C_{4}H_{4}O_{4} in the tablet.

**Other tests.** Complies with the tests stated under Tablets.

**Assay.** Complies with the tests stated under Tablets.

**Test solution.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Enalapril Maleate, add 150 ml of phosphate buffer pH 2.0, disperse with the aid of ultrasound for 15 minutes, shake for another 30 minutes and dilute with the buffer to 250.0 ml, mix and filter.

**Reference solution.** A 0.02 per cent w/v solution of enalapril maleate RS in phosphate buffer pH 2.0.

Follow the chromatographic procedure described under Uniformity of content.

Calculate the content of C_{20}H_{28}N_{2}O_{5},C_{4}H_{4}O_{4} in the tablet.

**Ephedrine**

\[
\text{C}_{10}\text{H}_{15}\text{NO} \quad \text{Mol. Wt. 165.2 (anhydrous)}
\]

\[
\text{C}_{10}\text{H}_{15}\text{NO}, \frac{1}{2}\text{H}_{2}\text{O} \quad \text{Mol. Wt. 174.2 (hemihydrate)}
\]

Ephedrine is (1R,2S)-2-methylamino-1-phenylpropan-1-ol, an alkaloid obtained from Ephedra or prepared synthetically. It may be anhydrous or a hemihydrate.

Ephedrine contains not less than 98.5 per cent and not more than 101.0 per cent of C_{10}H_{15}NO, calculated on the anhydrous basis.

**Description.** Colourless crystals or a white, crystalline powder. Gradually decomposes on exposure to light.

**Identification**

*Test A may be omitted if tests B, C, D and E are carried out.
Tests B and C may be omitted if tests A, D and E are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6) on discs prepared in the following manner. Separately dissolve 40 mg of the substance under examination and 50 mg of ephedrine hydrochloride RS in 1 ml of water, add 1 ml of 2 M sodium hydroxide, shake and dry the organic layer with 0.2 g of anhydrous sodium sulphate. Prepare the discs using 0.3 g of potassium bromide IR, apply dropwise to the discs 0.1 ml of the chloroform layer, allowing the solvent to evaporate between applications, and dry the discs at 50° for 2 minutes.

Compare the spectrum with that obtained with the base isolated from ephedrine hydrochloride RS or with the reference spectrum of ephedrine.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.05 per cent w/v solution in 0.1 M hydrochloric acid shows absorption maxima at about 251 nm, 259 nm and 265 nm; absorbance at about 251 nm, about 0.37; at about 259 nm, about 0.48; at about 265 nm about 0.36.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

D. Dissolve 10 mg in 1 ml of water, add 0.2 ml of dilute hydrochloric acid and add 0.1 ml of cupric sulphate solution followed by 1 ml of sodium hydroxide solution; a violet colour is produced. Add 2 ml of ether and shake; the ether layer is purple and the aqueous layer is blue.

E. Melting range (2.4.21). 40° to 43° (hydrated material), determined on the undried substance. The anhydrous material melts at about 36°.

**Tests**

**Appearance of solution.** A 2.5 per cent w/v solution is clear (2.4.1), and colourless (2.4.1).

**Specific optical rotation** (2.4.22). –41.0° to –43.0°, determined at 20° in a solution prepared by dissolving 2.25 g in 15 ml of dilute hydrochloric acid and diluting to 50.0 ml with water.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 80 volumes of 2-propanol, 15 volumes of strong ammonia solution and 5 volumes of chloroform.
**Test solution (a).** Dissolve 0.2 g of the substance under examination in 10 ml of methanol.

**Test solution (b).** Dilute 5 ml of test solution (a) to 50 ml with methanol.

**Reference solution (a).** Dilute 5 ml of test solution (b) to 100 ml with methanol.

**Reference solution (b).** A 0.25 per cent w/v solution of ephedrine hydrochloride RS in methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with ninhydrin solution and heat at 110° for 5 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). Ignore any spot of lighter colour than the background.

**Chlorides.** Dissolve 0.18 g in 10 ml of water; add 5 ml of 2 M nitric acid and 0.5 ml of silver nitrate solution and allow to stand for 2 minutes protected from bright light. Any opalescence produced is not more intense than that obtained by repeating the operation at the same time and in the same manner using 2.0 ml of chloride standard solution (25 ppm Cl) in place of the solution of the substance under examination (280 ppm).

**Sulphated ash (2.3.18).** Not more than 0.1 per cent.

**Water (2.3.43).** 4.5 to 5.5 per cent (for hemihydrate) and not more than 1.0 per cent (for anhydrous), determined on 0.3 g.

**Assay.** Weigh accurately about 0.5 g and dissolve in 5 ml of ethanol (95 per cent). Add 50.0 ml of 0.1 M hydrochloric acid and titrate with 0.1 M sodium hydroxide using methyl red solution as indicator until a yellow colour is obtained.

1 ml of 0.1 M hydrochloric acid is equivalent to 0.01652 g of C₁₀H₁₅NO,HCl.

**Storage.** Store protected from light.

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**Ephedrine Hydrochloride**

**C₁₀H₁₅NO,HCl**  
Mol. Wt. 201.7

Ephedrine Hydrochloride is (1R,2S)-2-methylamino-1-phenylpropan-1-ol hydrochloride.

Ephedrine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of C₁₀H₁₅NO,HCl calculated on the dried basis.

**Description.** Colourless crystals or a white, crystalline powder; odourless. It is affected by light.

**Identification**

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ephedrine hydrochloride RS or with the reference spectrum of ephedrine hydrochloride.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Dissolve 10 mg in 1 ml of water and add 0.1 ml of cupric sulphate solution and 1 ml of sodium hydroxide solution; a violet colour is produced. Add 2 ml of ether and shake; the ether layer is purple and the aqueous layer is blue.

D. A 5 per cent w/v solution gives reaction A of chlorides (2.3.1).

**Tests**

**Appearance of solution.** A 10.0 per cent w/v solution (Solution A) is clear (2.4.1), and colourless (2.4.1).

**Acidity or alkalinity.** To 10 ml of Solution A add 0.1 ml of methyl red solution and 0.2 ml of 0.01 M sodium hydroxide; the solution is yellow. Add 0.4 ml of 0.01 M hydrochloric acid; the solution is red.

**Specific optical rotation** (2.4.22). –33.5° to –35.5°, determined in a 5.0 per cent w/v solution.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 80 volumes of 2-propanol, 15 volumes of strong ammonia solution and 5 volumes of chloroform.

**Test solution (a).** Dissolve 0.2 g of the substance under examination in 10 ml of methanol.

**Test solution (b).** Dilute 5 ml of test solution (a) to 50 ml with methanol.

**Reference solution (a).** Dilute 5 ml of test solution (b) to 100 ml with methanol.

**Reference solution (b).** A 0.2 per cent w/v solution of ephedrine hydrochloride RS in methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with ninhydrin solution and heat at 110° for 5 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). Ignore any spot of lighter colour than the background.

**Sulphates (2.3.17).** 15 ml of solution A complies with the limit test for sulphates (100 ppm).

**Sulphated ash (2.3.18).** Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

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**Assay.** Weigh accurately about 0.17 g, dissolve in 10 ml of mercuric acetate solution, warming gently, add 50 ml of acetone and mix. Titrate with 0.1 M perchloric acid, using 1 ml of a saturated solution of methyl orange in acetone as indicator, until a red colour is obtained. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02017 g of C₁₀H₁₅NO,HCl.

**Storage.** Store protected from light.

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**Ephedrine Oral Solution**

Ephedrine Hydrochloride Oral Solution; Ephedrine Hydrochloride Elixir; Ephedrine Elixir

Ephedrine Oral Solution is a solution containing 0.3 per cent w/v of Ephedrine Hydrochloride in a suitable flavoured vehicle containing a sufficient volume of Ethanol (95 per cent) or of an appropriate dilute ethanol to give a final concentration of not more than 3 per cent v/v of ethanol.

Ephedrine Oral Solution contains not less than 0.27 per cent and not more than 0.33 per cent w/v of ephedrine hydrochloride, C₁₀H₁₅NO,HCl.

**Identification**

A. To 30 ml add 2 ml of 2 M hydrochloric acid, extract with two quantities, each of 20 ml, of ether and discard the ether. Add sufficient dilute ammonia solution to the aqueous phase to make it alkaline, extract with two quantities, each of 30 ml, of ether, wash the combined ether extracts with three quantities, each of 15 ml, of water, dry over anhydrous sodium sulphate, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ephedrine hydrochloride RS treated in the same manner or with the reference spectrum of ephedrine.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

**Tests**

**Ethanol content.** Not more than 3 per cent v/v, determined by gas chromatography (2.4.13).

**Test solution.** Use the preparation under examination.

**Reference solution (a).** Add sufficient of 1-propanol (internal standard) to the test solution to produce a solution containing 5.0 per cent v/v of 1-propanol.

**Chromatographic system**

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: 0.005 M dioctyl sodium sulphosuccinate

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with ninhydrin solution and heat at 110° for 5 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). Ignore any spot of lighter colour than the background.

**Other tests.** Complies with the tests stated under Oral Liquids.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dilute an accurately weighed quantity of the oral solution containing 60 mg of Ephedrine Hydrochloride to 50 ml with methanol.

**Reference solution.** A 0.12 per cent w/v solution of ephedrine hydrochloride RS in methanol.

**Chromatographic system**

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: 0.005 M dioctyl sodium sulphosuccinate

**Calculate the percentage content of ethanol from the areas of the peaks due to ethanol in the chromatograms obtained with reference solutions (a) and (b).**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 80 volumes of 2-propanol, 15 volumes of strong ammonia solution and 5 volumes of chloroform.

**Test solution (a).** Add sufficient 5 M ammonia to 50 ml of the oral solution to make it alkaline, extract with two quantities, each of 100 ml, of ether, wash the combined extracts with 10 ml of water, dry with anhydrous sodium sulphate, filter and evaporate the filtrate to dryness. Dissolve the oily residue in sufficient methanol to produce 5 ml.

**Test solution (b).** Dilute 1 ml of test solution (a) to 10 ml with methanol.

**Reference solution (a).** Dilute 1 ml of test solution (a) to 200 ml with methanol.

**Reference solution (b).** A 0.3 per cent w/v solution of ephedrine hydrochloride RS in methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with ninhydrin solution and heat at 110° for 5 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). Ignore any spot of lighter colour than the background.
in a mixture of 65 volumes of methanol, 35 volumes of water and 1 volume of glacial acetic acid,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 263 nm,
- a 20 µl loop injector.

Determine the weight per ml of the oral solution (2.4.29), and calculate the content of \( \text{C}_{10}\text{H}_{15}\text{NO},\text{HCl} \), weight in volume.

**Storage.** Store protected from light.

**Ephedrine Tablets**

**Ephedrine Hydrochloride Tablets**

Ephedrine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of ephedrine hydrochloride, \( \text{C}_{10}\text{H}_{15}\text{NO},\text{HCl} \).

**Identification**

A. Shake a quantity of the powdered tablets containing 0.1 g of Ephedrine Hydrochloride with 20 ml of \( 0.1 \text{ M hydrochloric acid} \), filter, wash the filtrate with two quantities, each of 20 ml, of chloroform and discard the chloroform. Make the aqueous layer alkaline with \( 5 \text{ M ammonia} \) and extract with two quantities, each of 30 ml, of a mixture of 3 volumes of chloroform and 1 volume of ethanol (95 per cent). Dry the combined extracts over anhydrous sodium sulphate, filter and evaporate to a low volume at a pressure of 2 kPa. Prepare a disc using 0.3 g of potassium bromide IR, apply dropwise to the disc 0.1 ml of the chloroform solution, allowing the solvent to evaporate between applications, and dry the disc at 50° for 2 minutes. The disc so obtained complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ephedrine hydrochloride RS treated in the same manner or with the reference spectrum of ephedrine.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Triturate a quantity of the powdered tablets containing about 0.4 g of Ephedrine Hydrochloride with 10 ml of chloroform and discard the chloroform. Repeat trituration with a further 10 ml of chloroform and again discard the chloroform. Shake the residue with 30 ml of warm ethanol (95 per cent) for 20 minutes, filter, evaporate the filtrate to dryness on a water-bath and dry the residue at 80° (residue R). Dissolve 10 mg of residue R in 1 ml of water and add 0.1 ml of cupric sulphate solution followed by 1 ml of sodium hydroxide solution; a violet colour is produced. Add 1 ml of ether and shake; the ether layer is purple and the aqueous layer is blue.

D. A 5 per cent w/v solution of residue R gives reaction A of chlorides (2.3.1).

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 80 volumes of 2-propanol, 15 volumes of strong ammonia solution and 5 volumes of chloroform.

**Test solution (a).** Extract a quantity of the powdered tablets containing 0.1 g of Ephedrine Hydrochloride with 5 ml of methanol and filter.

**Test solution (b).** Dilute 1 ml of test solution (a) to 10 ml with methanol.

**Reference solution (a).** Dilute 1 ml of test solution (a) to 200 ml with methanol.

**Reference solution (b).** A 0.2 per cent w/v solution of ephedrine hydrochloride RS in methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with ninhydrin solution and heat at 110° for 5 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). Ignore any spot of lighter colour than the background.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh 20 tablets and reduce to a fine powder. Weigh accurately a quantity of the powder containing about 0.15 g of Ephedrine Hydrochloride and add 30 ml of anhydrous glacial acetic acid and 10 ml of mercuric acetate solution. Warm gently to effect solution and cool. Titrate with 0.1 M perchloric acid, using 0.1 ml of crystal violet solution as indicator, until the violet colour changes to green-blue. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02017 g of \( \text{C}_{10}\text{H}_{15}\text{NO},\text{HCl} \).

**Storage.** Store protected from light.

**Ergocalciferol**

Calciferol; Vitamin D₂

![Ergocalciferol Structure](image)

Ergocalciferol is (5Z,7E,22E)-(3S)-9,10-secoergosta-5,7,10(19),22-tetraen-3-ol.
Ergocalciferol contains not less than 97.0 per cent and not more than 103.0 per cent of $\text{C}_28\text{H}_{44}\text{O}$.

**Description.** White or almost white crystals or a white or slightly yellowish, crystalline powder. It is sensitive to air, heat and light. A reversible isomerisation to pre-ergocalciferol may occur in solution, depending on temperature and time.

**Identification**

*Test A may be omitted if tests B, C and D are carried out.* *Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ergocalciferol RS.

B. Dissolve 1 mg in 1 ml of 1,2-dichloroethane and 4 ml of antimony trichloride solution; a yellowish-orange colour is produced.

C. In the test for Ergosterol, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution (a).

D. To a solution of about 0.5 mg in 5 ml of chloroform add 0.3 ml of acetic anhydride and 0.1 ml of sulphuric acid and shake vigorously; a bright red colour is produced which rapidly changes through violet and blue to green.

**Tests**

*Specific optical rotation (2.3.22).* $+103^\circ$ to $+107^\circ$, determined within 30 minutes of preparation, in a solution prepared by dissolving 0.2 g rapidly and without heating in sufficient aldehyde-free ethanol (95 per cent) to produce 25.0 ml.

*Light absorption.* Dissolve 10 mg rapidly and without heating in sufficient aldehyde-free ethanol (95 per cent) to produce 100.0 ml. Dilute 5.0 ml of this solution to 50.0 ml with aldehyde-free ethanol (95 per cent). Absorbance of the resulting solution at the maximum at about 265 nm (2.4.7), measured within 30 minutes of preparation, 0.45 to 0.50.

*Reducing substances.* To 10 ml of a 1 per cent w/v solution in aldehyde-free ethanol (95 per cent) add 0.5 ml of a 0.5 per cent w/v solution of blue tetrazolium in aldehyde-free ethanol (95 per cent) and 0.5 ml of a solution prepared by diluting 1 volume of tetramethylammonium hydroxide solution (10 per cent) with aldehyde-free ethanol (95 per cent) to make 10 volumes. Allow to stand for exactly 5 minutes and add 1 ml of glacial acetic acid. Measure the absorbance of the resulting solution at the maximum at about 525 nm (2.4.7), using as the blank a solution prepared by treating 10 ml of aldehyde-free ethanol (95 per cent) in the same manner. The absorbance is not more than that obtained by carrying out the procedure described above simultaneously using a solution containing 0.2 $\mu$g per ml of hydroquinone in aldehyde-free ethanol (95 per cent) and beginning at the words “add 0.5 ml of a 0.5 per cent w/v solution...”.

*Ergosterol.* Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

*Mobile phase.* A 0.01 per cent w/v solution of butylated hydroxytoluene in a mixture of equal volumes of cyclohexane and peroxide-free ether.

*Test solution.* Dissolve 0.25 g of the substance under examination in sufficient 1,2-dichloroethane containing 1 per cent w/v of squalane and 0.1 per cent w/v butylated hydroxytoluene (solvent A) to produce 5 ml.

*Reference solution (a).* A 5.0 per cent w/v solution of ergocalciferol RS in solvent A.

*Reference solution (b).* A 0.01 per cent w/v solution of ergosterol RS in solvent A.

*Reference solution (c).* Mix equal volumes of reference solutions (a) and (b).

Apply to the plate 10 $\mu$l of each solution. Develop the chromatograms immediately, protected from light. After development dry the plate in air and spray three times with antimony trichloride reagent. Examine the chromatograms for not more than 4 minutes after spraying. The principal spot in the chromatogram obtained with the test solution is initially orange-yellow but becomes brown later. In the chromatogram obtained with the test solution any violet spot with an R$_f$ value slightly lower than that of the principal spot (due to ergosterol and appearing slowly) is not more intense than the spot in the chromatogram obtained with reference solution (b). The chromatogram obtained with the test solution shows no spot that does not correspond to one of the spots in the chromatograms obtained with reference solutions (a) and (b). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

*Assay.* Carry out the following procedure as rapidly as possible in subdued light and protected from air.

Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh accurately about 50.0 mg of the substance under examination, dissolve in 10 ml of toluene without heating and dilute to 100.0 ml with the mobile phase; dilute 5.0 ml of this solution to 50.0 ml with the mobile phase; further dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

*Reference solution (a).* Dissolve 50.0 mg of ergocalciferol RS in 10 ml of toluene without heating and dilute to 100.0 ml with the mobile phase; dilute 5.0 ml of this solution to 50.0 ml with the mobile phase; further dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

*Reference solution (b).* Dissolve 50.0 mg of cholecalciferol RS in 10 ml of toluene without heating and dilute to 100.0 ml
with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase. Heat under a reflux condenser 5.0 ml of this solution, under nitrogen, using a water-bath for 60 minutes to obtain a solution of cholecalciferol, precholecalciferol and trans-cholecalciferol. Cool and dilute the refluxed solution to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with porous silica particles 3 to 10 µm (such as Nucleosil 50-S 5 µm),
- mobile phase: a mixture of 997 volumes of hexane and 3 volumes of 1-pentanol,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 10 or 20 µl loop injector.

Inject a suitable volume of reference solution (b) and adjust the sensitivity so that the height of the peak due to cholecalciferol is more than 50 per cent of full-scale deflection. The approximate relative retention times calculated with reference to cholecalciferol are 0.4 for precholecalciferol and 0.5 for trans-cholecalciferol. The resolution between precholecalciferol and trans-cholecalciferol should not be less than 1.0; if necessary adjust the proportions of the constituents and flow rate of the mobile phase to obtain the required resolution.

Inject a suitable volume of reference solution (a) and adjust the sensitivity so that height of the peak due to ergocalciferol is more than 50 per cent of full-scale deflection. The contents of an opened container should be used immediately.

Ergometrine Maleate

**Ergonovine Maleate**

![Chemical Structure](image)

C_{19}H_{23}N_{3}O_{4}, C_{4}H_{4}O_{4} Mol.Wt.4415

Ergometrine Maleate is 9,10-didehydro-N-[\((S)\]-2-hydroxy-1-methylethyl]-6-methylergoline-8\(\beta\)-carboxamide hydrogen maleate.

Ergometrine Maleate contains not less than 98.0 per cent and not more than 101.0 per cent of C_{19}H_{23}N_{3}O_{4},C_{4}H_{4}O_{4}, calculated on the dried basis.

**Description.** A white or faintly yellow, crystalline powder; odourless. It is affected by light.

**Identification**

Test A may be omitted if tests B, C, D and E are carried out. Tests B, D and E may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ergometrine maleate RS* or with the reference spectrum of ergometrine maleate.

B. Dissolve 30 mg in sufficient 0.01 M hydrochloric acid to produce 100 ml and dilute 10 ml of the solution to 100 ml with the same solvent. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 311 nm and a minimum at 265 nm to 272 nm; absorbance at about 311 nm, 0.52 to 0.58.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

D. Dissolve 0.1 g, without heating and protected from light, in sufficient carbon dioxide-free water to produce 10 ml (solution A). To 0.1 ml of solution A add 1 ml of glacial acetic acid, 1 drop of ferric chloride solution and 1 ml of phosphoric acid and heat on a water-bath at 80°; a blue or violet colour is produced after about 10 minutes.

E. To 1 ml of a 0.01 per cent w/v solution add 2 ml of 4-dimethylaminobenzaldehyde solution; a deep blue colour is produced after about 10 minutes.

**Tests**

**Appearance of solution.** Solution A is clear (2.4.1), and not more intensely coloured than reference solution YS5 or BYS5 (2.4.1).

**pH** (2.4.24). 3.6 to 4.4, determined in solution A.

**Specific optical rotation** (2.4.22). +50.0° to +56.0°, determined in solution A.

**Related substances.** Carry out the following operations as rapidly as possible, protected from light.

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*. 

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**Storage.** Store protected from light in hermetically-sealed containers under nitrogen in a refrigerator (2° to 8°). The contents of an opened container should be used immediately.
Mobile phase. A mixture of 75 volumes of chloroform, 25 volumes of methanol and 3 volumes of water.

Prepare the following solutions freshly.

Solvent mixture. A mixture of 1 volume of strong ammonia solution and 9 volumes of ethanol (80 per cent).

Test solution (a). Dissolve 0.1 g of the substance under examination in 10 ml with solvent mixture.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with the same solvent mixture.

Reference solution (a). A 0.1 per cent w/v solution of ergometrine maleate RS in the same solvent mixture.

Reference solution (b). A 0.01 per cent w/v solution of ergometrine maleate RS in the same solvent mixture.

Reference solution (c). A 0.005 per cent w/v solution of ergometrine maleate RS in the same solvent mixture.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 14 cm. Dry the plate in air and examine in ultraviolet light at 365 nm. Assess the intensities of any secondary spots in the chromatogram obtained with the test solution by reference to the spots in the chromatograms obtained with reference solutions (b), (c) and (d). The total of the intensities so assessed does not exceed 10 per cent of the intensity of the principal spot.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Carry out the following procedure protected from light.

Dilute a suitable volume, accurately measured, of the injection with sufficient water to produce a solution containing 1 mg of Ergometrine Maleate per ml. Titrate with 0.05 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.05 M perchloric acid is equivalent to 0.02207 g of C₁₉H₂₃N₃O₂.C₄H₄O₄.
0.004 per cent w/v of Ergometrine Maleate. To 3.0 ml add 6.0 ml of 4-dimethylaminobenzaldehyde solution, mix, cool to room temperature and allow to stand for 30 minutes (solution A). At the same time prepare solution B in the same manner but using 3.0 ml of a 0.004 per cent w/v solution of ergometrine maleate RS and beginning at the words “add 6.0 ml......”. Measure the absorbance of solution B at the maximum at about 545 nm (2.4.7), using as the blank a solution prepared by mixing 6.0 ml of 4-dimethylaminobenzaldehyde solution and 3.0 ml of water. Without delay replace solution B with solution A, using the same cell, and measure the absorbance of solution A at the same wavelength. Calculate the content of C₁₉H₂₃N₃O₂, C₄H₄O₄.

Storage. Store protected from light in single dose containers in a refrigerator (2° to 8°).

**Ergometrine Tablets**

Ergometrine Maleate Tablets; Ergonovine Tablets; Ergonovine Maleate Tablets

Ergometrine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ergometrine maleate, C₁₉H₂₃N₃O₂C₄H₄O₄. The tablets may be coated.

**Identification**

A. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution (a).

B. Extract a quantity of the powdered tablets containing 2 mg of Ergometrine Maleate with 20 ml of water, filter and wash the residue with sufficient water to produce 20 ml. The solution exhibits a blue fluorescence.

C. To 2 ml of the solution obtained in test B add 4 ml of 4-dimethylaminobenzaldehyde solution; a deep blue colour is produced after about 10 minutes.

**Tests**

**Related substances.** Carry out the following procedure in subdued light and protect from light any solutions not used immediately.

Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G slurred with 0.1 M sodium hydroxide.

**Mobile phase.** A mixture of 90 volumes of chloroform and 10 volumes of methanol.

**Test solution.** Triturate a quantity of the powdered tablets containing 1 mg of Ergometrine Maleate with 0.2 ml of a 1 per cent w/v solution of domiphen bromide, add 2 ml of methanol, centrifuge and remove the supernatant liquid. Extract the residue with two quantities, each of 1 ml, of methanol, evaporate the combined extracts to dryness at 20° at a pressure not exceeding 2 kPa and dissolve the residue in 0.25 ml of methanol, centrifuge if necessary.

**Reference solution (a).** A 0.4 per cent w/v solution of ergometrine maleate RS in methanol.

**Reference solution (b).** Dilute 5 ml of reference solution (a) to 50 ml with methanol.

**Reference solution (c).** Dilute 5 ml of reference solution (b) to 10 ml with methanol.

**Reference solution (d).** Dilute 5 ml of reference solution (c) to 10 ml with methanol.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. Assess the intensities of any secondary spots in the chromatogram obtained with the test solution by reference to the spots in the chromatograms obtained with reference solutions (b), (c) and (d). The total of the intensities so assessed does not exceed 10 per cent of the intensity of the principal spot.

**Uniformity of content.** Comply with the test stated under Tablets.

**Protect the solutions from light throughout the test.**

To one tablet add 10.0 ml of a 1 per cent w/v solution of tartaric acid, shake for 30 minutes, centrifuge and use the supernatant liquid. Dilute a suitable volume, accurately measured, with sufficient water to produce a solution containing 0.004 per cent w/v of Ergometrine Maleate. To 3.0 ml add 6.0 ml of 4-dimethylaminobenzaldehyde solution, mix, cool to room temperature and allow to stand for 30 minutes (solution A). At the same time prepare solution B in the same manner but using 3.0 ml of a 0.004 per cent w/v solution of ergometrine maleate RS and beginning at the words “add 6.0 ml......” Measure the absorbance of solution B at the maximum at about 545 nm (2.4.7), using as the blank a solution prepared by mixing 6.0 ml of 4-dimethylaminobenzaldehyde solution and 3.0 ml of water. Without delay replace solution B with solution A, using the same cell, and measure the absorbance of solution A at the same wavelength. Calculate the content of C₁₉H₂₃N₃O₂C₄H₄O₄ in the tablet.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 2 mg of Ergometrine Maleate, shake with 50.0 ml of a 1 per cent w/v solution of tartaric acid for 30 minutes, centrifuge and use the supernatant liquid. Carry out the procedure described under Uniformity of content beginning at the words “To 3.0 ml add 6 ml......”.

**Storage.** Store protected from light.
Ergotamine Tartrate

\[
(C_{33}H_{35}N_{5}O_{5})_2.C_4H_6O_6 \quad \text{Mol. Wt.} \quad 1313.4
\]

Ergotamine Tartrate is (5'S)-12'-hydroxy-2'-methyl-3',6',18-trioxo-5-benzylergotaman (2R,3R) tartrate.

Ergotamine Tartrate contains not less than 98.0 per cent and not more than 101.0 per cent of \((C_{33}H_{35}N_{5}O_{5})_2.C_4H_6O_6\), calculated on the dried basis.

**Description.** Colourless crystals, or a white or almost white, crystalline powder; odourless; slightly hygroscopic. It may contain two molecular equivalents of methanol of crystallisation.

**Identification**

Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Before trituration with potassium bromide IR during preparation of the disc, triturate first with 0.2 ml of methanol. Compare the spectrum with that obtained with ergotamine tartrate RS or with the reference spectrum of ergotamine tartrate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent solution in 0.01 M hydrochloric acid shows an absorption maximum at 311 nm to 321 nm and a minimum at 265 nm to 275 nm; absorbance at the maximum, 0.59 to 0.64, calculated on the dried basis.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a) when examined for not more than 1 minute in ultraviolet light at 365 nm or when examined in daylight after spraying with ethanolic 4-dimethylaminobenzaldehyde solution.

D. Dissolve 1 mg in a mixture of 5 ml of glacial acetic acid and 5 ml of ethyl acetate. To 1 ml of the solution add 1 ml of sulphuric acid, with continuous shaking and cooling; a blue colour with a red tinge develops. Add 0.1 ml of ferric chloride test solution previously diluted with an equal volume of water; the red tinge becomes less apparent and the blue colour more pronounced.

E. Dissolve 1 mg in 5 ml of a 1 per cent w/v solution of tartaric acid. To 1 ml of this solution add slowly 3 ml of 4-dimethylaminobenzaldehyde solution and mix; a deep blue colour is produced.

**Tests**

Carry out the following tests as rapidly as possible, protected from light.

**Appearance of solution.** Mix 50 mg with 25 mg of tartaric acid and dissolve in 20 ml of water. The solution is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

**pH** (2.4.24). 4.0 to 6.0, determined in a 0.25 per cent w/v suspension.

**Specific optical rotation** (2.4.22). The specific optical rotation of the ergotamine base, checked for purity by the method given below, is \(-154^\circ \) to \(-165^\circ \), determined by the following method. Dissolve 0.4 g in 40 ml of a 1 per cent w/v solution of tartaric acid, cautiously add 0.5 g of sodium bicarbonate in small portions and mix well. Wash 100 ml of chloroform by shaking with 5 quantities, each of 50 ml, of water and extract the solution of the substance under examination with 4 quantities, each of 10 ml, of the washed chloroform. Filter the combined chloroform extracts through a small filter moistened with the washed chloroform, dilute to 50 ml with the same solvent and measure the optical rotation.

To 25 ml of the chloroform solution add 50 ml of anhydrous glacial acetic acid. Titrate with \(0.05\) M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of \(0.05\) M perchloric acid is equivalent to 0.02908 g of ergotamine base, \(C_{33}H_{35}N_{5}O_{5}\).

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 70 volumes of ether, 15 volumes of dimethylformamide, 10 volumes of chloroform and 5 volumes of ethanol.

Prepare the following solutions immediately before use in the order stated.

**Solvent mixture.** A mixture of 9 volumes of chloroform and 1 volume of methanol.

**Test solution (a).** Dissolve 0.1 g of the substance under examination in 10 ml with solvent mixture.

**Test solution (b).** Dilute 5 ml of test solution (a) to 50 ml with the same solvent mixture.
Ergotamine Tartrate Injection

Ergotamine Injection is a sterile solution of Ergotamine Tartrate in Water for Injection containing Ethanol (95 per cent), Glycerin and sufficient Tartaric Acid to adjust the pH of the solution to 3.3.

Ergotamine Injection contains a quantity of total alkaloids, calculated as (C_{33}H_{35}N_{5}O_{5})_2.C_4H_6O_6, equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ergotamine tartrate, of which 50 to 70 per cent is present as ergotamine tartrate.

Description. A clear, colourless or almost colourless solution.

Identification

A. In the test for Ergot alkaloids and related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that due to ergotamine in the chromatogram obtained with the reference solution.

B. To a volume containing 0.2 mg of Ergotamine Tartrate add 1 ml of 4-dimethylaminobenzaldehyde solution; a deep blue colour is produced.

C. Mix a volume containing 2 mg of Ergotamine Tartrate with 2 ml of dilute sulphuric acid, dissolve a few mg of magnesium powder in the solution and add 25 mg of resorcinol. Shake to dissolve, carefully add 2 ml of sulphuric acid down the inside of the tube and warm gently; a red ring forms at the interface of the two liquid layers and spreads throughout the lower layer.

Tests

Carry out the following tests as rapidly as possible, protected from light.

pH (2.4.24). 2.8 to 3.8.

Ergot alkaloids and related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G slurried with 0.1 M sodium hydroxide.

Mobile phase. A mixture of 90 volumes of chloroform and 10 volumes of methanol.

Test solution. Add sufficient of a 10 per cent w/v solution of sodium bicarbonate to a volume of the injection containing 5 mg of Ergotamine Tartrate to make it distinctly alkaline to litmus paper. Extract with five quantities, each of 10 ml, of chloroform, filter the extracts through a small double filter paper, wash the filter with chloroform, evaporate the combined filtrates and washings to dryness at 20° at a pressure of about 1.5 kPa and dissolve the residue in 1 ml of a mixture of equal volumes of chloroform and methanol.

Reference solution. Dissolve 5 mg of ergotamine tartrate RS in 10 ml of a 1 per cent w/v solution of tartaric acid and complete the preparation described for the test solution beginning at the words “Extract with five quantities...”.

Apply without delay, to the plate 20 µl of the test solution and 14 µl, 10 µl, 7 µl and 2 µl of the reference solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. The chromatogram obtained with the test solution shows two principal spots, corresponding to ergotamine and, of higher Rf value, ergotaminine; a spot between the two principal spots and a number of spots of lower Rf values may also be seen. Compare the chromatogram obtained with the test solution with the chromatograms obtained with the reference solution. The spot corresponding to ergotaminine is not larger or more intense than the spot

Reference solution (a). A 0.1 per cent w/v solution of ergotamine tartrate RS in the same solvent mixture.

Reference solution (b). A 0.015 per cent w/v solution of ergotamine tartrate RS in the same solvent mixture.

Reference solution (c). A 0.005 per cent w/v solution of ergotamine tartrate RS in the same solvent mixture.

Apply to the plate 5 µl of each solution. Immediately after applying expose the plate to an atmosphere saturated with ammonia vapour for exactly 20 seconds, dry the plate at the line of application in a current of cold air and immediately start developing the chromatogram, allowing the mobile phase to rise 17 cm. Dry the plate in a current of dry air for 2 minutes and examine in ultraviolet light at 365 nm for not more than 1 minute. Spray abundantly with ethanolic 4-dimethylaminobenzaldehyde solution and dry in a current of warm air for about 2 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (c).

Loss on drying (2.4.19). Not more than 6.0 per cent, determined on 0.1 g by drying over phosphorus pentoxide at a pressure of 1.5 to 2.5 kPa for 6 hours.

Assay. Weigh accurately about 0.2 g and dissolve in 40 ml of anhydrous glacial acetic acid. Titrate with 0.05 M perchloric acid, determining the end-point potentiometrically (2.4.25).

Carry out a blank titration.

1 ml of 0.05 M perchloric acid is equivalent to 0.03284 g of (C_{33}H_{35}N_{5}O_{5})_2.C_4H_6O_6.

Storage. Store protected from light in sealed glass containers, under nitrogen and in a refrigerator (2° to 8°).
corresponding to ergotamine obtained with 7 µl of the reference solution. The spot corresponding to ergotamine is not smaller or less intense than the spot corresponding to ergotamine obtained with 10 µl of the reference solution and is not larger or more intense than the spot corresponding to ergotamine obtained with 14 µl of the reference solution, corresponding to not less than 50 per cent and not more than 70 per cent of ergotamine tartrate. Any other spots are not larger or more intense than the spot corresponding to ergotamine obtained with 2 µl of the reference solution.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. To an accurately measured volume add sufficient of a 0.25 per cent w/v solution of tartaric acid to produce a solution containing about 0.005 per cent w/v of Ergotamine Tartrate. Mix 3.0 ml of this solution with 6.0 ml of 4-dimethylaminobenzaldehyde solution, cool to room temperature and allow to stand for 30 minutes (solution A). At the same time mix 3.0 ml of a 0.003 per cent w/v solution of ergometrine maleate RS in a 0.25 per cent w/v solution of tartaric acid with 6.0 ml of 4-dimethylaminobenzaldehyde solution, cool to room temperature and allow to stand for 30 minutes (solution B). Prepare solution C by mixing 3.0 ml of a 0.25 per cent w/v solution of tartaric acid with 6.0 ml of 4-dimethylaminobenzaldehyde solution. Measure the absorbance of solution B at 545 nm (2.4.7), using solution C as the blank. Without delay replace solution B with solution A, using the same cell, and measure the absorbance of solution A at the same wavelength. Calculate the content of total alkaloids as \((C_{33}H_{35}N_{5}O_{5})_2C_4H_6O_6\) from the absorbances obtained.

1 mg of ergometrine maleate RS is equivalent to 1.488 mg of \((C_{33}H_{35}N_{5}O_{5})_2C_4H_6O_6\).

Storage. Store protected from light in single dose glass containers at a temperature not exceeding 30°.

Ergotamine Tablets

Ergotamine Tartrate Tablets

Ergotamine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ergotamine tartrate, \((C_{33}H_{35}N_{5}O_{5})_2C_4H_6O_6\). The tablets may be coated.

Identification

A. Triturate a quantity of the powdered tablets containing 5 mg of Ergotamine Tartrate with 10 ml of light petroleum (40° to 60°), allow to settle and discard the petroleum extract. To the residue add 10 ml of chloroform saturated with strong ammonia solution, triturate, filter and evaporate the filtrate to dryness on a water-bath. The residue so obtained complies with the following tests.

Dissolve 1 mg in a mixture of 5 ml of glacial acetic acid and 5 ml of ethyl acetate. To 1 ml of the solution add 1 ml of sulphuric acid, with continuous shaking and cooling; a blue colour with a red tinge develops. Add 0.1 ml of ferric chloride test solution previously diluted with an equal volume of water; the red tinge becomes less apparent and the blue colour more pronounced.

B. Dissolve 1 mg in 5 ml of a 1 per cent w/v solution of tartaric acid. To 1 ml of this solution add slowly 3 ml of 4-dimethylaminobenzaldehyde solution and mix; a deep blue colour is produced.

C. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

Tests

Carry out the following tests as rapidly as possible, protected from light.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 70 volumes of ether, 15 volumes of dimethylformamide, 10 volumes of chloroform and 5 volumes of ethanol.

Prepare the following solutions immediately before use in the order stated.

Test solution. Extract a quantity of the powdered tablets containing 1 mg of Ergotamine Tartrate with 2 ml of a mixture of equal volumes of chloroform and methanol and centrifuge. Remove the supernatant liquid, extract the residue with two quantities, each of 1 ml, of the solvent mixture, evaporate the combined extracts to dryness at 20° at a pressure of 2 kPa and dissolve the residue in 0.25 ml of a mixture of equal volumes of chloroform and methanol; centrifuge if necessary.

Reference solution (a). A 0.4 per cent w/v solution of ergotamine tartrate RS in the same solvent mixture.

Reference solution (b). A 0.04 per cent w/v solution of ergotamine tartrate RS in the same solvent mixture.

Reference solution (c). A 0.02 per cent w/v solution of ergotamine tartrate RS in the same solvent mixture.

Reference solution (d). A 0.01 per cent w/v solution of ergotamine tartrate RS in the same solvent mixture.

Apply to the plate 5 µl of each solution. Immediately after application expose the plate to an atmosphere saturated with
ammonia vapour for exactly 20 seconds, dry the plate at the line of application in a current of cold air and immediately start developing the chromatogram, allowing the mobile phase to rise 17 cm. Dry the plate in air and examine in ultraviolet light at 365 nm. Assess the intensity of any secondary spots in the chromatogram obtained with the test solution by reference to the spots in the chromatograms obtained with reference solutions (a), (b) and (c). The sum of the intensities so assessed in the chromatogram obtained with the test solution should not exceed 10 per cent of the intensity of the principal spot in the chromatogram obtained with the test solution. In addition, any single secondary spot in any chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (d).

**Uniformity of content.** Comply with the test stated under Tablets.

To one tablet add sufficient quantity of a 1 per cent w/v solution of tartaric acid to produce a solution containing 0.05 mg of Ergotamine Tartrate per ml. Shake for 30 minutes and centrifuge. Mix 3.0 ml of this solution with 6.0 ml of 4-dimethylaminobenzaldehyde solution, cool to room temperature and allow to stand for 30 minutes (solution A). At the same time mix 3.0 ml of a 0.003 per cent w/v solution of ergometrine maleate RS in a 0.25 per cent w/v solution of tartaric acid with 6.0 ml of 4-dimethylaminobenzaldehyde solution, cool to room temperature and allow to stand for 30 minutes (solution B). Prepare solution C by mixing 3.0 ml of a 0.25 per cent w/v solution of tartaric acid with 6.0 ml of 4-dimethylaminobenzaldehyde solution. Measure the absorbance of solution B at 545 nm (2.4.7), using solution C as the blank. Without delay replace solution B with solution A, using the same cell, and measure the absorbance of solution A at the same wavelength. Calculate the content of total alkaloids as (C33H35N5O5)2.C4H6O6 from the absorbances at the same wavelength. Calculate the content of total alkaloids as (C33H35N5O5)2.C4H6O6 from the absorbances at the same wavelength.

1 mg of ergometrine maleate RS is equivalent to 1.488 mg of (C33H35N5O5)2.C4H6O6.

Calculate the content of (C33H35N5O5)2.C4H6O6 in the tablet.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 5 mg of Ergotamine Tartrate and dissolve in 50 ml of a 1 per cent w/v solution of tartaric acid, allow to stand for 30 minutes with frequent shaking and dilute to 100.0 ml with water. Using 3.0 ml of the clear supernatant liquid, carry out the procedure described under Uniformity of content beginning at the words “Mix 3.0 ml of this solution with 6.0 ml of 4-dimethylaminobenzaldehyde solution...”.

**Storage.** Store protected from light at a temperature not exceeding 30°.

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**Erythromycin**

C_{37}H_{67}NO_{13}    
Mol. Wt. 733.9

Erythromycin is a mixture of macrolide antibiotics consisting largely of erythromycin A, (2R,3S,4S,5R,6R,8R,10R,11R,12S,13R)-5-(3-amino-3,4,6-trideoxy-N,N-dimethyl-α-D-xyl-o-hexopyranosylxy)-3-(2,6-dideoxy-3-C-3-dimethyl-α-L-ribo-hexopyranosyloxy)-13-ethyl-6,11,12-trihydroxy-2,4,6,8,10,12-hexamethyl-9-oxotridecan-13-olide, it is produced by the growth of certain strains of Streptomyces erythreus.

Erythromycin has a potency not less than 920 Units per mg, calculated on the anhydrous basis.

**Description.** Colourless or slightly yellow crystals or a white or slightly yellow powder; slightly hygroscopic.

**Identification**

Test A may be omitted if tests B, C and D are carried out. Tests B and D may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with erythromycin RS or with the reference spectrum of erythromycin.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** The upper layer obtained by shaking together 45 volumes of ethyl acetate, 40 volumes of a 15 per cent w/v solution of ammonium acetate previously adjusted to pH 9.6 with 10 M ammonia and 20 volumes of 2-propanol and allowing to separate.

**Test solution.** Dissolve 0.1 g of the substance under examination in 100 ml of methanol.

**Reference solution (a).** A 0.1 per cent w/v solution of erythromycin RS in methanol.

**Reference solution (b).** A 0.2 per cent w/v solution of spiramycin RS in methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with ethanolic anisaldehyde
solution, heat at 110° for 5 minutes and allow to cool. The
principal spot in the chromatogram obtained with the test
solution corresponds to that in the chromatogram obtained
with reference solution (a) and is different in position and
colour from the spots in the chromatogram obtained with
reference solution (b).

C. To about 5 mg add 5 ml of a 0.02 per cent w/v solution of
xanthydrol in a mixture of 1 volume of hydrochloric acid
and 99 volumes of 5 M acetic acid and heat on a water-bath; a red
colour is produced.

D. Dissolve about 10 mg in 5 ml of 7 M hydrochloric acid
and allow to stand for about 20 minutes; a yellow colour develops.

Tests

pH (2.4.24). 8.0 to 10.5, determined in a 0.066 per cent w/v
solution in carbon dioxide-free water.

Specific optical rotation (2.4.22). −71.0° to −78.0°, determined
in a 2.0 per cent w/v solution in ethanol. Measure the optical
rotation at least 30 minutes after preparing the solution.

Related substances. Determine by thin-layer chromatography
(2.4.17), coating the plate with silanised silica gel H.

Mobile phase. A mixture of 75 volumes of methanol and 45
volumes of a 5 per cent w/v solution of ammonium acetate.

Test solution. Dissolve 0.2 g of the substance under
examination in 100 ml of methanol.

Reference solution (a). A 0.2 per cent w/v solution of
erthyromycin RS in methanol.

Reference solution (b). A 0.01 per cent w/v solution of
erthyromycin RS in methanol.

Apply to the plate 10 µl of each solution. After development,
and after drying at a pressure not exceeding 0.7 kPa complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6).

Heavy metals (2.3.13). 1.0 g complies with the limit test for
heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 6.5 per cent, determined on 0.2
g using a 10 per cent w/v solution of imidazole in anhydrous
methanol as the solvent.

Assay. Determine by the microbiological assay of antibiotics,
Method A (2.2.10), using a solution prepared by dissolving
about 25 mg, accurately weighed, in 10 ml of methanol and
adding sufficient water to produce 100.0 ml. Express the results
as units per mg.

Storage. Store protected from light at a temperature not
exceeding 30°.

Erythromycin Tablets

Erythromycin Tablets contain not less than 90.0 per cent and
not more than 110.0 per cent of the stated amount of
erthyromycin, C_{37}H_{67}NO_{13}. The tablets are enteric-coated.

Identification

A. Shake a quantity of the powdered tablets containing 0.1 g
of Erythromycin with 5 ml of chloroform, decolourise if
necessary, with decolorising charcoal, filter and evaporate
the filtrate to dryness. The residue after drying at a pressure
not exceeding 0.7 kPa complies with the following test.

B. Dissolve a quantity of the powdered tablets containing 3
mg of Erythromycin as completely as possible in 2 ml of
acetone and add 2 ml of hydrochloric acid; an orange colour
is produced which changes to red and then to deep purplish
red. Add 2 ml of chloroform and shake; the chloroform layer
becomes purple.

Tests

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by the microbiological assay of antibiotics,
Method A (2.2.10) on a solution prepared in the following
manner.

Weigh and powder 20 tablets. Weigh accurately a quantity of
the powder containing about 0.4 g of Erythromycin and
triturate with 10 ml of sterile phosphate buffer pH 8.0 and add
sufficient sterile phosphate buffer pH 8.0 to produce 100.0 ml.

Calculate the content of erythromycin in the tablets, taking
each 1000 Units found to be equivalent to 1 mg of
erthyromycin.

Storage. Store protected from light at a temperature not
exceeding 30°.
Erythromycin Estolate

![Erythromycin Estolate structure](structure.png)

Erythromycin Estolate is

$$(2R,3S,4S,5R,6R,8R,10R,11R,12S,13R)-5-(3\text{-amino}-3,4,6\text{-}
trideoxy-N,N\text{-dimethyl-}\beta\text{-d-xylo-hexopyranosyloxy})\text{-}3\text{-}(2,6\text{-}
dideoxy-3-C,3-O\text{-dimethyl-}\alpha\text{-l-ribo-hexopyranosyloxy})\text{-}13\text{-}
ethyl-6,11,12-tri hydroxy-2,4,6,8,10,12\text{-hexamethyl-9-}
oxotridecan-13\text{-olide dodecyl sulphate.}

Erythromycin Estolate has a potency not less than 610 Units per mg, calculated on the anhydrous basis.

**Description.** A white, crystalline powder.

**Identification**

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *erythromycin estolate RS* or with the reference spectrum of erythromycin estolate.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated principal spots.

C. Suspend about 3 mg in 2 ml of *1 M sulphuric acid, add 0.1 ml of a 0.01 per cent w/v solution of methylene blue and 2 ml of chloroform and shake; the chloroform layer becomes blue.

D. Dissolve about 10 mg in 5 ml of *7 M hydrochloric acid and allow to stand for 20 minutes; a yellow colour is produced.

**Tests**

**pH (2.4.24).** 5.5 to 7.0, determined in the supernatant liquid obtained by suspending 0.4 g in 10 ml of carbon dioxide-free water; shaking for 5 minutes and allowing to stand.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silanised silica gel G.

*Mobile phase.* A mixture of 85 volumes of chloroform, 5 volumes of ethanol (95 per cent) and 1 volume of a 15 per cent w/v solution of ammonium acetate previously adjusted to pH 7.0.

*Test solution (a).* Dissolve 0.4 g of the substance under examination in 100 ml of acetone.

*Test solution (b).* Dilute 5 ml of test solution (a) to 20 ml with acetone.

*Reference solution (a).* A 0.1 per cent w/v solution of erythromycin estolate RS in acetone.

*Reference solution (b).* A solution containing 0.1 per cent w/v each of *erythromycin estolate RS* and *erythromycin ethylsuccinate RS* in acetone.

*Reference solution (c).* A 0.008 per cent w/v of *erythromycin RS* in acetone.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *anisaldehyde solution*, heat at 110° for 5 minutes and allow to cool. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the principal spot in the chromatogram obtained with reference solution (c).

**Content of dodecyl sulphate, C$_{12}$H$_{26}$O$_4$S.** 23.0 per cent to 25.5 per cent, calculated on the anhydrous basis and determined by the following method. Weigh accurately about 0.5 g and dissolve in 25 ml of dimethylformamide. Titrate with 0.1 M sodium methoxide, using 0.05 ml of a 0.3 per cent w/v solution of thymol blue in methanol as indicator. Carry out a blank titration.

1 ml of 0.1 M sodium methoxide is equivalent to 0.02664 g of C$_{12}$H$_{26}$O$_4$S.

**Sulphated ash (2.3.18).** Not more than 0.5 per cent, determined on 0.5 g.

**Water (2.3.43).** Not more than 4.0 per cent, determined on 0.3 g using a 10 per cent w/v solution of imidazole in anhydrous methanol as the solvent.

**Assay.** Determine by the microbiological assay of antibiotics, Method A (2.2.10) on a solution prepared in the following manner. Weigh accurately about 40 mg, dissolve in 40 ml of methanol and add 20 ml of phosphate buffer pH 7.0 and sufficient water to produce 100.0 ml. Maintain the solution at 60° for 3 hours and cool. Express the results as units per mg.
**Storage.** Store protected from light at a temperature not exceeding 30°.

**Erythromycin Estolate Tablets**

Erythromycin Estolate Tablets contain not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of erythromycin, C_{37}H_{67}NO_{13}. The tablets may be coated.

**Identification**

A. To a quantity of the powdered tablets containing 0.1 g of erythromycin add 10 ml of chloroform, shake well, decolorise if necessary, with decolorising charcoal, filter and evaporate the filtrate to dryness. The residue after drying at a pressure not exceeding 0.7 kPa complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with erythromycin estolate RS or with the reference spectrum of erythromycin estolate.

B. Dissolve a quantity of the powdered tablets containing 12 mg of erythromycin as completely as possible in 2 ml of acetone and add 2 ml of hydrochloric acid; an orange-red colour is produced which changes to red and then to deep purple. Add 2 ml of chloroform and shake; the chloroform layer becomes purple.

**Tests**

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by the microbiological assay of antibiotics, Method A (2.2.10) on a solution prepared in the following manner.

Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 25 mg of erythromycin, dissolve in 40 ml of methanol, add 20 ml of phosphate buffer pH 7.0 and sufficient water to produce 100.0 ml. Maintain the solution at 60° for 3 hours, cool and filter. Calculate the content of erythromycin in the tablets, taking each 1000 Units found to be equivalent to 1 mg of erythromycin.

**Storage.** Store at a temperature not exceeding 30°.

**Labelling.** The label states the strength in terms of the equivalent amount of erythromycin.

**Erythromycin Stearate**

C_{37}H_{67}NO_{13}, C_{18}H_{36}O_{2}  
Mol. Wt. 1018.4

Erythromycin Stearate is a mixture of the stearate of Erythromycin with an excess of stearic acid.

Erythromycin Stearate has a potency not less than 600 Units per mg, calculated on the anhydrous basis.

**Description.** Colourless or slightly yellow crystals or a white or slightly yellow, crystalline powder.

**Identification**

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. The upper layer of a mixture of 45 volumes of ethyl acetate, 40 volumes of a 15 per cent w/v solution of ammonium acetate, previously adjusted to pH 9.6 with 9 M ammonia, and 20 volumes of 2-propanol.

Test solution. Dissolve 0.28 g of the substance under examination in 100 ml of methanol.

Reference solution (a). A 0.2 per cent w/v solution of erythromycin RS in methanol.

Reference solution (b). A 0.1 per cent w/v solution of stearic acid in methanol.

Apply to the plate 5 µl of each solution. After development, dry the plate in air, spray with a solution containing 0.02 per cent w/v of 2,7-dichlorofluorescein and 0.01 per cent w/v of rhodamine B in ethanol (95 per cent), allow the plate to stand for a few seconds in the vapour above a water-bath and examine in ultraviolet light at 365 nm. The chromatogram obtained with the test solution exhibits two spots, one of which corresponds in position to the principal spot in the chromatogram obtained with reference solution (a) and the other to the principal spot in the chromatogram obtained with reference solution (b).

Spray the plate with ethanolic anisaldehyde solution, heat at 110° for 5 minutes and examine in daylight. The coloured spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a).

B. To about 5 mg add 5 ml of a 0.02 per cent w/v solution of xanthydrol in a mixture of 1 volume of hydrochloric acid and 99 volumes of 5 M acetic acid and heat on a water-bath; a red colour is produced.

C. Dissolve about 10 mg in 5 ml of 7 M hydrochloric acid and allow to stand for about 20 minutes; a yellow colour develops.

**Tests**

**pH** (2.4.24). 7.0 to 10.5, determined in a 1.0 per cent w/v suspension.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silanised silica gel H.

Mobile phase. A mixture of 100 volumes of methanol and 60 volumes of a 15 per cent w/v solution of ammonium acetate.
Test solution. Dissolve 0.28 g of the substance under examination in 100 ml of methanol.

Reference solution (a). A 0.2 per cent w/v solution of erythromycin RS in methanol.

Reference solution (b). A 0.01 per cent w/v solution of erythromycin RS in methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with ethanolic anisaldehyde solution, heat at 110° for 5 minutes and allow to cool. Any spot with an Rf value lower than that of the principal spot in the chromatogram obtained with reference solution (a) and any spot with an Rf value higher than that of the principal spot is not more intense than the corresponding spot in the chromatogram obtained with reference solution (b).

Erythromycin stearate. Not less than 84.0 per cent of C_{37}H_{67}NO_{13}, calculated on the anhydrous basis and determined by the following method. Weigh accurately about 0.4 g and dissolve in 30 ml of chloroform. If the solution is opalescent, filter and shake the residue with three quantities, each of 25 ml, of chloroform. Filter, if necessary, and wash the filter with chloroform. Evaporate the combined filtrate and washings on a water-bath to about 30 ml, add 50 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.1018 g of C_{37}H_{67}NO_{13},C_{18}H_{36}O_{2}.

Free stearic acid. Not more than 14.0 per cent of C_{18}H_{36}O_{2}, calculated on the anhydrous basis and determined by the following method. Weigh accurately about 0.4 g and dissolve in 50 ml of methanol. Titrate with 0.1 M sodium hydroxide, determining the end-point potentiometrically (2.4.25). Calculate the volume of 0.1 M sodium hydroxide required for each g of the substance and subtract the volume of 0.1 M perchloric acid required for each g of the substance in the test for Erythromycin stearate.

1 ml of the difference is equivalent to 0.02845 g of C_{18}H_{36}O_{2}.

Erythromycin stearate and free stearic acid. 98.0 to 103.0 per cent, calculated by adding together the percentages of erythromycin stearate and free stearic acid determined as described above.

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Water (2.3.43). Not more than 4.0 per cent, determined on 0.3 g using a 10 per cent w/v solution of imidazole in anhydrous methanol as the solvent.

Assay. Determine by the microbiological assay of antibiotics, Method A (2.2.10) using a solution prepared by dissolving about 50 mg accurately weighed in sufficient methanol to produce 100.0 ml. Express the results as units per mg.

Storage. Store protected from light at a temperature not exceeding 30°.

Erythromycin Stearate Tablets

Erythromycin Stearate Tablets contain not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of erythromycin, C_{37}H_{67}NO_{13}. The tablets may be coated.

Identification

A. To a quantity of the powdered tablets containing 0.1 g of erythromycin add 10 ml of water and shake well. Decant the supernatant liquid and discard. Extract the residue by shaking with 10 ml of methanol, filter the extract and evaporate to dryness. The residue after drying at a pressure not exceeding 0.7 kPa complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with erythromycin stearate RS or with the reference spectrum of erythromycin stearate.

B. Dissolve a quantity of the powdered tablets containing 3 mg of erythromycin as completely as possible in 2 ml of acetone and add 2 ml of hydrochloric acid; an orange colour is produced which changes to red and then to deep purplish red. Add 2 ml of chloroform and shake; the chloroform layer becomes purple.

C. Extract a quantity of the powdered tablets containing 50 mg of erythromycin with 10 ml of chloroform, filter and evaporate to dryness. Heat 0.1 g of the residue gently with 5 ml of 2 M hydrochloric acid and 10 ml of water until the solution boils; oily globules rise to the surface. Cool, remove the fatty layer, heat it with 3 ml of 0.1 M sodium hydroxide and allow to cool; the solution sets to a gel. Add 10 ml of hot water and shake; the solution froths. To 1 ml add a 10 per cent w/v solution of calcium chloride; a granular precipitate is produced which is insoluble in hydrochloric acid.

Tests

Disintegration (2.5.1). 90 minutes.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by the microbiological assay of antibiotics, Method A (2.2.10) on a solution prepared in the following manner.

Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 25 mg of erythromycin and dissolve as completely as possible in sufficient methanol to
produce 100.0 ml. Calculate the content of erythromycin in the tablets, taking each 1000 Units found to be equivalent to 1 mg of erythromycin.

**Storage.** Store protected from light.

**Labelling.** The label states the strength in terms of the equivalent amount of erythromycin.

### Ethacrynic Acid

**Etacrynic Acid**

![Chemical Structure](image)

C₁₃H₁₂Cl₂O₄  Mol. Wt. 303.1

Ethacrynic Acid is 2-[2,3-dichloro-4-(2-ethylacyloyl)phenoxy]acetic acid

Ethacrynic Acid contains not less than 98.0 per cent and not more than 102.0 per cent of C₁₃H₁₂Cl₂O₄, calculated on the dried basis.

**Description.** A white or almost white, crystalline powder.

*CAUTION* - As Ethacrynic Acid irritates the skin, eyes and the mucous membranes it should be handled with care.

**Identification**

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ethacrynic acid RS* or with the reference spectrum of ethacrynic acid.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution in a mixture of 99 volumes of methanol and 1 volume of 1 M hydrochloric acid shows a well defined absorption maximum at about 270 nm and a shoulder at about 285 nm; absorbance at about 270 nm, 0.55 to 0.60.

C. To 25 mg add 2 ml of 1 M sodium hydroxide and heat in a water-bath for 5 minutes, cool, add 0.25 ml of sulphuric acid (50 per cent v/v) and 0.5 ml of a 10 per cent w/v solution of chromotropic acid sodium salt and add cautiously 2 ml of sulphuric acid; a deep violet colour is produced.

D. On 20 mg determine by the oxygen-flask method (2.3.34), using 5 ml of *dilute sulphuric acid solution* as the absorbing liquid. When the process is complete, acidify with *dilute sulphuric acid* and boil gently for 2 minutes; the solution gives the reactions of chlorides (2.3.1).

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 60 volumes of chloroform, 50 volumes of ethyl acetate and 20 volumes of glacial acetic acid.

**Test solution.** Dissolve 0.2 g of the substance under examination in 10 ml of ethanol (95 per cent).

**Reference solution (a).** A 0.03 per cent w/v solution of the substance under examination in ethanol (95 per cent).

**Reference solution (b).** A 0.01 per cent w/v solution of the substance under examination in ethanol (95 per cent).

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Sulphated ash (2.3.18).** Not more than 0.1 per cent.

**Loss on drying (2.3.19).** Not more than 0.5 per cent, determined on 2.0 g by drying in an oven over phosphorus pentoxide at 60° at a pressure of 0.1 to 0.5 kPa.

**Assay.** Weigh accurately about 0.25 g, dissolve in 100 ml of methanol and add 5 ml of water. Titrate with 0.1 M sodium hydroxide solution, determining the end-point potentiometrically (2.4.25).

1 ml of 0.1 M sodium hydroxide is equivalent to 0.03031 g of C₁₃H₁₂Cl₂O₄.

### Ethacrynic Acid Tablets

**Etacrynic Acid Tablets**

Ethacrynic Acid Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ethacrynic acid, C₁₃H₁₂Cl₂O₄.

**Identification**

Mix a quantity of the powdered tablets containing 50 mg of Ethacrynic Acid with 0.1 M hydrochloric acid and extract with two quantities, each of 40 ml, of dichloromethane. Dry the combined extracts with anhydrous sodium sulphate, filter and evaporate to dryness with the aid of gentle heat. The residue complies with the following tests.
A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ethacrynic acid RS or with the reference spectrum of ethacrynic acid.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution in a mixture of 99 volumes of methanol and 1 volume of 1 M hydrochloric acid shows a well defined absorption maximum at about 270 nm and a shoulder at about 285 nm; absorbance at about 270 nm, 0.55 to 0.60.

C. To 25 mg add 2 ml of 1 M sodium hydroxide and heat in a water-bath for 5 minutes, cool, add 0.25 ml of sulphuric acid (50 per cent v/v) and 0.5 ml of a 10 per cent w/v solution of chromotropic acid sodium salt and add cautiously 2 ml of sulphuric acid; a deep violet colour is produced.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 60 volumes of chloroform, 50 volumes of ethyl acetate and 20 volumes of glacial acetic acid.

Test solution. Shake a quantity of the powdered tablets containing 0.2 g of Ethacrynic Acid with 10 ml of ethanol (95 per cent) and filter.

Reference solution (a). Dilute 3 volumes of the test solution to 200 volumes with ethanol (95 per cent).

Reference solution (b). Dilute 1 volume of the test solution to 200 volumes with ethanol (95 per cent).

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution (a). Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing about 50 mg of Ethacrynic Acid with 0.5 ml of glacial acetic acid and 50.0 ml of acetonitrile for 15 minutes and filter.

Test solution (b). Prepare in the same manner as test solution (a) but using 0.5 ml of glacial acetic acid, 45 ml of acetonitrile and 5.0 ml of a 0.15 per cent w/v solution of propyl hydroxybenzoate (internal standard) in acetonitrile.

Reference solution. Dissolve 50 mg of ethacrynic acid RS in 5.0 ml of the internal standard solution and dilute to 50.0 ml with a mixture of 70 volumes of acetonitrile and 30 volumes of water.

Chromatographic system
- a stainless steel column 20 cm x 4 mm, packed with octadecylsilyl silica gel (10 µm),
- mobile phase: a mixture of 60 volumes of water, 40 volumes of acetonitrile and 1 volume of glacial acetic acid,
- flow rate. 2 ml per minute,
- spectrophotometer set at 270 nm,
- a 20 µl loop injector.

Inject the test and reference solution. Calculate the content of C₁₃H₁₂Cl₂O₄ in the tablets.

Ethambutol Hydrochloride

\[
\text{C}_{10}\text{H}_{24}\text{N}_{2}\text{O}_{2},2\text{HCl}
\]

Mol. Wt. 277.2

Ethambutol Hydrochloride is \((S,S)-(N,N')\text{-ethylenebis}(2\text{-aminobutan-1-ol})\) dihydrochloride.

Ethambutol Hydrochloride contains not less than 97.0 per cent and not more than 101.0 per cent of \(\text{C}_{10}\text{H}_{24}\text{N}_{2}\text{O}_{2},2\text{HCl}\), calculated on the dried basis.

Description. A white, crystalline powder; almost odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ethambutol hydrochloride RS or with the reference spectrum of ethambutol hydrochloride.

B. In the test for 2-Aminobutanol, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. A 5 per cent w/v solution gives reaction A of chlorides (2.3.1).

Tests

pH (2.4.24). 3.4 to 4.0, determined in a 2.0 per cent w/v solution.

Specific optical rotation (2.4.22). +6.0° to +6.6°, determined in a 10.0 per cent w/v solution.
2-Aminobutanol. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 75 volumes of methanol, 15 volumes of water and 10 volumes of strong ammonia solution.

Test solution (a). Dissolve 0.5 g of the substance under examination in 10 ml of methanol.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with methanol.

Reference solution (a). A 0.05 per cent w/v solution of 2-aminobutanol RS in methanol.

Reference solution (b). A 0.5 per cent w/v solution of ethambutol hydrochloride RS in methanol.

Apply to the plate 2 μl of each solution. After development, dry the plate in air, heat at 110° for 10 minutes, cool, spray with ninhydrin solution and heat at 110° for 5 minutes. Any spot corresponding to 2-aminobutanol in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

Meso ethambutol (RS isomer). Determine by either of the following methods.

Method A. Determine by differential scanning calorimetry (DSC)(2.4.31).

Test preparation: Weigh between 4 and 6 mg of the sample in the 40 μl aluminium DSC crucible. Carry out the test by heating at a rate of 10° per minute from 25° to 250°, under nitrogen purging (20 ml/min) and record the thermogram. Observe the endotherms at 42° ± 2° and 77° ± 2° corresponding to the transitions of the RS isomer and SS isomer, respectively. There should not be any endothermic peak at 42° ± 2° in the thermogram.

Method B. Determine by liquid chromatography (2.4.14)

Test solution. Suspend 4.0 mg of the substance under examination in 4.0 ml of acetonitrile and 100 μl of triethylamine. Stir the mixture with the aid of ultrasound for 5 minutes. Add 15 μl of R-(+)-phenyl isocyanate and heat the mixture for 20 minutes at 70° in a water-bath.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with acetonitrile.

Reference solution (b). Suspend 4.0 mg of ethambutol for system suitability RS (containing RS isomer) 4.0 ml of acetonitrile and 100 μl of triethylamine. Mix the mixture with the aid of ultrasound for 5 minutes. Add 15 μl of R-(+)-phenyl isocyanate and heat the mixture for 20 minutes at 70° in a water-bath.

Chromatographic system
- a column 10 cm x 4.6 mm, packed with octadecylsilane bonded to silica (3 μm)
- column temperature 40°,
Inject the reference solution. The test is not valid unless the tailing factor is not more than 3.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of C_{10}H_{24}N_{2}O_{2}.2HCl.

**Storage.** Store protected from moisture.

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**Ethambutol Tablets**

**Ethambutol Hydrochloride Tablets**

Ethambutol Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of ethambutol hydrochloride, C_{10}H_{24}N_{2}O_{2}.2HCl. The tablets may be coated.

**Identification**

A. Extract a quantity of the powdered tablets containing 50 mg of Ethambutol Hydrochloride with 5 ml of methanol, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ethambutol hydrochloride RS or with the reference spectrum of ethambutol hydrochloride.

B. Shake a quantity of the powdered tablets containing 0.1 g of Ethambutol Hydrochloride with 10 ml of water, filter, and to the filtrate add 2 ml of a 1 per cent w/v solution of copper sulphate and 1 ml of 1 M sodium hydroxide; a distinct blue colour is produced.

**Tests**

**2-Aminobutanol.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 75 volumes of methanol, 15 volumes of water and 10 volumes of strong ammonia solution.

Test solution. Shake a quantity of the powdered tablets containing 0.5 g of Ethambutol Hydrochloride for 5 minutes with sufficient methanol to produce 10 ml and filter.

Reference solution. A 0.05 per cent w/v solution of 2-aminobutanol RS in methanol.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, heat at 110° for 5 minutes, cool, spray with ninhydrin solution and heat at 110° for 5 minutes. Any spot corresponding to 2-aminobutanol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Dissolution.**

Apparatus. No 1

Medium. 900 ml of freshly distilled water

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Dilute suitably with water to produce a solution containing about 0.030 per cent w/v of ethambutol hydrochloride. Using the resulting solution as the test solution carry out the procedure described under Assay.

Calculate the content of C_{10}H_{24}N_{2}O_{2}.2HCl.

D. Not less than 75 per cent of the stated amount of C_{10}H_{24}N_{2}O_{2}.2HCl.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14).

Prepare the following solutions freshly:

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 30 mg of Ethambutol Hydrochloride, add 50 ml of water and shake for about 15 minutes and add sufficient water to produce 100.0 ml. Filter and discard the first 10 ml of the filtrate. Use the clear filtrate.

Standard solution. A 0.03 per cent w/v solution of ethambutol hydrochloride RS in water.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica particles (5 µm) (such as Zorbax SB-CN),
- column. temperature 30°,
- mobile phase: a mixture of equal volumes of a buffer consisting of 1 ml of triethylamine in sufficient water to produce 1000 ml adjusted to pH 7.0 with orthophosphoric acid, and acetonitrile,
- spectrophotometer set at 200 nm,
- a 50 µl loop injector.

Inject alternately the test solution and the reference solution.

Calculate the content of C_{10}H_{24}N_{2}O_{2}.2HCl in the tablets.

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**Ethambutol and Isoniazid Tablets**

**Ethambutol Hydrochloride and Isoniazid Tablets**

Ethambutol and Isoniazid Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of ethambutol hydrochloride, C_{10}H_{24}N_{2}O_{2}.2HCl and isoniazid, C_{6}H_{7}N_{3}O.
**Identification**

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to ethambutol hydrochloride RS in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to isoniazid RS in the chromatogram obtained with the reference solution.

**Tests**

2-Aminobutanol. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 75 volumes of methanol, 15 volumes of water and 10 volumes of strong ammonia solution.

**Test solution.** Shake a quantity of the powdered tablets containing 0.5 g of ethambutol hydrochloride for 5 minutes with sufficient methanol to produce 10 ml and filter.

**Reference solution.** A 0.05 per cent w/v solution of 2-aminobutanol RS in methanol.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, heat at 110° for 5 minutes, cool, spray with ninhydrin solution and heat at 110° for 5 minutes. Any spot corresponding to 2-aminobutanol in the chromatogram obtained with the test solution is not more intense than the corresponding spot in the chromatogram obtained with the reference solution.

**Dissolution** (2.5.2).

**Apparatus.** No 1

Medium 900 ml of water

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 10 ml of the filtrate.

On the filtrate determine by liquid chromatography (2.4.14).

For Ethambutol Hydrochloride —

**Test solution.** Dilute the filtrate to obtain 0.044 per cent w/v solution in the dissolution medium.

**Reference solution.** A 0.044 per cent w/v solution of ethambutol hydrochloride RS in the dissolution medium.

Determine the content of C_{10}H_{24}N_{2}O_{2}.2HCl by the procedure given under Assay of Ethambutol Hydrochloride.

Calculate the content of C_{10}H_{24}N_{2}O_{2}.2HCl in the medium.

For Isoniazid — Determine the amount of C_{6}H_{7}N_{3}O dissolved by measuring the absorbance of the filtrate, suitably diluted with the dissolution medium to obtain a solution containing about 0.015 mg of isoniazid per ml, at the maximum at about 263 nm (2.4.7). Calculate the content of C_{6}H_{7}N_{3}O in the medium from the absorbance obtained by repeating the determination using a 0.0015 per cent w/v solution of isoniazid RS in place of the filtrate.

D. Not less than 75 per cent of the stated amount of C_{6}H_{7}N_{3}O.2HCl and C_{6}H_{7}N_{3}O.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** For isoniazid - Determine by liquid chromatography (2.4.14).

**Diluent.** Dissolve 1.4 g of disodium hydrogen orthophosphate anhydrous in water, adjust the pH to 6.8 ± 0.05 with dilute phosphoric acid and add sufficient water to produce 1000 ml.

**Test solution.** Weigh and powder 20 tablets. Weigh accurately a quantity of powdered tablets containing about 40 mg of Isoniazid, dissolve in 50.0 ml of methanol and dilute to 500.0 ml with the diluent.

**Reference solution.** Weigh accurately about 40 mg of isoniazid RS, dissolve in 50.0 ml of methanol and dilute to 500.0 ml with the diluent.

**Chromatographic system**

- a stainless steel column 15 cm x 4.6 mm, packed with octadecyldimethylsilicongel (5 µm) (such as Intersil ODS-3),
- column temperature 30°,
- mobile phase: 96 volumes of buffer solution pH 6.8 prepared by dissolving 1.4 g disodium hydrogen orthophosphosphate anhydrous in 1000 ml of water, the pH of which is adjusted to 6.8 ± 0.05 with dilute phosphoric acid and 4 volumes of acetonitrile,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency determined from the isoniazid peak is not more than 1500 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of C_{6}H_{7}N_{3}O in the tablets.

For ethambutol hydrochloride — Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 60 mg of Ethambutol Hydrochloride and dissolve in 100.0 ml of the diluent.

**Reference solution.** A 0.06 per cent w/v solution of ethambutol hydrochloride RS in the diluent.

**Chromatographic system**

- a stainless steel column 15 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica particles (such as Zorbax SB CN 5 µm),
– mobile phase: a mixture of 50 volumes of buffer pH 7.0 prepared by mixing 1 ml of triethylamine in 1000 ml of water the pH of which is adjusted to 7.0 ± 0.05 with phosphoric acid and 50 volumes of acetonitrile.
– flow rate. 1 ml per minute,
– spectrophotometer set at 200 nm,
– a 50 µl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 3.0, the column efficiency determined from Isoniazid peak is not more than 1500 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and reference solution.
Calculate the content of C₁₀H₂₄N₂O₂·₂HCl the tablets.

Storage. Store protected from moisture.

**Ethanol**

Absolute Alcohol; Dehydrated Alcohol

CH₃CH₂OH  
Mol. Wt. 46.1

Ethanol contains not less than 99.0 per cent w/w and not more than 100.0 per cent w/w, corresponding to not less than 99.4 per cent v/v and not more than 100.0 per cent v/v, at 15.56°C, of C₂H₆O.

**Description.** A clear, colourless, mobile and volatile liquid; odour, characteristic and spirituous; hygroscopic. Readily volatilises even at low temperature; boils at 78°C; flammable, burning with a blue, smokeless flame.

**Identification**

A. Mix 0.25 ml in a small beaker with 1 ml of potassium permanganate solution and 0.25 ml of dilute sulphuric acid and cover the beaker immediately with a filter paper moistened with a solution freshly prepared by dissolving 0.1 g of sodium nitroprusside and 0.5 g of piperazine hydrate in 5 ml of water; an intense blue colour is produced on the filter paper, the colour becoming lighter after a few minutes.

B. To 5 ml of a 0.5 per cent v/v solution add 1 ml of 1 M sodium hydroxide followed by slow addition of 2 ml of iodine solution; the odour of iodoform develops and a yellow precipitate is produced.

**Tests**

**Relative density** (2.4.29). 0.7871 to 0.7902, determined at 25°C.

**Appearance of solution.** Dilute 5.0 ml to 100.0 ml with water. The solution is clear (2.4.1). Cool to 10°C for 30 minutes; the solution remains clear.

**Acidity or alkalinity.** To 20 ml add 0.25 ml of phenolphthalein solution; the solution remains colourless and requires not more than 0.2 ml of 0.1 M sodium hydroxide to produce a pink colour.

**Methanol.** To 1 drop add 1 drop of water, 1 drop of dilute phosphoric acid and 1 drop of potassium permanganate solution. Mix, allow to stand for 1 minute and add sodium bisulphite solution dropwise until the permanganate colour is discharged. If a brown colour remains, add 1 drop of dilute phosphoric acid. To the colourless solution add 5 ml of freshly prepared chromotropic acid solution and heat on a water-bath at 60°C for 10 minutes; no violet colour is produced.

**Foreign organic substances.** Clean a glass-stoppered cylinder thoroughly with hydrochloric acid, rinse with water and finally rinse with the substance under examination. Put 20 ml in the cylinder, cool to about 15°C and then add from a carefully cleaned pipette 0.1 ml of 0.1 M potassium permanganate. Mix at once by inverting the stoppered cylinder and allow to stand at 15°C for 5 minutes; the pink colour does not entirely disappear.

**2-Propanol and 2-methyl-2-propanol.** To 1 ml add 3 ml of water and 10 ml of mercuric sulphate solution and heat in a boiling water-bath; no precipitate is formed within 3 minutes.

**Aldehydes.** Not more than 10 ppm, determined by the following method. To 5.0 ml add 5 ml of water and 1 ml of decolorised magenta solution and allow to stand for 30 minutes. Any colour produced is not more intense than that produced by treating in the same manner 5.0 ml of a 0.001 per cent w/v solution of redistilled acetaldehyde in aldehyde-free ethanol (95 per cent).

**Benzene and related substances.** Determine by gas chromatography (2.4.13).

**Test solution.** The substance under examination.

**Reference solution (a).** A 0.1 per cent v/v solution of 2-butanol reagent in the test solution.

**Reference solution (b).** A solution containing 0.1 per cent v/v each of 2-butanol reagent and 1-propanol in the test solution.

**Reference solution (c).** A 0.0002 per cent v/v solution of benzene in the test solution.

**Chromatographic system**

- a glass column 1.8 m x 2 mm, packed with acid-washed diatomaceous support (80 to 100 mesh) coated with 15 per cent w/w of polyethylene glycol 400,
- temperature: column, 50°C,
- inlet port, 150°C,
- flame ionisation detector at 250°C,
- flow rate. 30 ml per minute of the carrier gas (nitrogen).

Inject separately 2 µl of each of the test solution and reference solution (a). The chromatogram obtained with the test solution
shows no peak with a retention time similar to the peak due to 2-butanol (retention time relative to isopropyl alcohol, about 1.5) obtained with reference solution (a). Inject 2 µl of reference solution (b) and adjust the sensitivity of the system so that the heights of the peaks due to 2-butanol and propanol in the chromatogram obtained with reference solution (b) are not less than 50 per cent of the full scale of the recorder.

The test is not valid unless the resolution between the peaks due to 2-butanol and propanol in the chromatogram obtained with reference solution (b) is at least 1.2.

Inject alternately 2 µl each of the test solution and reference solution (c). The area of any peak due to benzene in the chromatogram obtained with the test solution is not greater than the difference between the area of the peak due to benzene in the chromatogram obtained with reference solution (c) and that of the peak due to benzene in the chromatogram obtained with the test solution.

In the chromatogram obtained with reference solution (a) the sum of the areas of any peaks other than the principal peak and the peaks due to 2-butanol is not greater than 3 times the area of the peak due to 2-butanol (0.3 per cent).

**Fusel oil constituents.** Place 25 ml in a porcelain dish protected from dust and allow the liquid to evaporate on a water-bath until a little of the liquid remains. Remove the dish from the water-bath and allow the liquid to evaporate at room temperature till the dish is almost dry. No foreign odour is perceptible. Add 1 ml of sulphuric acid; no red or brown colour is produced.

**Non-volatile matter.** Evaporate 100.0 ml in a tared dish on a water-bath and dry the residue at 105°; the residue weighs not more than 5 mg.

**Storage.** Store in tightly-closed containers at a temperature not exceeding 30°, away from fire and protected from moisture.

**Labelling.** The label states that it is flammable.

**Ethanol (95 Per Cent)**

**Alcohol (95 per cent)**

Ethanol (95 per cent) is a mixture of Ethanol and Water.

Ethanol (95 per cent) contains not less than 92.0 per cent w/w and not more than 92.7 per cent w/w, corresponding to not less than 94.7 per cent v/v and not more than 95.2 per cent v/v, at 15.56°, of C2H6O.

**Description.** A clear, colourless, mobile and volatile liquid; odour, characteristic and spirituous. It is readily volatilised even at low temperatures; boils at about 78°; flammable, burning with a blue, smokeless flame.

**Identification**

A. Mix 0.25 ml in a small beaker with 1 ml of potassium permanganate solution and 0.25 ml of dilute sulphuric acid and cover the beaker immediately with a filter paper moistened with a solution freshly prepared by dissolving 0.1 g of sodium nitroprusside and 0.5 g of piperazine hydrate in 5 ml of water; an intense blue colour is produced on the filter paper, the colour becoming lighter after a few minutes.

B. To 5 ml of a 0.5 per cent v/v solution add 1 ml of 1 M sodium hydroxide followed by slow addition of 2 ml of iodine solution; the odour of iodoform develops and a yellow precipitate is produced.

**Tests**

**Relative density** (2.4.29). 0.8084 to 0.8104, determined at 25°.

**Appearance of solution.** Dilute 5.0 ml to 100.0 ml with water. The solution is clear (2.4.1). Cool to 10° for 30 minutes; the solution remains clear.

**Acidity or alkalinity.** To 20 ml add 0.25 ml of phenolphthalein solution; the solution remains colourless and requires not more than 0.2 ml of 0.1 M sodium hydroxide to produce a pink colour.

**Methanol.** To 1 drop add 1 drop of water, 1 drop of dilute phosphoric acid and 1 drop of potassium permanganate solution. Mix, allow to stand for 1 minute and add sodium bisulphite solution dropwise until the permanganate colour is discharged. If a brown colour remains, add 1 drop of dilute phosphoric acid. To the colourless solution add 5 ml of freshly prepared chromotropic acid solution and heat on a water-bath at 60° for 10 minutes; no violet colour is produced.

**Foreign organic substances.** Clean a glass-stoppered cylinder thoroughly with hydrochloric acid, rinse with water and finally rinse with the substance under examination. Put 20 ml in the cylinder, cool to about 15° and then add from a carefully cleaned pipette 0.1 ml of 0.1 M potassium permanganate. Mix at once by inverting the stoppered cylinder and allow to stand at 15° for 5 minutes; the pink colour does not entirely disappear.

**2-Propanol and 2-methyl-2-propanol.** To 1 ml add 3 ml of water and 10 ml of mercuric sulphate solution and heat in a boiling water-bath; no precipitate is formed within 3 minutes.

**Aldehydes.** Not more than 10 ppm, determined by the following method. To 5.0 ml add 5 ml of water and 1 ml of decolorised magenta solution and allow to stand for 30 minutes. Any colour produced is not more intense than that produced by treating in the same manner 5 ml of a 0.001 per cent w/v solution of redistilled acetaldehyde in aldehyde-free ethanol (95 per cent).

**Benzene and related substances.** Determine by gas chromatography (2.4.13).
Test solution. The substance under examination.

Reference solution (a). A 0.1 per cent v/v solution of 2-butanol reagent in the test solution.

Reference solution (b). A solution containing 0.1 per cent v/v each of 2-butanol reagent and 1-propanol in the test solution.

Reference solution (c). A 0.0002 per cent v/v solution of benzene in the test solution.

Chromatographic system
- a glass column 1.8 m x 2 mm, packed with acid-washed diatomaceous support (80 to 100 mesh) coated with 15 per cent w/w of polyethylene glycol 400,
- temperature: column, 50°; inlet port, 150°,
- flame ionisation detector at 250°,
- flow rate: 30 ml per minute of the carrier gas (nitrogen).

Inject alternately 2 µl of each of the test solution and reference solution (a). The chromatogram obtained with the test solution shows no peak with a retention time similar to the peak due to 2-butanol (retention time relative to isopropyl alcohol, about 1.5) obtained with reference solution (a). Inject 2 µl of reference solution (b) and adjust the sensitivity of the system so that the heights of the peaks due to 2-butanol and propanol in the chromatogram obtained with reference solution (b) are not less than 50 per cent of the full scale of the recorder.

The test is not valid unless the resolution between the peaks due to 2-butanol and propanol in the chromatogram obtained with reference solution (b) is at least 1.2.

Inject alternately 2 µl each of the test solution and reference solution (c). The area of any peak due to benzene in the chromatogram obtained with the test solution is not greater than the difference between the area of the peak due to benzene in the chromatogram obtained with reference solution (c) and that of the peak due to benzene in the chromatogram obtained with the test solution.

In the chromatogram obtained with reference solution (a) the sum of the areas of any peaks other than the principal peak and the peaks due to 2-butanol is not greater than 3 times the area of the peak due to 2-butanol (0.3 per cent).

Fusel oil constituents. Place 25 ml in a porcelain dish protected from dust and allow the liquid to evaporate on a water-bath until a little of the liquid remains. Remove the dish from the water-bath and allow the liquid to evaporate at room temperature till the dish is almost dry. No foreign odour is perceptible. Add 1 ml of sulphuric acid; no red or brown colour is produced.

Non-volatile matter. Evaporate 100.0 ml in a tared dish on a water-bath and dry the residue at 105°; the residue weighs not more than 5 mg.

Storage. Store in tightly-closed containers at a temperature not exceeding 30° and away from fire.

Labelling. The label states that it is flammable.

Anaesthetic Ether

CH₃CH₂OCH₂CH₃

C₇H₁₄O Mol. Wt. 74.1

Anaesthetic Ether is diethyl ether to which a suitable non-volatile stabiliser in a proportion not greater than 0.002 per cent w/v may have been added.

Description. A clear, colourless, very mobile liquid; odour, characteristic; highly flammable.

NOTE — It is absolutely essential that a preservative of the type of sodium pyrogallate, hydroquinone or propyl gallate in suitable concentrations shall be added in Anaesthetic Ether intended for use in tropical climates unless the Anaesthetic Ether is stored in a copper container or in a container copper-plated internally. The preservative used and its concentration shall be declared on the label.

Tests

Relative density (2.4.29). 0.714 to 0.716, determined at 20°.

Boiling range (2.4.8). 34° to 35°.

CAUTION — It is dangerous to determine the boiling range if the sample does not comply with the test for peroxides.

Acidity. To 20 ml of ethanol (95 per cent) add 0.25 ml of bromothymol blue solution add dropwise 0.02 M sodium hydroxide until the blue colour persists for 30 seconds. Add 25 ml of the substance under examination, shake and again add dropwise 0.2 M sodium hydroxide until the blue colour reappears and persists for 30 seconds. Not more than 0.4 ml of 0.02 M sodium hydroxide is required.

Peroxides. Place 8 ml of potassium iodide and starch solution in a 12-ml glass-stoppered cylinder of about 1.5 cm diameter. Fill completely with the substance under examination, insert the stopper, shake vigorously and allow to stand in the dark for 30 minutes; no colouration is produced.

Acetone and aldehydes. Place 2 ml of alkaline potassium mercuri-iodide solution in a 12-ml glass-stoppered cylinder of about 1.5 cm diameter and fill completely with the substance under examination, insert the stopper and shake vigorously for 15 seconds and set aside for 5 minutes, protected from light; no colour or turbidity, except for slight opalescence, is produced.

If the ether does not comply with the test, distil 40 ml (after ensuring that it complies with the test for peroxides) until only 5 ml remains and repeat the test using 10 ml of the distillate.
Foreign odour. Pour 10 ml in successive portions on to a clean filter paper and allow to evaporate spontaneously; no foreign odour is detectable at any stage of evaporation.

Non-volatile matter. Evaporate 50 ml in a tared dish on a water-bath and dry at 105° (after ensuring that the sample complies with the test for peroxides); the residue weighs not more than 1.5 mg.

Methanol. To 10 ml, add 5 ml of ethanol (20 per cent) and 5 ml of water, in a separator, shake vigorously, set aside and allow the mixture to separate and draw off the lower layer. To 5 ml of the lower layer add 2.0 ml of potassium permanganate and phosphoric acid solution, set aside for 10 minutes and add 2.0 ml of oxalic acid and sulphuric acid solution and 5 ml of decolorised magenta solution. Set aside for 30 minutes; no colour is produced.

Water (2.3.43). Not more than 0.2 per cent, determined on 20.0 ml.

Storage. Store protected from light at a temperature not exceeding 30°. Ether remaining in a partly used container may deteriorate rapidly.

Labelling. The label states that (1) it is very flammable and should not be used near a naked flame; (2) the name and proportion of any stabiliser added.

Ethinyloestradiol

Ethynylestradiol

\[
\text{C}_{20}\text{H}_{24}\text{O}_{2} \quad \text{Mol. Wt. 296.4}
\]

Ethynylestradiol is 19-nor-17α-pregna-1,3,5(10)-triene-20yn-3,17β-diol.

Ethinyloestradiol contains not less than 97.0 per cent and not more than 102.0 per cent of \(\text{C}_{20}\text{H}_{24}\text{O}_{2}\), calculated on the dried basis.

Description. A white or slightly yellowish-white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Test C may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ethinyloestradiol RS or with the reference spectrum of ethinyloestradiol.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Dissolve about 1 mg in 1 ml of sulphuric acid; an orange-red colour develops which exhibits a greenish fluorescence when examined in ultraviolet light at 365 nm. Add the solution to 10 ml of water; the colour changes to violet and a violet precipitate is produced.

Tests

Appearance of solution. A 5.0 per cent w/v solution in ethanol is clear (2.4.1), and not more intensely coloured than reference solution BYS6 (2.4.1).

Specific optical rotation (2.4.22). −27.0° to −30.0°, determined at 20° in a 5.0 per cent w/v solution in pyridine.

Light absorption (2.4.7). Absorbance of a 0.01 per cent w/v solution in ethanol (95 per cent) at about 281 nm, 0.69 to 0.73.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 90 volumes of toluene and 10 volumes of ethanol (95 per cent).

Test solution (a). Dissolve 0.2 g of the substance under examination in 10 ml of a mixture of 9 volumes of chloroform and 1 volume of methanol.

Test solution (b). Dilute 5 ml of test solution (a) to 100 ml with the same solvent mixture.

Reference solution (a). Dilute 5 ml of test solution (b) to 25 ml with the same solvent mixture.

Reference solution (b). A 0.1 per cent w/v solution of ethinyloestradiol RS in the same solvent mixture.

Reference solution (c). A 0.02 per cent w/v solution of estrone RS in the same solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable, heat at 110° for 10 minutes and spray the hot plate with ethanolic sulphuric acid (20 per cent v/v). Heat again at 110° for 10 minutes and examine in ultraviolet light at 365 nm. In the chromatogram obtained with test solution (a) any spot corresponding to estrone is not more intense than the spot in the chromatogram obtained with reference solution (c) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a).

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 0.5 g by drying in an oven at 105° for 3 hours.
**Assay.** Dissolve 0.2 g in 40 ml of tetrahydrofuran, add 5 ml of a 10 per cent w/v solution of silver nitrate and titrate with 0.1 M sodium hydroxide, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.02964 g of C$_{20}$H$_{24}$O$_2$.

**Storage.** Store protected from light.

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**Ethinyloestradiol Tablets**

Ethinyloestradiol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ethinyloestradiol, C$_{20}$H$_{24}$O$_2$.

**Identification**

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 90 volumes of toluene and 10 volumes of ethanol (95 per cent).

**Test solution.** Shake a quantity of the powdered tablets containing 0.25 mg of Ethinyloestradiol with four quantities, each of 20 ml of chloroform, filter each extract in turn, evaporate the combined filtrates to dryness on a water-bath in a current of nitrogen and dissolve the residue in 0.25 ml of chloroform.

**Reference solution.** A 0.1 per cent w/v solution of ethinyloestradiol RS in chloroform.

Apply to the plate 20 µl of each solution. After development, dry the plate in air, spray with ethanolic sulphuric acid (20 per cent v/v), heat at 110° for 10 minutes and examine in ultraviolet light at 365 nm and in daylight. By both methods of visualisation, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Triturate a quantity of the powdered tablets containing 0.1 mg of Ethinyloestradiol with 0.5 ml of 0.1 M sodium hydroxide and 5 ml of water, allow to stand for 5 minutes, filter, acidify the filtrate with 0.15 ml of sulphuric acid, add 3 ml of ether, shake and allow to separate. Evaporate the ether layer to dryness and heat the residue on a water-bath for 5 minutes with 0.2 ml of glacial acetic acid and 2 ml of phosphoric acid; a pink colour with an intense orange fluorescence is produced.

**Tests**

**Uniformity of content.** Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

**Test solution.** Finely crush one tablet, add 20.0 ml of mobile phase, shake for 10 minutes, dilute to 100 ml with mobile phase and filter. Take 1 ml of this solution dilute 10 ml with the same solvent.

**Reference solution.** A 0.0025 per cent w/v solution of ethinylestradiol RS in mobile phase.

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 60 volume of acetonitrile and 40 volumes of water.
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- a 20 µl loop injector.

Inject the test solution and reference solution.

Calculate the content of C$_{20}$H$_{24}$O$_2$ in the tablet.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh and powder 20 tablets. Weight accurately a quantity of the powder containing 2.5 mg of ethinylestradiol, add 20.0 ml of mobile phase, shake for 10 minutes, dilute to 100 ml and filter. Take 1 ml of this solution and dilute to 10 ml with the same solvent.

**Reference solution.** A 0.0025 per cent w/v solution of ethinylestradiol RS in mobile phase.

Carry out the chromatographic procedure described under Uniformity of content. Calculate the content of C$_{20}$H$_{24}$O$_2$ in the tablets.

**Storage.** Store protected from light.

---

**Ethionamide**

\[
\begin{align*}
\text{C}_8\text{H}_{10}\text{N}_2\text{S} & \quad \text{Mol. Wt. 166.2} \\
\text{N} & \quad \text{S} \\
\text{CH}_3 & \quad \text{NH}_2 \\
\end{align*}
\]

Ethionamide is 2-ethylpyridine-4-carbothioamide.

Ethionamide contains not less than 98.5 per cent and not more than 101.0 per cent of C$_8$H$_{10}$N$_2$S, calculated on the dried basis.

**Description.** A yellow crystalline powder or small yellow crystals.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ethionamide RS or with the reference spectrum of ethionamide.
B. Dissolve about 10 mg in 5 ml of methanol and add 5 ml of 0.1 M silver nitrate; a dark brown precipitate is produced.

C. Melting point (2.4.21). 158° to 164°.

Tests

Appearance of solution. Dissolve 0.5 g in 10 ml of methanol, heating to about 50° and allow to cool to room temperature. The solution is not more opalescent than opalescence standard OS2 (2.4.1).

Acidity. Dissolve 2.0 g in 20 ml of methanol, heating to about 50°, and add 20 ml of water. Cool slightly, shake until crystallisation occurs and allow to cool to room temperature. Add 60 ml of water and titrate with 0.1 M sodium hydroxide using 0.2 ml of cresol red solution as indicator. Not more than 0.2 ml is required to change the colour of the solution to red.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 100 ml of the mobile phase.

Reference solution. Dissolve 25 mg of the ethionamide RS in 100 ml of the mobile phase. Dilute 1 ml of this solution to 100 ml with the mobile phase.

Chromatographic system
– a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm) (such as Intersil ODS-3),
– mobile phase: a mixture of 60 volumes of a buffer prepared by dissolving 2 ml of triethylamine in water, adjusting the pH to 6.0 with orthophosphoric acid and diluting to 1000 ml, and 40 volumes of acetonitrile and filtered,
– flow rate. 1 ml per minute,
– spectrophotometer set at 290 nm,
– a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent, the tailing factor is not more than 2.0 and the column efficiency in not less than 5000 theoretical plates.

Inject alternately the test solution and the reference solution. Calculate the content of C₈H₁₀N₂S.

Storage. Store protected from light and moisture.

Ethionamide Tablets

Ethionamide Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of ethionamide, C₈H₁₀N₂S. The tablets may be coated.

Identification

A. Extract a quantity of the powdered tablets containing 25 mg of Ethionamide with 5 ml of methanol, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ethionamide RS or with the reference spectrum of ethionamide.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14) as given under Assay using the following solutions.

Test solution. Weigh accurately a quantity of the powder containing 50 mg of Ethionamide and dissolve in 100 ml of the mobile phase.

Reference solution. Dissolve 25 mg of the ethionamide RS in 100 ml of the mobile phase. Dilute 1 ml of this solution to 100 ml with the mobile phase.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent, the tailing factor is not more than 2.0 per cent.

Inject the test solution. Any individual impurity is not more than 0.5 per cent and the sum of all impurities found is not more than 1.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14) as given under the test for Related substances using the following solutions.
Dissolution (2.5.2).
Apparatus. No 2
Medium. 900 ml of 0.1 M hydrochloric acid
Speed and time. 100 rpm and 45 minutes.
Withdraw a suitable volume of the medium, filter and dilute a suitable volume of the filtrate with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 274 nm (2.7.4). Calculate the content of $C_8H_{10}N_2S$ from the absorbance of a solution of known concentration of ethionamide RS.
D. Not less than 75 per cent of the stated amount of $C_8H_{10}N_2S$.
Other tests. Comply with the tests stated under Tablets.
Assay. Determine by liquid chromatography (2.4.14).
Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Ethionamide in 100.0 ml of the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.
Reference solution. Dissolve 50 mg of the ethionamide RS in 100 ml of the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.
Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm) (such as Intersil ODS-3),
- mobile phase: a mixture of 40 volumes of acetonitrile and 60 volumes of buffer pH 6.0 prepared by mixing 2 ml of triethylamine to 1000 ml with water and adjusting the pH to 6.0 with phosphoric acid,
- flow rate. 1 ml per minute,
- spectrophotometer set at 290 nm,
- a 20 µl loop injector.
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent, the tailing factor is not more than 2.0 and the column efficiency in not less than 5000 theoretical plates. Inject alternately the test solution and the reference solution. Calculate the content of $C_8H_{10}N_2S$ in the tablets.
Storage. Store protected from light and moisture.

Ethopropazine Hydrochloride

$$\text{H}_3\text{C} - \text{N} - \text{CH}_3$$

$$\text{N} - \text{CH}_3$$

$$\text{S} - \text{HCl}$$

$C_{19}H_{24}N_2S\text{HCl}$ Mol. Wt. 348.9

Ethopropazine Hydrochloride is 10-[(diethylamino)propyl]phenothiazine hydrochloride.
Ethopropazine Hydrochloride contains not less than 99.0 per cent and not more than 101.5 per cent of $C_{19}H_{24}N_2S\text{HCl}$, calculated on the dried basis.

Identification
A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ethopropazine hydrochloride RS.
B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0005 per cent w/v solution in ethanol (95 per cent) shows an absorption maximum at about 252 nm and a less well-defined maximum at about 303 nm; absorbance at about 252 nm, about 0.42.
C. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (b).
D. Gives reaction A of chlorides (2.3.1).

Tests
Acidity or alkalinity. Dissolve 0.15 g in 50 ml of carbon dioxide-free water and add 0.15 ml of methyl red solution; the solution is yellow and not more than 0.2 ml of 0.01 M hydrochloric acid is required to change the colour of the solution to red.
Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.
Mobile phase. A freshly prepared mixture of equal volumes of ether and ethyl acetate saturated with strong ammonia solution.
Test solution. Dissolve 0.5 g of the substance under examination in 100 ml of methanol.
Reference solution (a). Dilute 1 volume of the test solution to 100 volumes with methanol.
Reference solution (b). A 0.5 per cent w/v solution of ethopropazine hydrochloride RS in methanol.
Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a).
Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).
Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.7 g, dissolve in 200 ml of acetone, add 15 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid, using 0.15 ml of a saturated solution of methyl orange in acetone as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.03489 g of C19H24N2S,HCl.

Storage. Store protected from light.

Ethopropazine Tablets

Ethopropazine Hydrochloride Tablets

Ethopropazine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of ethopropazine hydrochloride, C19H24N2S,HCl. The tablets may be coated.

Identification

A. Extract a quantity of the powdered tablets containing 50 mg of Ethopropazine Hydrochloride with 20 ml of chloroform, filter, evaporate the filtrate to dryness and dry the residue at 60° at a pressure not exceeding 0.7 kPa. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ethopropazine hydrochloride RS.

B. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

C. To a quantity of the powdered tablets containing 5 mg of Ethopropazine Hydrochloride add 5 ml of sulphuric acid and allow to stand for 5 minutes; a red colour is produced.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A freshly prepared mixture of equal volumes of ether and ethyl acetate saturated with strong ammonia solution.

Test solution. Shake a quantity of the powdered tablets containing 0.1 g of Ethopropazine Hydrochloride with 50 ml of chloroform for 15 minutes, centrifuge and use the supernatant liquid.

Reference solution (a). A 0.2 per cent w/v solution of ethopropazine hydrochloride RS in chloroform.

Reference solution (b). A 0.002 per cent w/v solution of ethopropazine hydrochloride RS in chloroform.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

Other tests. Comply with the tests stated under Tablets.

Assay. Protect the solution from light throughout the test.

Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Ethopropazine Hydrochloride, extract with four quantities, each of 20 ml, of ethanol (95 per cent). Filter and dilute the filtrate to 100.0 ml with ethanol (95 per cent). Dilute 10.0 ml of this solution to 100.0 ml with ethanol (95 per cent). Dilute 10.0 ml of this solution further to 100.0 ml and measure the absorbance of the resulting solution at the maximum at about 252 nm (2.4.7). Calculate the content of C19H24N2S,HCl, taking 845 as the specific absorbance at 252 nm.

Storage. Store protected from light.

Ethosuximide

Ethosuximide is (RS)-2-ethyl-2-methylsuccinimide.

Ethosuximide contains not less than 98.0 per cent and not more than 101.0 per cent of C7H11NO2, calculated on the anhydrous basis.

Description. A white or almost white powder or waxy solid.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Melt a sufficient quantity at 50°, prepare a thin film between two previously warmed bromide plates and record the spectrum immediately. Compare the spectrum with that obtained with ethosuximide RS.

C7H11NO2

Mol. Wt. 141.2

Ethosuximide contains not less than 98.0 per cent and not more than 101.0 per cent of C7H11NO2, calculated on the anhydrous basis.
B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.1 per cent w/v solution in ethanol (95 per cent) shows an absorption maximum at about 248 nm; absorbance at 248 nm, about 0.85.

C. Dissolve 0.1 g in 3 ml of methanol and add 0.05 ml of a 10 per cent w/v solution of cobalt chloride, 0.05 ml of a 10 per cent w/v solution of calcium chloride and 0.1 ml of 2 M sodium hydroxide; a purple colour develops and no precipitate is produced.

**Tests**

**Appearance of solution.** Dissolve 2.5 g in sufficient water to produce 25 ml. The solution is clear (2.4.1) and colourless (2.4.1).

**Acidity.** Dissolve 5.0 g in 50 ml of water by warming on a water-bath for 5 minutes. Cool and titrate with 0.1 M sodium hydroxide using bromocresol green solution as indicator. Not more than 0.7 ml of 0.1 M sodium hydroxide is required.

**Cyanide.** Dissolve 1.0 g in 10 ml of ethanol (90 per cent) and add 0.5 ml of ferrous sulphate solution, 1 ml of 2 M sodium hydroxide and 0.1 ml of ferric chloride solution. Heat to boiling, cool and acidify using 3 ml of 1 M sulphuric acid. After 15 minutes, there is no blue colour and no blue precipitate is produced.

**Related substances.** Determine by gas chromatography (2.4.13).

**Test solution (a).** Dissolve 1 g of the substance under examination in sufficient chloroform to produce 10 ml.

**Test solution (b).** Dilute 5 ml of test solution (a) to 10 ml with a 0.01 per cent w/v solution of anthracene (internal standard) in chloroform.

**Reference solution (a).** Dissolve 10 mg of 2-ethyl-2-methylsuccinic acid in sufficient chloroform to produce 10 ml.

**Reference solution (b).** Dilute 1 ml of test solution (a) to 100 ml with chloroform. To 1 ml of this solution add 5 ml of the internal standard solution and sufficient chloroform to produce 10 ml.

**Reference solution (c).** Dilute 1 ml of test solution (b) to 50 ml with chloroform. Add 1 ml of this solution to 1 ml of reference solution (a), add 5 ml of the internal standard solution and sufficient chloroform to produce 10 ml.

**Chromatographic system**

- a glass column 2 m x 2 mm, packed with silanised diatomaceous support (125 to 180 mesh) impregnated with 3 per cent w/w polycyanopropylmethylphenylmethyl siloxane,
- temperature:
  - column: 165°C,
  - inlet port and detector: 240°C,
- flow rate: 30 ml per minute of the carrier gas.

Inject 1 µl of reference solution (c) and adjust the sensitivity of the detector so that the heights of the three principal peaks are not less than 70 per cent of full-scale deflection. The peaks in order of emergence, are due to 2-ethyl-2-methylsuccinic acid, ethosuximide, and anthracene.

The test is not valid unless the resolution factor between the peaks corresponding to 2-ethyl-2-methylsuccinic acid and ethosuximide in the chromatogram obtained with test solution (a) is at least 4.

Inject 1 µl of test solution (a) and verify that there is no peak with the same retention time as the internal standard. Inject separately 1 µl of test solution (b) and reference solution (b) and record the chromatogram for twice the retention time of ethosuximide. Calculate the ratio (R) of the area of the peak due to ethosuximide to the area of the peak due to the internal standard in the chromatogram obtained with reference solution (b). In the chromatogram obtained with test solution (b) the ratio of the sum of the areas of any secondary peaks to the area of the peak due to the internal standard is not greater than R.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

**Assay.** Weigh accurately about 0.12 g, dissolve 20 ml of dimethylformamide and add 0.2 ml of a 0.5 per cent w/v solution of thymolphthalein in dimethylformamide. Titrate with 0.1 M tetrabutylammonium hydroxide, until a distinct blue colour is produced.

1 ml of 0.1 M tetrabutylammonium hydroxide is equivalent to 0.01412 g of C₇H₁₁NO₂.

**Storage.** Store protected from light.

**Ethosuximide Capsules**

Ethosuximide Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of ethosuximide, C₇H₁₁NO₂.

**Identification**

A. Heat a quantity of the contents of the capsules containing 0.1 g of Ethosuximide with 0.2 g of resorcinol and 0.1 ml of sulphuric acid at 140° for 5 minutes, add 5 ml of water; make alkaline with 5 M sodium hydroxide and add 0.2 ml to a large volume of water; a bright green fluorescence is produced.

B. Shake a quantity of the contents of the capsules containing 0.25 g of Ethosuximide with 80 ml of ethanol (95 per cent) for
a few minutes, add sufficient ethanol (95 per cent) to produce 100 ml, mix and filter. Dilute 20 ml of the filtrate to 100 ml with ethanol (95 per cent). Absorbance of the resulting solution at the maximum at about 248 nm, about 0.43 (2.4.7).

Tests

Other tests. Comply with the tests stated under Capsules.

Assay. Weigh accurately a quantity of the contents of the capsules containing about 0.2 g of Ethosuximide and dissolve in 30 ml of dimethylformamide. Titrate with 0.1 M tetrabutylammonium hydroxide, using a 0.1 per cent w/v solution of azo violet in dimethylformamide as indicator. Carry out a blank titration.

1 ml of 0.1 M tetrabutylammonium hydroxide is equivalent to 0.01412 g of C7H11NO2.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Ethosuximide Syrup

Ethosuximide Oral Solution

Ethosuximide Syrup is a solution of Ethosuximide in a suitable flavoured vehicle.

Ethosuximide Syrup contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of ethosuximide, C7H11NO2.

Identification

A. Extract a quantity of the syrup containing 0.5 g of Ethosuximide with two quantities, each of 30 ml, of chloroform, filter the combined extracts through a plug of cotton and evaporate the filtrate to dryness. Heat 100 mg of the residue with 0.2 g of resorcinol and 0.1 ml of sulphuric acid at 140° for 5 minutes, cool, add 5 ml of water, make alkaline with 5 M sodium hydroxide and add 0.2 ml to a large volume of water; a bright green fluorescence is produced.

B. In the Assay, the principal peak in the chromatogram obtained with test solution (b) has the same retention time as that of the peak due to ethosuximide in the chromatogram obtained with reference solution (a).

Tests

Other tests. Complies with the tests stated under Oral liquids.

Assay. Determine by gas chromatography (2.4.13).

Test solution (a). Add 10 ml of water and 2 g of sodium bicarbonate to a weighed quantity of the syrup containing about 0.25 g of Ethosuximide and extract with five quantities, each of 25 ml, of chloroform, washing each extract with the same 10 ml of water. To the combined extracts add 10 ml of a 3.0 per cent w/v solution of dimethyl phthalate (internal standard) in chloroform, shake with 10 g of anhydrous sodium sulphate and filter.

Test solution (b). Prepare in the same manner as test solution (a) but omit the internal standard.

Reference solution. Add 2 ml of the internal standard solution to 25.0 ml of a 0.2 per cent w/v solution of ethosuximide RS in chloroform.

Chromatographic system

– a glass column 1.5 m x 4 mm, packed with acid-washed silanised diatomaceous support (80 to 100 mesh) impregnated with 3 per cent w/w of cyanopropylmethyl phenyl methyl silicone fluid (OV-225) of cyanopropylmethyl phenyl methyl silicone fluid (such as OV-225),
– temperature: column.165°, inlet port and detector. 240°,
– flow rate. 30 ml per minute of the carrier gas.

Determine the weight per ml of the syrup (2.4.29), and calculate the content of C7H11NO2, weight in volume.

Storage. Store at a temperature not exceeding 30°.

Ethylcellulose

Cellulose ethyl ether

Ethylcellulose is an ethyl ether of cellulose.

Ethylcellulose contains not less than 44.0 per cent and not more than 51.0 per cent of ethoxy (-OC2H5) groups, calculated on the dried basis.

Description. A white to light tan powder; almost odourless.

Identification

Dissolve 15 mg of the dried sample in 10 ml of dried dichloromethane. Grind 0.5 ml of this solution to dryness with 0.3 g of potassium bromide. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ethylcellulose RS.

Tests

pH (2.4.24). 5.5 to 8.0, determined in a solution prepared in the following manner. Stir 1.0 g with 50 ml of carbon dioxide-free water previously heated to 90°, then cool and dilute with sufficient carbon dioxide-free water to produce 100 ml.
Apparent viscosity. 90.0 to 110.0 per cent of that stated on the label for viscosity types of 10 millipascal seconds or more; 80.0 to 120.0 per cent of that stated on the label for viscosity types of 6 to 10 millipascal seconds; 75.0 to 140.0 per cent of that stated on the label for viscosity types of 6 millipascal seconds or less, determined by the following method. Weigh accurately about 0.5 g, calculated on the dried basis and dissolve in 95.0 ± 0.05 g of a mixture of 80 parts of toluene and 20 parts of ethanol by weight. For ethylcellulose containing less than 46.5 per cent of ethoxy groups use a mixture of 60 parts of toluene and 40 parts of ethanol. Determine the viscosity at 25°C by Method A (2.4.28).

Arsenic (2.3.10). Mix 1.0 g with 5 ml of sulphuric acid AsT, add a few glass beads and digest in a fume hood, preferably on a hot plate at a temperature not exceeding 120°C, until charring begins. (Additional acid may be necessary to wet some samples completely but the total volume added should not exceed 10 ml). Cautiously add, dropwise, hydrogen peroxide solution (30 per cent) allowing the reaction to subside and again heating between additions of drops. Add the first few drops very slowly with sufficient mixing to prevent a rapid reaction. Discontinue heating if foaming becomes excessive. When the reaction has abated, heat cautiously, rotating the flask occasionally to prevent the sample from caking on glass exposed to the heating unit. Maintain oxidising conditions at all times during the digestion by adding small quantities of the hydrogen peroxide solution whenever the mixture turns brown or darkens. Continue the digestion until the organic matter is destroyed, gradually raising the temperature of the heating unit until fumes of sulphur trioxide are copiously evolved and the solution becomes colourless or retains only a light straw colour. Cool, add cautiously 10 ml of water, mix, and again evaporate till strong fuming, repeating this procedure to remove any trace of hydrogen peroxide. Cool, add cautiously 10 ml of water, wash the sides of the flask with a few ml of water, and dilute with water to 35 ml. The resulting solution complies with the limit test for arsenic (3 ppm).

Heavy metals (2.3.13). 0.5 g complies with the limit test for heavy metals, Method B (40 ppm).

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Loss on drying (2.4.19). Not more than 3.0 per cent, determined on 1.0 g by drying in an oven at 105°C for 2 hours.

Assay. Weigh accurately about 50 mg in an empty, tared Hard Gelatin Capsule Shell and carry out the determination of methoxyl (2.3.29).

1 ml of 0.1 M sodium thiosulphate is equivalent to 0.0007510 of ethoxy (-OC₂H₅) groups.

Labelling. The label states the apparent viscosity in mPa s of a 2.0 per cent w/v solution and its ethoxy content.

Ethyl Chloride

CH₂CH₂Cl

C₂H₅Cl  Mol. Wt. 64.5

Ethyl Chloride is chloroethane.

Description. Gaseous at ambient temperatures and pressures but is generally compressed to a colourless, mobile, flammable and very volatile liquid; odour, pleasant and ethereal.

Identification

A. Burns with a luminous flame with the production of hydrogen chloride.

B. Hydrolyse a few ml with 5 M sodium hydroxide; the resulting solution gives the reactions of chlorides (2.3.1), and after the addition of iodoform solution and warming, crystals of iodoform are produced.

Tests

Acidity or alkalinity. Shake 10 ml with 10 ml of ice-cold water and allow the ethyl chloride to evaporate at room temperature; the residual liquid (liquid A) is neutral to litmus solution.

Ionisable chlorides. 5 ml of liquid A gives no turbidity with silver nitrate solution.

Ethanol. Warm 5 ml of liquid A with iodine solution and sodium carbonate; no iodoform is produced.

Distillation range. Into a dry 100-ml measuring cylinder insert a stopper carrying a short exit tube not less than 6 mm in internal diameter and an accurately standardised short-bulb thermometer covering the range –20° to +30° and graduated in tenths of a degree. Cover the bulb of the thermometer with a piece of very fine muslin, free from grease and sizing materials, so that one end hangs down about 10 mm below the bulb. Cool the cylinder in ice-water, transfer to it 100 ml of the sample, previously cooled in ice-water, insert the stopper and adjust the thermometer so that the end of the muslin dips into the liquid and the bulb is above the surface. Replace the ice-water with water at 24° to 26° and observe the temperature when 5 ml of sample has evaporated and again when 5 ml remains. Continually lower the thermometer to maintain its position relative to the liquid surface throughout the test. Correct the observed temperature by adding 0.26° for every kPa that the barometric pressure is below 101.3 kPa or by subtracting 0.26° for every kPa above. The corrected temperature is not lower than 12.0° and not higher than 12.5°.

Other organic compounds. On evaporation, no foreign odour is detectable at any stage.

Non-volatile matter. Not more than 0.01 per cent w/w, when evaporated and dried at 105°.
**Storage.** Store protected from light in a refrigerator (2° to 8°).

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**Ethyl Oleate**

\[
\text{C}_{20}\text{H}_{38}\text{O}_2 \quad \text{Mol. Wt. 310.5}
\]

Ethyl Oleate consists of the ethyl esters of (Z)-oleic and related acids.

Ethyl Oleate contains not less than 100.0 per cent w/w and not more than 105.0 per cent w/w of the ethyl esters of (Z)-oleic and related acids, calculated as \(\text{C}_{20}\text{H}_{38}\text{O}_2\).

**Description.** A pale yellow oil; odour, slight but not rancid.

**Tests**

- **Weight per ml** (2.4.29). 0.869 g to 0.874 g, determined at 20°.
- **Acid value** (2.3.23). Not more than 0.5.
- **Peroxides.** Dissolve 5.0 g in 15 ml of chloroform, add 20 ml of glacial acetic acid and 0.5 ml of a saturated solution of potassium iodide, mix and allow to stand for exactly 1 minute in the dark. Add 30 ml of water and titrate with 0.01 M sodium thiosulphate using starch solution as indicator; not more than 2.5 ml of 0.01 M sodium thiosulphate is required.
- **Iodine value** (2.3.28). 75 to 85.

**Assay.** Boil a suitable volume of ethanol (95 per cent) to expel carbon dioxide and neutralise it to phenolphthalein solution. Weigh accurately about 2.0 g of the substance under examination, dissolve in 5 ml of the neutralised ethanol contained in a hard-glass flask and neutralise the free acid in the solution with 0.1 M ethanolic potassium hydroxide using 0.0 ml of phenolphthalein solution as indicator. Add 25.0 ml of 0.5 M ethanolic potassium hydroxide and boil under a reflux condenser on a water-bath for 1 hour and continue boiling for 2 hours over a flame. Add 20 ml of water and titrate the excess of alkali with 0.5 M hydrochloric acid using a further 0.2 ml of phenolphthalein solution as indicator. Repeat the operation without the substance under examination. The difference between the titres represents the alkali required to saponify the substance under examination.

1 ml of 0.5 M ethanolic potassium hydroxide is equivalent to 0.1553 g of \(\text{C}_{20}\text{H}_{38}\text{O}_2\).

**Storage.** Store protected from light in small, well-filled and well-closed containers under an atmosphere of nitrogen.

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**Ethylenediamine Hydrate**

\[
\text{H}_2\text{N}\text{—CH}_2\text{—NH}_2\text{—H}_2\text{O} \quad \text{Mol. Wt. 78.1}
\]

Ethylenediamine Hydrate is ethane-1,2-diamine monohydrate.

Ethylenediamine Hydrate contains not less than 97.5 per cent w/w and not more than 101.5 per cent w/w of \(\text{C}_2\text{H}_4\text{N}_2\cdot\text{H}_2\text{O}\).

**Description.** A clear, colourless or slightly yellow liquid; odour, ammoniacal.

**Identification**

A. Dilute 1 ml to 6 ml with water. To 3 drops of the solution add 2 ml of a 1 per cent w/v solution of copper sulphate and shake; a purple-blue colour is produced.

B. It is strongly alkaline.

**Tests**

- **Ammonia and other bases.** Weigh accurately about 1.5 ml and transfer with the aid of ethanol (95 per cent) to a small dish. Add, with stirring, 20 ml of dilute hydrochloric acid. Evaporate the solution to dryness on a water-bath, breaking up any cake formed with a glass rod, and dry at 105° for 1 hour.

1 g of residue is equivalent to 0.5872 g of \(\text{C}_2\text{H}_4\text{N}_2\cdot\text{H}_2\text{O}\).

Calculate the percentage of \(\text{C}_2\text{H}_4\text{N}_2\cdot\text{H}_2\text{O}\); the result is within 0.5 per cent of the percentage of ethylenediaminehydrate determined in the Assay.

- **Heavy metals** (2.3.13). Evaporate 5.0 ml on a water-bath to dryness, add to the residue 1 ml of hydrochloric acid and 0.5 ml of nitric acid and evaporate to dryness. Dissolve the residue in 20 ml of warm water, cool, add sufficient water to produce 100 ml and mix. 20 ml of the resulting solution complies with the limit test for heavy metals, Method A (20 ppm).

- **Iron** (2.3.14). To the residue obtained in the test for Non-volatile matter add 1 ml of hydrochloric acid and 0.5 ml of nitric acid and evaporate to dryness on a water-bath. Dissolve the residue in 20 ml of warm water and dilute with water to 100 ml. 40 ml of the solution complies with the limit test for iron (20 ppm).

- **Non-volatile matter.** Not more than 0.02 per cent w/v, determined on 5.0 ml by evaporating to dryness on a water-bath and drying at 105° for 1 hour.

**Assay.** Weigh accurately about 1.0 g, dissolve in 75 ml of water and titrate with 1 M hydrochloric acid using bromophenol blue solution as indicator until a yellow colour is produced.
1 ml of 1 M hydrochloric acid is equivalent to 0.03906 g of C_{2}H_{8}N_{2}H_{2}O.

**Storage.** Store protected from light.

**Ethyloestrenol**

[Chemical structure of Ethyloestrenol]

C_{20}H_{32}O  Mol. Wt. 288.5

Ethyloestrenol is 17α-ethylestr-4-en-17β-ol containing a variable amount of methanol of crystallisation.

Ethyloestrenol contains not less than 95.0 per cent and not more than 103.0 per cent of C_{20}H_{32}O, calculated on the anhydrous and methanol-free basis.

**Description.** A white or almost white, crystalline powder; almost odourless.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ethyloestrenol RS or with the reference spectrum of ethyloestrenol.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 80 volumes of heptane and 20 volumes of acetone.

**Solvent mixture.** A mixture of 90 volumes of chloroform and 10 volumes of methanol.

**Test solution.** Dissolve 0.25 g of the substance under examination in 100 ml with solvent mixture.

**Reference solution (a).** A 0.25 per cent w/v solution of ethyloestrenol RS in the same solvent mixture.

**Reference solution (b).** A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 2 µl of each solution. After development, dry the plate in air, heat it at 105° for 10 minutes, spray with ethanolic sulphuric acid (20 per cent), heat at 105° for a further 10 minutes and allow to cool. Any spot corresponding to 17α-ethylestran-17β-ol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

**Related substances.** Carry out Identification test B but using 10 µl of the following solutions.

**Test solution.** A 1 per cent w/v solution of the substance under examination.

**Reference solution (a).** A 0.01 per cent w/v solution of the substance under examination.

**Reference solution (b).** A 0.005 per cent w/v solution of the substance under examination.

Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Methanol.** Not more than 4.0 per cent w/w, determined by the following method.

**Determine by gas chromatography (2.4.13).**

**Test solution (a).** A solution containing 10.0 per cent w/v of the substance under examination in acetone.

**Test solution (b).** A solution containing 10.0 per cent w/v of the substance under examination and 0.4 per cent v/v of ethanol (internal standard) in acetone.

**Reference solution.** A solution containing 0.4 per cent v/v of methanol and 0.4 per cent v/v of the internal standard in acetone.

**Tests**

**Specific optical rotation** (2.4.22). +29.0° to +33.0°, determined in a 1.0 per cent w/v solution in dioxan.

**17α-Ethyloestran-17β-ol.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G containing 20 per cent w/v of silver nitrate.

**Mobile phase.** A mixture of 75 volumes of toluene and 25 volumes of nonan-5-one.

**Solvent mixture.** A mixture of 9 volumes of chloroform and 1 volume of methanol.

**Test solution.** Dissolve 0.4 g of the substance under examination in 10 ml with solvent mixture.

**Reference solution (a).** A 0.08 per cent w/v solution of 17α-ethyloestran-17β-ol RS in the same solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate, heat it at 105° for 10 minutes, spray with ethanolic sulphuric acid (20 per cent), heat at 105° for a further 10 minutes and allow to cool. Any spot corresponding to 17α-ethyloestran-17β-ol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Related substances.** Carry out Identification test B but using 10 µl of the following solutions.

**Test solution.** A 1 per cent w/v solution of the substance under examination.

**Reference solution (a).** A 0.01 per cent w/v solution of the substance under examination.

**Reference solution (b).** A 0.005 per cent w/v solution of the substance under examination.

Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Methanol.** Not more than 4.0 per cent w/w, determined by the following method.

**Determine by gas chromatography (2.4.13).**

**Test solution (a).** A solution containing 10.0 per cent w/v of the substance under examination in acetone.

**Test solution (b).** A solution containing 10.0 per cent w/v of the substance under examination and 0.4 per cent v/v of ethanol (internal standard) in acetone.

**Reference solution.** A solution containing 0.4 per cent v/v of methanol and 0.4 per cent v/v of the internal standard in acetone.
Chromatographic system
- a glass column 2.0 m x 0.4 mm, packed with porous polymer beads (100 to 120 mesh) (such as Porapak Q),
- temperature: column.170°, inlet port and detector. 240°,
- flame ionisation detector,
- flow rate. 30 ml per minute of the carrier gas (nitrogen).

Calculate the percentage w/w of methanol, assuming its weight per ml at 20° to be 0.792 g.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 0.5 per cent, determined on 5.0 g.

**Assay.** Determine by gas chromatography (2.4.13).

**Test solution (a).** A solution containing 0.2 per cent w/v of the substance under examination in chloroform.

**Test solution (b).** A solution containing 0.2 per cent w/v of the substance under examination and 0.1 per cent w/v of arachidic alcohol (internal standard) in chloroform.

**Reference solution.** A 0.2 per cent w/v solution of ethyloestrenol RS in chloroform.

Chromatographic system
- a glass column 1.0 m x 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) (such as OV-17),
- temperature: column.200°, inlet port and detector. 280°,
- flame ionisation detector,
- flow rate. 30 ml per minute of the carrier gas (nitrogen).

Calculate the content of C20H32O.

**Storage.** Store protected from light in a refrigerator (2° to 8°).

**Ethyloestrenol Tablets**

Ethyloestrenol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ethyloestrenol, C20H32O.

**Identification**

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G

**Mobile phase.** A mixture of 80 volumes of heptane and 20 volumes of acetone.

**Solvent mixture.** A mixture of 90 volumes of chloroform and 10 volumes of methanol.

**Tests**

**17α-Ethyloestran-17β-ol.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G containing 20 per cent w/v of silver nitrate.

**Mobile phase.** A mixture of 75 volumes of toluene and 25 volumes of nonan-5-one.

**Solvent mixture.** A mixture of 90 volumes of chloroform and 10 volumes of methanol.

**Test solution.** Dissolve 20 mg of the residue obtained in the test for Related substances in 0.5 ml with solvent mixture.

**Reference solution.** A 0.08 per cent w/v solution of 17α-ethyloestran-17β-ol RS in the same solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in air, heat it at 105° for 10 minutes, spray with ethanolic sulphuric acid (20 per cent) and heat at 105° for a further 10 minutes. Allow to cool and examine in daylight and in ultraviolet light at 365 nm. Any spot corresponding to 17α-ethyloestran-17β-ol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Related substances.** Carry out Identification test A but using 10 µl of the following solutions.

**Solvent mixture.** A mixture of 90 volumes of chloroform and 10 volumes of methanol.

**Test solution.** Extract a quantity of the powdered tablets containing 1 mg of Ethyloestrenol with chloroform, filter, evaporate the filtrate to dryness at room temperature at a pressure not exceeding 0.2 kPa and dissolve the residue in 0.4 ml with solvent mixture.

**Reference solution (a).** A 0.25 per cent w/v solution of ethyloestrenol RS in the same solvent mixture.

**Reference solution (b).** A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 2 µl of each solution. After development, dry the plate in air, heat it at 105° for 10 minutes, spray with ethanolic sulphuric acid (20 per cent) and heat at 105° for a further 10 minutes. Allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

B. In the Assay, the principal peak the chromatogram obtained with the test solution has the same retention time as that of the peak due to ethyloestrenol RS in the chromatogram obtained with the reference solution (a).

**Tests**

**ααααα-ααααα-Ethyloestran-17βββββ-ol**. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G containing 20 per cent w/v of silver nitrate.

**Mobile phase.** A mixture of 75 volumes of toluene and 25 volumes of nonan-5-one.

**Solvent mixture.** A mixture of 90 volumes of chloroform and 10 volumes of methanol.

**Test solution.** Dissolve 20 mg of the residue obtained in the test for Related substances in 0.5 ml with solvent mixture.

**Reference solution.** A 0.08 per cent w/v solution of 17α-ethyloestran-17β-ol RS in the same solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in air, heat it at 105° for 10 minutes, spray with ethanolic sulphuric acid (20 per cent) and heat at 105° for a further 10 minutes and allow to cool. Any spot corresponding to 17α-ethyloestran-17β-ol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.
**Etosposide**

A white or almost white crystalline powder.

---

**Identification**

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with etosposide RS.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel H.

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**Assay**

Determine by gas chromatography (2.4.13).

*Test solution.* Weigh and powder 20 tablets. Extract a quantity of the powdered tablets containing about 8 mg of ethyloestrenol with 20 ml of acetone, filter, evaporate the filtrate to dryness on a water-bath and dissolve the residue in 4.0 ml of chloroform.

*Reference solution (a).* A solution containing 0.2 per cent w/v of ethyloestrenol RS and 0.1 per cent w/v of arachidic alcohol (internal standard) in chloroform.

*Reference solution (b).* Prepared in a similar manner as solution (1) but extracting with 20 ml of a 0.02 per cent w/v solution of arachidic alcohol in acetone.

**Chromatographic system**

- a glass column 1.0 m x 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/v of phenyl methyl silicone fluid (50 per cent phenyl) (such as OV-17),
- temperature: column 200°,
- inlet port and detector. 280°,
- flame ionisation detector,
- flow rate. 30 ml per minute of the carrier gas (nitrogen).

Calculate the content of C_{29}H_{32}O_{13} in the tablets.

**Storage.** Store protected from light in a refrigerator (2° to 8°).
Test solution. Dissolve 50 mg of the substance under examination in 10 ml of the solvent mixture.

Reference solution. A 0.5 per cent w/v solution of etoposide RS in the solvent mixture.

Apply to the plate 5µl of each solution as bands 10 mm by 3 mm. Allow the mobile phase to rise 17 cm. Dry the plate in warm air, spray it with a mixture of 1 volume of sulphuric acid and 9 volumes of ethanol and heat at 140ºC for 15 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Dissolve 5 mg in 5 ml of glacial acetic acid and add 0.05 ml of ferric chloride solution. Mix and cautiously add 2 ml of sulphuric acid. Avoid mixing the 2 layers. Allow to stand for about 30 minutes; a pink to reddish-brown ring develops at the interface and the upper layer is yellow.

Tests

Appearance of solution. A 3.0 per cent w/v solution in a mixture of 1 volume of methanol and 9 volumes of dichloromethane is clear (2.4.1) and not more intensely coloured than reference solution Y6 or BY6 (2.4.1).

Specific optical rotation. – 106.0º to -114.0º, determined in a 0.5 per cent w/v solution in a mixture of 1 volume of methanol and 9 volumes of dichloromethane.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of mobile phase A and mobile phase B.

Test solution (a). Dissolve 40 mg of the substance under examination in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Test solution (b). Dissolve 50.0 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a). Dilute 1.0 ml of test solution (a) to 10.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 20.0 ml with the solvent mixture.

Reference solution (b). Dilute 4.0 ml of reference solution (a) to 10.0 ml with the solvent mixture.

Reference solution (c) A 0.1 per cent w/v solution of etoposide RS in the solvent mixture.

Reference solution (d) To 10 ml of test solution (b), add 0.1 ml of a 4 per cent v/v solution of glacial acetic acid and 0.1 ml of phenolphthalein solution. Add 1 M sodium hydroxide until the solution becomes faintly pink (about 0.15 ml). After 15 minutes, add 0.1 ml of a 4 per cent v/v solution of glacial acetic acid.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 40ºC,
- mobile phase: A. a mixture of 1 volume of triethylamine, 1 volume of anhydrous formic acid and 998 volumes of water,
- B. a mixture of 1 volume of triethylamine, 1 volume of anhydrous formic acid and 998 volumes of acetonitrile,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 285 nm,
- a 10 µl loop injector.

Time Mobile Mobile Comment
(min) phase A phase B (per cent v/v) (per cent v/v)
0 75 25 isocratic
7 75 25 begin linear gradient
23 27 73 end chromatogram, return to 75A end equilibration, begin next chromatogram
25 75 25

Inject reference solution (d). Continue the chromatography until the peak due to phenolphthalein is eluted. The chromatogram shows two principal peaks corresponding to etoposide and to cis-etoposide. Ignore any peak due to phenolphthalein.

Inject test solution (a) and reference solutions (a), (b) and (d). The resolution between the peaks due to etoposide and to cis-etoposide is not less than 3.0. The area of any impurity peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and not more than two such peaks have an area more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent). Ignore any peak that is less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 6.0 per cent, determined on 0.25 g.

Assay. Determine by liquid chromatography (2.4.14).
Follow the chromatographic procedure described under Related substances.

Inject reference solution (c). The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately test solution (b) and reference solution (c).

Calculate the content of C$_{29}$H$_{32}$O$_{13}$.

**Storage.** Store protected from moisture.

**Etoposide Capsules**

Etoposide Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of etoposide, C$_{29}$H$_{32}$O$_{13}$.

**CAUTION —**Etoposide is potentially cytotoxic. Great care should be taken in handling the powder and preparing solutions.

**Identification**

Add a quantity of the contents of the capsules containing 0.1 g of Etoposide to a separating funnel containing 100 ml of water, extract with two quantities, each of 20 ml, of dichloromethane, dry the combined organic extracts over anhydrous sodium sulphate and filter. Extract the filtrate with 30 ml of water, filter the dichloromethane layer through anhydrous sodium sulphate and evaporate to dryness at 25º to 35º under reduced pressure. Dissolve the oily residue in 5 ml of water, shake gently and allow to stand for 30 minutes. Filter through a sintered-glass funnel, wash the precipitate in the funnel with three quantities, each of 20 ml, of water and dry the precipitate in the funnel at 40º at a pressure of 2 kPa for 90 minutes. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with etoposide RS.

**Tests**

**cis-Etoposide.** Determine by liquid chromatography (2.4.14).

**Test solution (a).** Weigh accurately a quantity of the mixed contents of 20 capsules containing about 0.5 g of Etoposide, dissolve in the mobile phase and dilute to 100 ml with the mobile phase; use immediately.

**Test solution (b).** Dilute 1 ml of test solution (a) to 50 ml with the mobile phase.

**Reference solution.** A 0.5 per cent w/v solution of etoposide RS in a mixture of 50 volumes of acetonitrile, 50 volumes of water and 0.1 volume of triethylamine and allow to stand for 40 minutes.

Use the chromatographic system described under Dissolution.

Inject the reference solution. The test is not valid unless the resolution between the principal peak and the peak immediately following the principal peak (cis-etoposide) is at least 1.0.

Inject test solution (a). The area of any peak corresponding to cis-etoposide is not more than the area of the peak in the chromatogram obtained with test solution (b) (2 per cent).

**Dissolution (2.5.2).**

**Apparatus No. 2**

Medium. 900 ml of a pH 4.5 buffer prepared by dissolving 2.99 g of sodium acetate and 14 ml of 2 M acetic acid in 1000 ml of water.

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Determine by liquid chromatography (2.4.14).

**Test solution.** Use the filtrate.

**Reference solution (a).** A 0.005 per cent w/v solution of etoposide RS in the dissolution medium.

**Reference solution (b).** A solution containing 0.005 per cent w/v of etoposide RS and 0.00025 per cent w/v of ethyl parahydroxybenzoate in the dissolution medium.

**Chromatographic system**

- a stainless steel column 30 cm × 3.9 mm, packed with phenyl silica gel for chromatography (10 μm)(such as Bondapak phenyl),
- mobile phase: a mixture of 26 volumes of acetonitrile and 74 volumes of a 0.272 per cent w/v solution of sodium acetate adjusted to pH 4.0 with glacial acetic acid,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

D. Not less than 80 per cent of the stated amount of C$_{29}$H$_{32}$O$_{13}$.

**Other tests.** Comply with the tests stated under Capsules.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh accurately a quantity of the mixed contents of 20 capsules containing about 40 mg of Etoposide dissolve in the mobile phase and dilute to 100.0 ml with the mobile phase; use immediately.

**Reference solution (a).** A 0.04 per cent w/v solution of etoposide RS in the mobile phase.

**Reference solution (b).** A solution containing 0.005 per cent w/v of etoposide RS and 0.00025 per cent w/v of ethyl parahydroxybenzoate in the mobile phase.

Use the chromatographic system described under Dissolution.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is at least 2.0.

Inject alternately the test solution and reference solution (a).
Calculate the content of \( C_{29}H_{32}O_{13} \) in the capsules.

**Storage.** Store protected from moisture at a temperature not exceeding 30\(^\circ\). The capsules should not be stored in a refrigerator.

### Etoposide Injection

Etoposide Injection is a sterile material consisting of Etoposide Concentrate. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections in accordance with the manufacturer’s instructions, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Infusions).*

**Storage.** The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

### Etoposide Concentrate

Etoposide Concentrate is a sterile solution of Etoposide in a suitable ethanolic vehicle.

*The concentrate complies with the requirements stated under Parenteral Preparations (Concentrated Solutions for Injection) and with the following requirements.*

Etoposide Concentrate contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of etoposide, \( C_{29}H_{32}O_{13} \).

### Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

*Mobile phase.* A mixture of 80 volumes of dichloromethane, 25 volumes of acetone, 2.5 volumes of ethanol (95 per cent) and 0.5 volume of water.

*Test solution.* Dilute a volume containing 20 mg of Etoposide to 25 ml with a mixture of 9 volumes of dichloromethane and 1 volume of methanol.

*Reference solution.* A 0.08 per cent w/v solution of etoposide RS in a mixture of 9 volumes of dichloromethane and 1 volume of methanol.

Apply to the plate 10 \( \mu l \) of each solution. Allow the mobile phase to rise 17 cm. Dry the plate in air, and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

### Tests

**pH** (2.4.24). 3.0 to 4.0, determined in a solution prepared by diluting a volume of the concentrate containing 0.1 g of Etoposide to 50 ml with carbon dioxide-free water.

**cis-Etoposide.** Determine by liquid chromatography (2.4.14).

*Test solution.* Dilute a volume of the concentrate containing 0.5 g of Etoposide to 100 ml with the mobile phase.

*Reference solution (a).* Dilute 1.0 ml of the test solution to 50 ml with the mobile phase.

*Reference solution (b).* A 0.5 per cent w/v solution of etoposide RS in the mobile phase.

Use the chromatographic system described under Assay.

Inject reference solution (b). The test is not valid unless the resolution between the principal peak and the peak immediately after the principal peak (cis-etoposide) is not less than 1.0.

Inject the test solution and reference solution (a). The area of any peak corresponding to cis-etoposide is not more than 1.0.

**Assay.** Determine by liquid chromatography (2.4.14).

*Test solution.* Dilute a volume of the concentrate containing about 40 mg of Etoposide to 100.0 ml with the mobile phase.

*Reference solution.* A 0.04 per cent w/v solution of etoposide RS in the mobile phase.

**Chromatographic system.**

- a stainless steel column 30 cm x 3.9 mm, packed with phenyl silica gel (10 \( \mu m \)) (such as Bondapak phenyl),
- mobile phase: a mixture of 26 volumes of acetonitrile and 74 volumes of a 0.272 per cent w/v solution of sodium acetate adjusted to pH 4.0 with glacial acetic acid,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 \( \mu l \) loop injector.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of \( C_{29}H_{32}O_{13} \) in the concentrate.

**Storage.** Store protected from light.

**Labelling.** The label states: (1) the directions for dilution of the contents; (2) that the diluted injection is to be given by
intravenous injection; (3) that the concentrate should be protected from light.
Ferrous Fumarate
Ferrous Fumarate Tablets
Ferrous Gluconate
Ferrous Gluconate Tablets
Ferrous Sulphate
Dried Ferrous Sulphate
Ferrous Sulphate Tablets
Fludrocortisone Acetate
Fludrocortisone Tablets
Fluocinolone Acetonide
Fluocinolone Cream
Fluorescein Sodium
Fluorescein Eye Drops
Fluorouracil
Fluorouracil Injection
Fluphenazine Decanoate
Fluphenazine Decanoate Injection
Fluphenazine Hydrochloride
Fluphenazine Hydrochloride Injection
Fluphenazine Tablets
Fluticasone Propionate
Fluticasone Propionate Inhalation
Fluticasone Propionate powder for Inhalation
Flurbiprofen
Flurbiprofen Tablets
Folic Acid
Folic Acid Tablets
Formeterol Fumarate Dihydrate
Formeterol Fumarate and Budesonide powder for Inhalation
Framycetin Sulphate
Fructose
Fructose Injection
Frusemide
Frusemide Injection
Frusemide Tablets
Furazolidone
Furazolidone Oral Suspension
Furazolidone Tablets
Fusidic Acid
Fusidic Acid Oral Suspension
Ferrous Fumarate

\[
\text{C}_4\text{H}_2\text{FeO}_4 \quad \text{Mol. Wt. 169.9}
\]

Ferrous Fumarate contains not less than 93.0 per cent and not more than 101.0 per cent of C\(_4\)H\(_2\)FeO\(_4\), calculated on the dried basis.

Description. A reddish orange to reddish brown, fine powder; may contain soft lumps that produce a yellow streak when crushed; odour, slight.

Identification

A. Heat 1 g with 25 ml of a mixture of equal volumes of hydrochloric acid and water on a water-bath for 15 minutes, cool and filter. Reserve the precipitate for test B; the filtrate gives reaction A of ferrous salts (2.3.1).

B. Wash the precipitate reserved in test A with a mixture of 1 g gives reaction A of ferrous salts (2.3.1). cool and filter. Reserve the precipitate for test B; the filtrate is a brownish solution.

C. Mix 0.5 g with 1 g of resorcinol. To 0.5 g of the mixture in a crucible, add a few drops of sulphuric acid and heat gently; a deep red semi-solid mass is formed. Add the mass to a large volume of water; an orange-yellow solution without any fluorescence is obtained.

Tests

Arsenic (2.3.10). Mix 2.0 g with 1.5 g of anhydrous sodium carbonate, add 10 ml bromine water and mix thoroughly. Evaporate to dryness on a water-bath, ignite gently and dissolve the cooled residue in 20 ml of brominated hydrochloric acid and 10 ml of water. Transfer to a small flask, add sufficient stannous chloride solution As\(T\) to remove the yellow colour, connect to a condenser and distil 22 ml. The distillate complies with the limit test for arsenic (5 ppm).

Heavy metals. Not more than 20 ppm, determined by the following method. Ignite 1.0 g gently until free from carbon, dissolve in 5 ml of hydrochloric acid by heating on a water-bath and evaporate to dryness. Dissolve the residue in a mixture of 15 ml of hydrochloric acid, 4 ml of nitric acid and 6 ml of water. Boil gently for minute, cool and extract with three quantities, each of 20 ml of ether. If the aqueous layer is more than slightly yellow, extract with a fourth quantity of 20 ml of ether and reject the ether extracts, heat the aqueous solution gently to remove the dissolved ether, add 1 g of citric acid, make alkaline with 5 M ammonia and add 1 ml of potassium cyanide solution. Dilute to 50 ml with water and add 0.1 ml of sodium sulphide solution. Any brown colour produced is not more intense than that produced by treating 1.0 ml of lead standard solution (20 ppm Pb) in a similar manner.

Sulphates (2.3.17). Boil 0.15 g with 10 ml of 2 M hydrochloric acid and 20 ml of freshly boiled and cooled water, cool in ice filter; the filtrate complies with the limit test for sulphates (0.1 per cent).

Ferric iron. Not more than 2.0 per cent, determined by the following method. Weigh accurately about 3.0 g and dissolve in a mixture of 200 ml of water and 20 ml of hydrochloric acid by heating rapidly to boiling point. Boil for 15 seconds, cool rapidly, add 3 g of potassium iodide, close the flask, allow to stand in the dark for 15 minutes and titrate the liberated iodine with 0.1 M sodium thiosulphate using starch solution, added towards the end of the titration, as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of iodine liberated by the ferric iron.

1 ml of 0.1 M sodium thiosulphate is equivalent to 0.005585 g of ferric iron.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105\(^\circ\)C.

Assay. Weigh accurately about 0.3 g and dissolve in 15 ml of dilute sulphuric acid with the aid of gentle heat. Cool, add 50 ml of water and immediately titrate with 0.1 M ceric ammonium sulphate using ferroin sulphate solution as indicator.

1 ml of 0.1 M ceric ammonium sulphate is equivalent to 0.01699 g of C\(_4\)H\(_2\)FeO\(_4\).

Ferrous Fumarate Tablets

Ferrous Fumarate Tablets contain not less than 90.0 per cent and not more than 105.0 per cent of the stated amount of ferrous fumarate, C\(_4\)H\(_2\)FeO\(_4\). The tablets may be coated.

Identification

The powdered tablets comply with the following tests.

A. Heat 1 g with 25 ml of a mixture of equal volumes of hydrochloric acid and water on a water-bath for 15 minutes, cool and filter. Reserve the precipitate for test B; the filtrate gives reaction A of ferrous salts (2.3.1).

B. Wash the precipitate reserved in test A with a mixture of 1 volume of dilute hydrochloric acid and 9 volumes of water and dry at 105\(^\circ\)C. Suspend 0.1 g of the residue in 2 ml of sodium carbonate solution and add dilute potassium permanganate
solution dropwise; the permanganate is decolorised and a brownish solution is obtained.

C. Mix 0.5 g with 1 g of resorcinol. To 0.5 g of the mixture in a crucible, add a few drops of sulphuric acid and heat gently; a deep red semi-solid mass is formed. Add the mass to a large volume of water; an orange-yellow solution without any fluorescence is obtained.

Tests

Ferric iron. Weigh accurately a quantity of the powder prepared for the Assay, containing about 1.5 g of Ferrous Fumarate, in a stoppered flask, dissolve as completely as possible with the aid of heat in a mixture of 100 ml of freshly boiled and cooled water and 10 ml of hydrochloric acid, boil for 15 seconds, cool rapidly, add 3 g of potassium iodide, close the flask and allow to stand in the dark for 15 minutes. Titrate the liberated iodine with 0.1 M sodium thiosulphate using starch solution, added towards the end of the titration, as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of iodine liberated by the ferric iron. Not more than 13.5 ml of 0.1 M sodium thiosulphate is required.  

Disintegration (2.5.1). 60 minutes.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.3 g of Ferrous Fumarate and dissolve in 15 ml of dilute sulphuric acid with the aid of gentle heat. Cool, add 50 ml of water and immediately titrate with 0.1 M ceric ammonium sulphate using ferroin sulphate solution as indicator.

1 ml of 0.1 M ceric ammonium sulphate is equivalent to 0.01699 g of C4H2FeO4.

Labelling. The label states the quantity of the active ingredient both as the amount of Ferrous Fumarate and in terms of the equivalent amount of ferrous iron in each tablet.

Ferrous Gluconate

\[
\text{Fe}^{2+} + \text{H}_2\text{O} \rightleftharpoons \text{Fe}^{3+} + \text{H}^+ + \text{OH}^-
\]

\[
\begin{align*}
\text{C}_{12}\text{H}_{22}\text{FeO}_{14}\text{xH}_{2}\text{O} & \quad \text{Mol. Wt. 446.1 (anhydrous)} \\
\text{Ferrous Gluconate is ferrous di(D-gluconate).}
\end{align*}
\]

Ferrous Gluconate contains not less than 95.0 per cent and not more than 102.0 per cent of C12H22FeO14, calculated on the dried basis.

Description. A yellowish grey or pale greenish-yellow, fine powder or granules; odour, slight, resembling that of burnt sugar.

Identification

A. Dissolve 5 g in carbon dioxide-free water at 60°, cool and dilute to 50 ml with water. 1 ml of the resulting solution gives reaction A of ferrous salts (2.3.1).

B. To 0.75 g in a test-tube add 7.5 ml of warm water, add 1 ml of glacial acetic acid and 1 ml of freshly distilled phenylhydrazine. Heat the mixture on a water-bath for 30 minutes. Cool and scratch the inner surface of the test tube with a glass rod until crystals of gluconic acid phenylhydrazide begin to form. Set aside for 10 minutes, filter, dissolve the precipitate in hot water, mix a small amount of decolorising charcoal and filter into a test tube. Allow the filtrate to cool, and scratch the inner surface of the test tube; white crystals are obtained which melt at about 202°, with decomposition (2.4.21).

Tests

Appearance of solution. Dissolve 5.0 g in carbon dioxide-free water at 60°, cool and dilute to 50 ml with the same solvent (solution A). Dilute 2 ml of solution A to 10 ml with water. When examined against the light, the resulting solution is clear (2.4.1).

pH (2.4.24). 4.0 to 5.5, determined in solution A, 3 to 4 hours after preparation.

Arsenic (2.3.10). To 5.0 g add 15 ml of water and 15 ml of stannated hydrochloric acid, distil 22 ml and add to the distillate 40 ml of water and 0.2 ml of stannous chloride solution AsT. The resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals (2.3.13). Warm 2.0 g gently with 10 ml of nitric acid until reaction begins and allow to stand until the evolution of nitrous fumes subsides. Boil gently to complete oxidation, adding a further 5 ml of nitric acid, if necessary, and continue boiling until the volume is reduced to about 5 ml. Add 20 ml of hydrochloric acid, boil gently for 1 minute, cool and extract with three quantities, each of 20 ml, of ether. If the acid solution is still more than faintly yellow, extract with a fourth quantity of 20 ml of ether and discard the ether extracts. Transfer the acid solution to a narrow-necked flask, rinse the separator with 5 ml of water, and add the rinsings to the flask. Heat to remove the dissolved ether and part of the hydrochloric acid. Cool and dilute to 50 ml with water. 25 ml of the resulting solution complies with the limit test for heavy metals, Method A (20 ppm).
Chlorides (2.3.12). 0.4 g complies with the limit test for chlorides (625 ppm).

Sulphates (2.3.17). 0.3 g complies with the limit test for sulphates (500 ppm).

Barium. Dissolve 0.1 g in 50 ml of distilled water, and 5 ml of dilute sulphuric acid, and allow to stand for 5 minutes. The solution is not more opalescent than a mixture of 10 ml of solution A and 45 ml of distilled water, when examined against the light.

Ferric iron. Not more than 1.0 per cent, determined by the following method. Weigh accurately about 5.0 g, transfer to a glass-stoppered flask and dissolve in a mixture of 100 ml of freshly boiled and cooled water and 10 ml of hydrochloric acid. Add 3 g of potassium iodide, shake well and allow to stand in the dark for 5 minutes. Titrate the liberated iodine with 0.1 M sodium thiosulphate using starch solution, added towards the end of the titration, as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of iodine liberated by the ferric iron.

1 ml of 0.1 M sodium thiosulphate is equivalent to 0.005585 g of ferric iron.

Oxalic acid. Dissolve 1 g in 5 ml of water, add 2 ml of hydrochloric acid and transfer to a separator. Extract with two quantities, each of 20 ml, of ether. Evaporate the combined ether extracts to dryness on a water-bath and dissolve the residue in 5 ml of water. Add 0.05 ml of acetic acid and 3 ml of calcium chloride solution; no turbidity is produced.

Reducing sugars. Dissolve 0.5 g in 10 ml of water and make alkaline with dilute ammonia solution. Pass hydrogen sulphide into the solution and allow to stand for 30 minutes. Filter and wash the precipitate with two quantities, each of 5 ml, of water. Combine the filtrate and the washings and acidify with dilute hydrochloric acid. Add 2 ml of dilute hydrochloric acid in excess. Boil the solution until the vapours no longer darken lead acetate paper and, if necessary, boil further to concentrate the solution to about 10 ml. Cool and add 10 ml of sodium carbonate solution, set aside for 5 minutes, filter and dilute the filtrate to 100 ml with water. To 5 ml of the filtrate add 2 ml of potassium cupri-tartrate solution and boil for 1 minute; no red precipitate is formed.

Loss on drying (2.4.19). 5.0 per cent to 10.0 per cent, determined on 1.0 g by drying in an oven at 105 °C.

Assay. Dissolve 0.5 g of sodium bicarbonate in a mixture of 70 ml of water and 30 ml of 1 M sulphuric acid. When effervescence ceases, add about 1.0 g, accurately weighed, of the substance under examination, shake gently to dissolve and titrate with 0.1 M ceric ammonium nitrate, using 0.1 ml of ferroin solution as indicator, until the red colour disappears.

1 ml of 0.1 M ceric ammonium nitrate is equivalent to 0.04461 g of C₁₂H₂₂FeO₁₄.

Storage. Store protected from light.

Ferrous Gluconate Tablets

Ferrous Gluconate Tablets contain not less than 90.0 per cent and not more than 105.0 per cent of the stated amount of ferrous gluconate, C₁₂H₂₂FeO₁₄·2H₂O. The tablets may be coated.

Identification

Dissolve a quantity of the powdered tablets containing about 1 g of Ferrous Gluconate in 10 ml of water and filter; the filtrate complies with the following tests.

A. 1 ml of the filtrate gives reaction A of ferrous salts (2.3.1).
B. To 7.5 ml of the filtrate add 1 ml of glacial acetic acid and 1 ml of freshly distilled phenylhydrazine. Heat the mixture on a water-bath for 30 minutes. Cool and scratch the inner surface of the test tube with a glass rod until crystals of gluconic acid phenylhydrazide begin to form. Set aside for 10 minutes, filter, dissolve the precipitate in hot water, mix a small amount of decolorising charcoal and filter into a test tube. Allow the filtrate to cool, and scratch the inner surface of the test tube; white crystals are obtained which melt at about 202 °C, with decomposition (2.4.21).
C. Shake a quantity of the powdered tablets containing 0.5 g of Ferrous Gluconate with 10 ml of dilute hydrochloric acid, filter and add to the filtrate 1 ml of barium chloride solution; an opalescence may be produced but no precipitate is formed.

Tests

Ferric iron. Weigh accurately a quantity of the powder prepared for the Assay, containing about 5.0 g of Ferrous Gluconate, in a stoppered flask, dissolve as completely as possible without the aid of heat in a mixture of 100 ml of freshly boiled and cooled water and 10 ml of hydrochloric acid, add 3 g of potassium iodide, close the flask and allow to stand in the dark for 5 minutes. Titrate the liberated iodine with 0.1 M sodium thiosulphate using starch solution, added towards the end of the titration, as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of iodine liberated by the ferric iron. Not more than 11.2 ml of 0.1 M sodium thiosulphate is required.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 1.0 g of Ferrous
Gluconate, dissolve in a mixture of 140 ml of water and 60 ml of 1 M sulphuric acid and titrate with 0.1 M ceric ammonium sulphate, using ferroin solution as indicator, until the red colour disappears.

1 ml of 0.1 M ceric ammonium sulphate, is equivalent to 0.04822 g of C₁₂H₂₄FeO₄₂H₂O.

Storage. Store protected from light.

Labelling. The label states the quantity of the active ingredient both as the amount of Ferrous Gluconate and in terms of the equivalent amount of ferrous iron in each tablet.

**Ferrous Sulphate**

FeSO₄·7H₂O  Mol. Wt. 278.0

Ferrous Sulphate contains not less than 98.0 per cent and not more than 105.0 per cent of FeSO₄·7H₂O.

**Description.** Bluish green crystals or a light green, crystalline powder; odourless. Efflorescent in air. On exposure to moist air, the crystals rapidly oxidise and become brown.

**Identification**

Gives reaction A of ferrous salts and the reactions of sulphates (2.3.1).

**Tests**

**Appearance of solution.** Dissolve 2.5 g in carbon dioxide-free water; add 0.5 ml of 1 M sulphuric acid and dilute to 50.0 ml with water (solution A). The solution is not more opalescent than opalescence standard OS2 (2.4.1).

**pH** (2.4.24). 3.0 to 4.0, determined in solution A.

**Arsenic** (2.3.10). Dissolve 5.0 g in 10 ml of water, add 15 ml of stannated hydrochloric acid and distil 20 ml. To the distillate add a few drops of bromine solution, remove the excess of bromine with a few drops of stannous chloride solution AsT and add 40 ml of water. The resulting solution complies with the limit test for arsenic (2 ppm).

**Copper.** Dissolve 8.0 g in 40 ml of hydrochloric acid, add 10 ml of nitric acid and 15 ml of water, boil gently for 5 minutes and cool. Shake with four quantities, each of 30 ml, of ether and discard the ether extracts. Heat the acid solution on a water-bath to remove the dissolved ether, cool and add sufficient water to produce 100.0 ml (solution B). To 10.0 ml of solution B add 1 g of citric acid, make alkaline with dilute ammonia solution, add 25 ml of water and 5 ml of sodium diethyldithiocarbamate solution. Extract successively with 5, 3 and 2-ml quantities of carbon tetrachloride, mix the carbon tetrachloride extracts and add sufficient carbon tetrachloride to produce 100.0 ml. The resulting solution is not more intensely coloured than a solution prepared by treating 4.0 ml of copper standard solution (10 ppm Cu) and 7.5 ml of water in the same manner (50 ppm).

**Lead.** Make 25.0 ml of solution B alkaline with dilute ammonia solution, add 1 ml of potassium cyanide solution and sufficient water to produce 50.0 ml. Add 0.1 ml of sodium sulphide solution; the solution is not more intensely coloured than a mixture of 10 ml of hydrochloric acid, 0.5 ml of nitric acid, 5.0 ml of lead standard solution (20 ppm Pb), 0.1 ml of sodium sulphide solution and sufficient water to produce 50.0 ml (50 ppm).

**Zinc.** To 2.5 ml of solution B add 1 g of citric acid and 1 g of resorcinol, neutralise the solution with dilute ammonia solution using thymol blue solution as indicator and shake for 1 minute with two quantities, each of 20 ml, of dithizone solution. To the combined extracts add 10 ml of 0.1 M hydrochloric acid and shake for 1 minute. Separate the acid layer, add 3 ml of 1 M hydrochloric acid and 20 ml of ammonium chloride solution and adjust the volume to 50.0 ml with water. Add 1.0 ml of potassium ferrocyanide solution and allow to stand for 15 minutes. Any turbidity produced is not greater than that developed in 15 minutes by the addition of 1.0 ml of potassium ferrocyanide solution to a freshly prepared mixture of 10.0 ml of zinc standard solution (10 ppm Zn), 4 ml of 1 M hydrochloric acid, 20 ml of ammonium chloride solution and sufficient water to produce 50.0 ml (500 ppm).

**Manganese.** Dissolve 1.0 g in 40 ml of water, add 10 ml of nitric acid and boil until red fumes are evolved. Add 0.5 g of ammonium persulphate and boil for 10 minutes. Discharge any pink colour by the dropwise addition of a 5 per cent w/v solution of sodium sulphone and boil until any odour of sulphur dioxide is eliminated. Add 10 ml of water, 5 ml of phosphoric acid and 0.5 g of sodium periodate, boil for 1 minute and allow to cool. The resulting solution is not more intensely coloured than that of a solution prepared at the same time and in the same manner using 1.0 ml of 0.02 M potassium permanganate in place of the substance under examination (0.1 per cent).

**Chlorides** (2.3.12). 20 ml of solution A complies with the limit test for chlorides (250 ppm).

**Assay.** Dissolve 2.5 g of sodium bicarbonate in a mixture of 150 ml of water and 10 ml of sulphuric acid. When effervescence ceases, add about 0.5 g of the substance under examination, accurately weighed, shake gently to dissolve and titrate with 0.1 M ceric ammonium nitrate, using 0.1 ml of ferroin solution as indicator, until the red colour disappears.

1 ml of 0.1 M ceric ammonium nitrate is equivalent to 0.02780 g of FeSO₄·7H₂O.
Dried Ferrous Sulphate

Dried Ferrous Sulphate is Ferrous Sulphate from which a part of the water of crystallisation has been removed by drying at a temperature of 40°F.

Dried Ferrous Sulphate contains not less than 86.0 per cent and not more than 90.0 per cent of FeSO₄.

Description. A greyish white to buff coloured powder.

Identification

Gives reaction A of ferrous salts and the reactions of sulphates (2.3.1).

Tests

Copper. Dissolve 8.0 g in 40 ml of hydrochloric acid, add 10 ml of nitric acid and 15 ml of water, boil gently for 5 minutes and cool. Shake with four quantities, each of 30 ml, of ether and discard the ether extracts. Heat the acid solution on a water-bath to remove the dissolved ether, cool and add sufficient water to produce 100.0 ml (solution A). To 10.0 ml of solution A add 1 g of citric acid, make alkaline with dilute ammonia solution, add 25 ml of water and 5 ml of sodium diethyldithiocarbamate solution. Extract successively with 5, 3 and 2 ml quantities of carbon tetrachloride, mix the carbon tetrachloride extracts and add sufficient carbon tetrachloride to produce 100.0 ml. The resulting solution is not more intensely coloured than a solution prepared by treating 4.0 ml of Dried Ferrous Sulphate, FeSO₄. The tablets are coated.

Lead. Make 25.0 ml of solution A alkaline with dilute ammonia solution, add 1 ml of potassium cyanide solution and sufficient water to produce 50.0 ml. Add 0.1 ml of sodium sulphide solution; the solution is not more intensely coloured than a mixture of 10 ml of hydrochloric acid, 0.5 ml of nitric acid, 5.0 ml of lead standard solution (20 ppm Pb), 0.1 ml of sodium sulphide solution and sufficient water to produce 50.0 ml (50 ppm).

Zinc. To 2.5 ml of solution A add 1 g of citric acid and 1 g of resorcinol, neutralise the solution with dilute ammonia solution using thymol blue solution as indicator and shake for 1 minute with two quantities, each of 20 ml, of dithizone solution. To the combined extracts add 10 ml of 0.1 M hydrochloric acid and shake for 1 minute. Separate the acid layer, add 3 ml of 1 M hydrochloric acid and 20 ml of ammonium chloride solution and adjust the volume to 50.0 ml with water. Add 1.0 ml of potassium ferrocyanide solution and allow to stand for 15 minutes. Any turbidity produced is not greater than that developed in 15 minutes by the addition of 1.0 ml of potassium ferrocyanide solution to a freshly prepared mixture of 10.0 ml of zinc standard solution (10 ppm Zn), 4 ml of 1 M hydrochloric acid, 20 ml of ammonium chloride solution and sufficient water to produce 50.0 ml (500 ppm).

Manganese. Dissolve 1.0 g in 40 ml of water, add 10 ml of nitric acid and boil until red fumes are evolved. Add 0.5 g of ammonium persulphate and boil for 10 minutes. Discharge any pink colour by the dropwise addition of a 5 per cent w/v solution of sodium sulphite and boil until any odour of sulphur dioxide is eliminated. Add 10 ml of water, 5 ml of phosphoric acid and 0.5 g of sodium periodate, boil for 1 minute and allow to cool. The resulting solution is not more intensely coloured than that of a solution prepared at the same time and in the same manner using 1.0 ml of 0.02 M potassium permanganate in place of the substance under examination (0.1 per cent).

Arsenic (2.3.10). Dissolve 3.3 g in 10 ml of water, add 15 ml of stannated hydrochloric acid and distil 20 ml. To the distillate add a few drops of bromine solution, remove the excess of bromine with a few drops of stannous chloride solution AsT and add 40 ml of water. The resulting solution complies with the limit test for arsenic (3 ppm).

Basic sulphate. 2.0 g dissolves slowly in a mixture of 7.5 ml of freshly boiled and cooled water and 0.5 ml of 0.5 M sulphuric acid, producing a solution that is not more than faintly turbid.

Assay. Weigh accurately about 0.5 g, dissolve in a mixture of 30 ml of water and 20 ml of 1 M sulphuric acid and titrate with 0.1 M ceric ammonium sulphate using ferroin solution as indicator.

1 ml of 0.1 M ceric ammonium sulphate is equivalent to 0.01519 g of FeSO₄.

Ferrous Sulphate Tablets

Ferrous Sulphate Tablets contain not less than 80.0 per cent and not more than 90.0 per cent of the stated amount of dried ferrous sulphate, FeSO₄. The tablets are coated.

Identification

A. The powdered tablets give reaction A of ferrous salts (2.3.1).

B. Extract the powdered tablets with 2 M hydrochloric acid and filter. The filtrate gives reaction A of sulphates (2.3.1).

Tests

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.5 g of Dried Ferrous Sulphate, dissolve in a mixture of 30 ml of water and 20 ml of
1 M sulphuric acid and titrate with 0.1 M ceric ammonium sulphate using ferroin solution as indicator.

1 ml of 0.1 M ceric ammonium sulphate is equivalent to 0.01519 g of FeSO₄₃.

**Labelling.** The label states the quantity of the active ingredient both as the amount of Dried Ferrous Sulphate and in terms of the equivalent amount of ferrous iron in each tablet.

**Fludrocortisone Acetate**

![Fludrocortisone Acetate structure](image)

C₂₃H₃₁FO₆  Mol. Wt. 422.5

Fludrocortisone Acetate is 9α-fluoro-11β,17α,21-trihydroxy-pregn-4-ene-3,20-dione 21-acetate.

Fludrocortisone Acetate contains not less than 96.0 per cent and not more than 104.0 per cent of C₂₃H₃₁FO₆, calculated on the dried basis.

**Description.** A white or almost white, crystalline powder; odourless or almost odourless; hygroscopic.

**Identification**

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with fludrocortisone acetate RS or with the reference spectrum of fludrocortisone acetate.

B. To a warm 1 per cent w/v solution in methanol add an equal volume of potassium cupri-tartrate solution; a red precipitate is produced.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Solvent mixture.** A mixture of 90 volumes of acetone and 10 volumes of formamide.

**Mobile phase.** A mixture of 30 volumes of toluene and 10 volumes of chloroform.

*Test solution.* Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

**Reference solution (a).** Dissolve 25 mg of fludrocortisone RS in 10 ml of the solvent mixture.

**Reference solution (b).** Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with ethanolic sulphuric acid (20 per cent v/v). Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

**Tests**

**Specific optical rotation** (2.4.22). +148° to +156°, determined in a 1.0 per cent w/v solution in dioxan.

**Light absorption** (2.4.7). Absorbance of a 0.001 per cent w/v solution in ethanol at the maximum at about 240 nm, 0.39 to 0.42.

**Related substances.** Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 20 mg of the substance under examination in the mobile phase and dilute to 10 ml with the mobile phase.

**Reference solution (a).** Dissolve 2 mg of fludrocortisone acetate RS and 2 mg of hydrocortisone acetate RS in the mobile phase and dilute to 50 ml with the mobile phase.

**Reference solution (b).** Dilute 1 ml of the test solution to 50 ml with the mobile phase.

**Chromatographic system**

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 35 volumes of tetrahydrofuran and 65 volumes of water,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Equilibrate the column with the mobile phase for about 30 minutes.
Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is 70 per cent to 90 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are: hydrocortisone acetate, about 8.5 minutes and fludrocortisone acetate about 10 minutes. The test is not valid unless the resolution between the peaks corresponding to hydrocortisone acetate and fludrocortisone acetate is at least 1.0. If this is not achieved, adjust the concentration of tetrahydrofuran in the mobile phase. Increasing the concentration of tetrahydrofuran reduces the retention times.

Inject the test solution and reference solution (b). Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with the test solution: the area of any peak other than the principal peak is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of the areas of all such peaks is not greater than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent). Ignore any peak due to the solvent and any peak with an area less than 0.025 times that of the principal peak in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 10 mg, dissolve in 50 ml of ethanol and add sufficient ethanol to produce 100.0 ml. Dilute 5.0 ml of this solution to 50.0 ml with ethanol and mix. Measure the absorbance of the resulting solution (2.4.7) at the maximum at about 238 nm. Calculate the content of C_{23}H_{31}FO_{6} taking 405 as the specific absorbance at 238 nm.

Storage. Store protected from light at a temperature not exceeding 30°.

**Fludrocortisone Tablets**

Fludrocortisone Acetate Tablets

Fludrocortisone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fludrocortisone acetate, C_{23}H_{31}FO_{6}.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Solvent mixture. A mixture of 90 volumes of acetone and 10 volumes of formamide.

**Mobile phase.** A mixture of 30 volumes of toluene and 10 volumes of chloroform.

Test solution. Shake a quantity of the powdered tablets containing 1 mg of Fludrocortisone Acetate with 20 ml of chloroform for 5 minutes, filter and evaporate the filtrate to dryness. Dissolve the residue in 4 ml of a mixture of 90 volumes of chloroform and 10 volumes of methanol.

Reference solution (a). Dissolve 25 mg of fludrocortisone RS in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid* (20 per cent v/v). Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

**Tests**

Uniformity of content. Comply with the test stated under Tablets.

Powder one tablet, disperse in 10 ml of water and extract with three quantities, each of 5 ml, of chloroform. Filter the extracts through a plug of cotton wool moistened with chloroform. Evaporate the chloroform on a water-bath just to dryness. Cool and dissolve the residue in 10.0 ml of ethanol. Measure the absorbance of the resulting solution at the maximum at about 240 nm (2.4.7). Calculate the content of C_{23}H_{31}FO_{6} taking 405 as the specific absorbance at 240 nm.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution (a). Weigh and powder 20 tablets. Shake a weighed quantity of the powdered tablets containing about 0.5 mg of Fludrocortisone Acetate with 2 ml of water for one minute, add 8 ml of acetonitrile and shake on a mechanical shaker for 40 minutes. Dilute the mixture to 20.0 ml with acetonitrile, centrifuge and use the supernatant liquid.

Test solution (b). Prepare in the same manner as test solution (a) but using 4.0 ml of a solution containing 0.01 per cent w/v...
of norethisterone RS (internal standard) in acetonitrile and 4.0 ml of acetonitrile in place of 8 ml of acetonitrile.

Reference solution. Mix 20.0 ml of internal standard, 25.0 ml of a 0.01 per cent w/v solution of fludrocortisone acetate RS in acetonitrile and 10 ml of water and dilute to 100.0 ml with acetonitrile.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylylsilyl silica gel (such as Spherisorb ODS 1),
- mobile phase: a mixture of 40 volumes of acetonitrile and 60 volumes of water,
- flow rate. 2 ml per minute,
- spectrophotometer set at 240 nm,
- a 20 µl loop injector.

Calculate the content of C_{23}H_{31}FO_{6} in the tablets.

Storage. Store protected from light.

Fluocinolone Acetonide

C_{24}H_{30}F_{2}O_{6}  Mol. Wt. 452.5

Fluocinolone Acetonide is 6α,9α-difluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxypregna-1,4-diene-3,20-dione.

Fluocinolone Acetonide contains not less than 96.0 per cent and not more than 104.0 per cent of C_{24}H_{30}F_{2}O_{6}, calculated on the dried basis.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with fluocinolone acetonide RS.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Solvent mixture. A mixture of 90 volumes of acetone and 10 volumes of formamide.

Mobile phase. A mixture of 30 volumes of toluene and 10 volumes of chloroform.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

Reference solution (a). Dissolve 25 mg of fludrocortisone RS in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with ethanolic sulphuric acid (20 per cent v/v). Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. Carry out the procedure given in Test B but using solutions prepared in the following manner.

Test solution. Dissolve 10 mg in 1.5 ml of glacial acetic acid in a separating funnel, add 0.5 ml of a 2 per cent w/v solution of chromium trioxide and allow to stand for 30 minutes. Add 5 ml of water and 2 ml of dichloromethane and shake vigorously for 2 minutes. Allow to separate and use the lower layer.

Reference solution (a). Prepare in the same manner as the test solution but using 10 mg of fluocinolone acetonide RS.

The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

D. Heat 0.5 ml of chromic-sulphuric acid in a test-tube (5 cm x about 6 mm) in a naked flame until white fumes are evolved; the solution wets the sides of the tube readily and there is no greasiness. Add 2 or 3 mg of the substance under examination and again heat in a naked flame until white fumes appear; the solution does not wet the sides of the tube and does not pour easily from the tube.

Tests

Specific optical rotation (2.4.22). +92.0° to +96.0°, determined in a 1.0 per cent w/v solution in dioxan.
Light absorption. Dissolve 15 mg in about 50 ml of ethanol and dilute to 100.0 ml with ethanol. Dilute 10.0 ml of the solution to 100.0 ml with ethanol. Absorbance of the resulting solution at the maximum at about 239 nm, 0.52 to 0.56 (2.4.7).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in acetonitrile and dilute to 10 ml with the same solvent.

Reference solution (a). Dissolve 2.5 mg of fluocinolone acetonide RS and 2.5 mg of triamcinolone acetonide RS in 45 ml of acetonitrile and dilute to 100 ml with water.

Reference solution (b). Dilute 1 ml of the test solution to 100 ml with acetonitrile.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with base-deactivated end-capped octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 450 ml of acetonitrile and 500 ml of water, allowed to equilibrate, the volume adjusted to 1000 ml with water and mixed again,
- flow rate. 1 ml per minute,
- spectrophotometer set at 238 nm,
- a 20 µl loop injector.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is not less than 50 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are: triamcinolone acetonide about 8.5 minutes and fluocinolone acetonide about 10 minutes. The test is not valid unless the resolution between the peaks corresponding to triamcinolone acetonide and fluocinolone acetonide is not less than 3.0.

Inject the test solution and reference solution (b). Continue the chromatography for 4 times the retention time of fluocinolone acetonide. In the chromatogram obtained with the test solution the area of any peak other than the principal peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of the areas of all such peaks is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent). Ignore any peak due to the solvent and any peak with an area less than 0.05 times that of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Weigh accurately about 50 mg, dissolve in ethanol, add sufficient ethanol to produce 50.0 ml and mix. Dilute 2.0 ml of this solution to 100.0 ml with ethanol. Measure the absorbance of the resulting solution at the maximum at about 238 nm. Calculate the content of C₂₅H₃₀F₂O₆ taking 355 as the specific absorbance at 238 nm.

Storage. Store protected from light.

Fluocinolone Acetonide Cream

Fluocinolone Acetonide Cream contains Fluocinolone Acetonide in a suitable base.

Fluocinolone Cream contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluocinolone acetonide, C₂₅H₃₀F₂O₆.

Identification
A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 60 volumes of n-hexane, 40 volumes of chloroform, 10 volumes of methanol and 1 volume of triethylamine.

Test solution. Disperse, by shaking a quantity of the cream containing 0.25 mg of Fluocinolone Acetonide in 2 ml of chloroform, add 10 ml of methanol, shake vigorously, cool in ice for 15 minutes, centrifuge at 3000 rpm for 15 minutes, decant the clear supernatant liquid, evaporate to dryness on a water-bath in a current of nitrogen and dissolve the residue in 1 ml of chloroform.

Reference solution. A 0.025 per cent w/v solution of fluocinolone acetonide RS in chloroform.

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable, heat at 105° for 5 minutes and spray whilst hot with blue tetrazolium solution. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution has the same retention time as that of the peak due to Fluocinolone Acetonide in the chromatogram obtained with the reference solution.

Tests
Other tests. Complies with the tests stated under Creams.

Assay. Determine by liquid chromatography (2.4.14).

For creams containing 0.025 per cent to 0.2 per cent w/w of fluocinolone acetonide:
Test solution (a). Weigh accurately a quantity of the cream containing about 2.5 mg of Fluocinolone Acetonide, add 60 ml of a solution prepared by adding 80 ml of methanol to 20 ml of a 25 per cent w/v solution of lithium chloride and disperse by shaking vigorously. Add 100 ml of cyclohexane, shake gently for 2 minutes and separate the lower, aqueous methanolic layer, taking care to exclude any solid matter that separates at the interface. Repeat the extraction using a further 25 ml of the lithium chloride solution. To the combined extracts add a solution containing 11 g of alum in 214 ml of water followed by 50 ml of chloroform, shake vigorously for about 3 minutes, allow the layers to separate and filter the chloroform extract through filter paper (such as Whatman No 1), previously moistened with chloroform, again excluding any solid matter at the interface. Repeat the extraction with 50- and 10-ml quantities of chloroform, filtering the extracts as before. Evaporate the combined extracts to dryness on a water-bath in a current of nitrogen, dissolve the residue in 5 ml of chloroform, transfer to a 10-ml volumetric flask with the aid of chloroform and add sufficient chloroform to produce 10.0 ml.

Test solution (b). Prepare in the same manner as test solution (a) but adding 1.0 ml of a 0.05 per cent w/v solution of phenacetin (internal standard) to the chloroform solution before dilution to 10.0 ml.

Reference solution. A solution containing 0.025 per cent w/v of fluocinolone acetonide RS and 0.005 per cent w/v of phenacetin in chloroform.

For creams containing 0.01 per cent w/w of fluocinolone acetonide:

Test solution (a). Prepare as described above but using a quantity of the cream containing about 1 mg of Fluocinolone Acetonide.

Test solution (b). Prepare in the same manner as test solution (a) but adding 1.0 ml of a 0.02 per cent w/v solution of phenacetin (internal standard) to the chloroform solution before diluting to 10.0 ml.

Reference solution. A solution containing 0.01 per cent w/v of fluocinolone acetonide RS and 0.002 per cent w/v of phenacetin in chloroform.

For creams containing 0.00625 per cent w/w of fluocinolone acetonide RS and 0.00125 per cent w/v of phenacetin in chloroform.

Test solution (a). Prepare as described above but using a quantity of the cream containing about 0.25 mg of Fluocinolone Acetonide.

Test solution (b). Prepare in the same manner as test solution (a) but adding 1.0 ml of a 0.005 per cent w/v solution of phenacetin (internal standard) to the chloroform solution before diluting to 10.0 ml.

Reference solution. A solution containing 0.0025 per cent w/v of fluocinolone acetonide RS and 0.0005 per cent w/v of phenacetin in chloroform.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with porous silica particles (5 µm),
- mobile phase: a mixture of 58 volumes of n-hexane, 40 volumes of chloroform, 2 volumes of methanol and 0.1 volume of glacial acetic acid,
- flow rate. 1.8 ml per minute,
- spectrophotometer set at 243 nm,
- a 20 µl loop injector.

The assay is not valid unless the resolution between the peaks due to fluocinolone acetonide and phenacetin is more than 2, and the capacity factors of fluocinolone acetonide and phenacetin are about 3 and 2, respectively. If these conditions are not achieved, adjust the concentration of methanol and chloroform in the mobile phase. Repeat the adjustment of chloroform and methanol concentration until correct values for both resolution and capacity factors have been obtained.

Calculate the content of $C_{24}H_{30}F_2O_6$ in the cream.

Storage. Store at a temperature not exceeding 30°.

**Fluorescein Sodium**

Soluble Fluorescein

\[
\text{NaO} \quad \text{O} \quad \text{O} \\
\text{COONa} \quad \text{COONa}
\]

$C_{20}H_{10}Na_2O_5$ Mol. Wt. 376.3

Fluorescein Sodium is disodium 2-(3-oxo-6-oxido-3H-xanthen-9-yl)benzoate.
Fluorescein Sodium contains not less than 98.5 per cent and not more than 100.5 per cent of C₂₀H₁₀Na₂O₅, calculated on the dried basis.

**Description.** An orange-red powder; odourless or almost odourless; hygroscopic.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with fluorescein sodium RS or with the reference spectrum of fluorescein sodium.

B. A solution is strongly fluorescent, even in extreme dilutions. The fluorescence disappears when the solution is made acidic and reappears when it is made alkaline.

C. A drop of a 0.05 per cent w/v solution, absorbed on a piece of filter paper, colours the paper yellow. On exposing the moist paper to the vapours of bromine for 1 minute and then to the vapours of ammonia, the yellow colour becomes deep pink.

D. The residue after incineration gives the reactions of sodium salts (2.3.1).

**Tests**

**pH** (2.4.24). 7.0 to 9.0, determined in a 2.0 per cent w/v solution.

**Related substances.** Determine by thin-layer chromatography (2.4.17), using a silica gel F₂₅₄ precoated plate (such as Merck silica gel 60 F₂₅₄ plate).

**Mobile phase.** A mixture of 80 volumes of chloroform and 20 volumes of methanol.

**Test solution.** Dissolve 0.1 g of the substance under examination in 10 ml of 0.1 M methanolic hydrochloric acid.

**Reference solution.** A 0.002 per cent w/v solution of the substance under examination in 10 ml of 0.1 M methanolic hydrochloric acid.

Apply to the plate 5 μl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Chloroform-soluble matter.** Dissolve 0.2 g in 10 ml of 0.1 M sodium hydroxide and extract with 10 ml of chloroform. Dry the chloroform layer with anhydrous sodium sulphate and filter. Absorbance of the resulting solution at about 480 nm, using chloroform as the blank, not more than 0.10 (2.4.7).

**Zinc.** Dissolve 0.1 g in 10 ml of water, add 2 ml of hydrochloric acid, filter and add 0.1 ml of potassium ferrocyanide solution; no turbidity or precipitate is produced immediately.

**Chlorides** (2.3.12). Dissolve 75 mg in 20 ml of water, add 2 ml of nitric acid and filter; the filtrate complies with the limit test for chlorides (0.33 per cent).

**Sulphates** (2.3.17). Dissolve 62.5 mg in 100 ml of water. To 20 ml add 2.5 ml of dilute hydrochloric acid and filter; the filtrate complies with the limit test for sulphates (1.2 per cent).

**Dimethylformamide.** Determine by gas chromatography (2.4.13).

**Test solution (a).** Dissolve 1.0 g of the substance under examination in 10 ml of water, add, with stirring, 10 ml of 0.6 M hydrochloric acid, allow to stand for 15 minutes and centrifuge. To 5 ml of the supernatant liquid add 0.1 g of trisodium phosphate and shake to dissolve.

**Test solution (b).** Prepare in the same manner as test solution (a) but using 10 ml of a 0.02 per cent w/v solution of dimethylacetamide (internal standard) in place of water.

**Reference solution.** Mix 10 ml of a 0.02 per cent w/v solution of dimethylformamide with 10 ml of the internal standard.

**Chromatographic system**

- a glass column 1.5m x 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 10 per cent w/w of polyethylene glycol 1000,
- temperature: column.120°,
- inlet port and detector. 180°,
- flame ionisation detector,
- flow rate. 30 ml per minute of the carrier gas (nitrogen).

In the chromatogram obtained with test solution (b) the ratio of the area of any peak corresponding to dimethylformamide to the area of the peak due to the internal standard is not greater than the corresponding ratio in the chromatogram obtained with the reference solution.

**Resorcinol.** Determine by thin-layer chromatography (2.4.17), using a silica gel F₂₅₄ precoated plate (such as Merck silica gel 60 F₂₅₄ plate).

**Mobile phase.** A mixture of 60 volumes of hexane and 40 volumes of ethyl acetate.

**Test solution.** Dissolve 1 g of the substance under examination in 10 ml of water, add slowly with constant stirring, 10 ml of 0.6 M hydrochloric acid, allow to stand for 15 minutes, centrifuge and use the supernatant liquid.

**Reference solution.** A 0.025 per cent w/v solution of resorcinol in water.

Apply to the plate 5 μl of each solution. After development, dry the plate in air and expose to iodine vapour for 30 minutes. Any spot corresponding to resorcinol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

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**Sulphates** (2.3.17). Dissolve 62.5 mg in 100 ml of water. To 20 ml add 2.5 ml of dilute hydrochloric acid and filter; the filtrate complies with the limit test for sulphates (1.2 per cent).
Acriflavin. Dissolve 10 mg in 5 ml of water, and add a few drops of sodium salicylate solution; no precipitate is formed.

**Loss on drying** (2.4.19). Not more than 10.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.5 g, dissolve in 20 ml of water, add 5 ml of dilute hydrochloric acid, and extract with four quantities, each of 20 ml, of a mixture of equal volumes of 2-methyl-1-propanol and chloroform. Wash the combined extracts with 10 ml of water, extract the washings with 5 ml of the mixture of 2-methyl-1-propanol and chloroform and add to the combined extracts. Evaporate the combined extracts to dryness on a water-bath in a current of air, dissolve the residue in 10 ml of ethanol (95 per cent), evaporate to dryness on a water-bath and dry to constant weight at 105°.

1 g of the residue is equivalent to 1.132 g of C₂₀H₁₀Na₂O₅.

**Storage.** Store protected from light.

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**Fluorescein Eye Drops**

**Fluorescein Sodium Eye Drops**

Fluorescein Eye Drops are a sterile solution of Fluorescein Sodium in Purified Water.

Fluorescein Eye Drops contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluorescein sodium, C₂₀H₁₀Na₂O₅.

**Identification**

A. Evaporate a volume of the eye drops containing 20 mg of Fluorescein Sodium and dry at 105° for 30 minutes. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with fluorescein sodium RS or with the reference spectrum of fluorescein sodium.

B. Dilute the eye drops with water to produce a solution containing 0.05 per cent w/v of Fluorescein Sodium. One drop of the solution, absorbed by a piece of filter paper, colours the paper yellow. On exposing the moist paper to the vapours of bromine for 1 minute and then to vapours of ammonia, the yellow colour becomes deep pink.

C. The eye drops are strongly fluorescent, even in extreme dilution. The fluorescence disappears when the solution is made acidic and reappears when it is made alkaline.

**Tests**

**pH** (2.4.24). 7.0 to 9.0.

**Related substances.** Determine by thin-layer chromatography (2.4.17), using a silica gel F254 precoated plate (such as Merck silica gel 60 F254 plate).

**Mobile phase.** A mixture of 80 volumes of chloroform and 20 volumes of methanol.

**Test solution.** Dilute a suitable volume of the eye drops, if necessary, with an equal volume of 0.1 M methanolic hydrochloric acid so as it give a concentration of 1.0 per cent w/v of fluorescein sodium.

**Reference solution.** Dilute 1 volume of the test solution to 500 volumes with 0.1 M methanolic hydrochloric acid.

Apply to the plate 5 μl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. Expose the plate to iodine vapour for 30 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Chloroform-soluble matter.** To a volume of the eye drops containing 0.1 g of Fluorescein Sodium add 1 ml of 2 M sodium hydroxide, extract with 10 ml of chloroform, dry the chloroform layer with anhydrous sodium sulphate and filter; absorbance of the resulting solution at about 480 nm, using chloroform as the blank, not more than 0.05 (2.4.7).

**Dimethylformamide.** Determine by gas chromatography (2.4.13).

**Test solution (a).** Dilute the eye drops with water, if necessary, to produce a solution containing 1.0 per cent w/v of Fluorescein Sodium. To 5 ml of this solution add, with stirring, 0.3 ml of 1 M hydrochloric acid, allow to stand for 15 minutes and centrifuge; dissolve 10 mg of trisodium phosphate in 2 ml of the supernatant liquid.

**Test solution (b).** Prepare in the same manner as test solution (a) but adding 1.0 ml of a 0.01 per cent v/v solution of dimethylacetamide (internal standard) before the hydrochloric acid.

**Reference solution.** A solution containing 0.002 per cent v/v of dimethylformamide and 0.002 per cent v/v of the internal standard.

**Chromatographic system**

- a glass column 1.5 m x 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 10 per cent w/w of polyethylene glycol 1000,
- temperature: column 120°, inlet port and detector 180°,
- flame ionisation detector,
- flow rate. 30 ml per minute of the carrier gas (nitrogen).

In the chromatogram obtained with test solution (b) the ratio of the area of any peak corresponding to dimethylformamide to the area of the peak due to the internal standard is not
Fluorouracil contains not less than 98.0 per cent and not more than 101.0 per cent of C₄H₃FN₂O₂, calculated on the dried basis.

**Description.** A white or almost white, crystalline powder. **CAUTION - Great care should be taken to avoid inhaling particles of Fluorouracil and exposing the skin to it.**

**Identification**

**Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with fluorouracil RS or with the reference spectrum of fluorouracil.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in acetate buffer pH 4.7 shows an absorption maximum only at about 266 nm.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

D. To 5 ml of a 1 per cent w/v solution add 1 ml of bromine water; the colour of bromine is discharged.

**Tests**

**Appearance of solution.** A 1.0 per cent w/v solution in carbon dioxide-free water is clear (2.4.1), and not more intensely coloured than reference solution YS7 or BYS7 (2.4.1).

**pH (2.4.24).** 4.5 to 5.0, determined in a 1.0 per cent w/v solution.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 70 volumes of ethyl acetate, 15 volumes of methanol and 15 volumes of water.

**Test solution (a).** Dissolve 0.1 g of the substance under examination in 10 ml of methanol (50 per cent).

**Test solution (b).** Dilute 5 ml of the test solution to 25 ml with methanol (50 per cent).

**Reference solution (a).** A 0.2 per cent w/v solution of fluorouracil RS in methanol (50 per cent).

**Reference solution (b).** A 0.0025 per cent w/v solution of fluorouracil RS in methanol (50 per cent).

**Reference solution (c).** A 0.0025 per cent w/v solution of 5-hydroxyuracil in methanol (50 per cent).

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b). Spray the plate with a freshly prepared 0.5 per cent w/v solution of fast blue.
blue B salt and then with 0.1 M sodium hydroxide. Any spot corresponding to 5-hydroxyuracil in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (c). Ignore any secondary spot on or near the line of application.

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent, determined on 1.0 g in a platinum crucible.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over phosphorus pentoxide in an oven at 80° at a pressure of 1.5 to 2.5 kPa for 4 hours.

**Assay.** Weigh accurately about 0.2 g, dissolve in 80 ml of dimethylformamide with the aid of gentle heat and cool. Titrate with 0.1 M tetrabutylammonium hydroxide in methanol, using 0.25 ml of a 1 per cent w/v solution of thymol blue in dimethylformamide as indicator. Carry out a blank titration. 1 ml of 0.1 M tetrabutylammonium hydroxide is equivalent to 0.01301 g of C₄H₃FN₂O₂.

**Storage.** Store protected from light.

### Fluorouracil Injection

Fluorouracil Injection is a sterile solution in Water for Injections of fluorouracil sodium, prepared by the interaction of Fluorouracil and Sodium Hydroxide.

Fluorouracil Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluorouracil, C₄H₃FN₂O₂.

**Description.** A colourless or almost colourless solution.

**Identification.**

A. Acidify carefully a volume of the injection containing 0.1 g of Fluorouracil with glacial acetic acid, stir, cool and filter. Wash the precipitate with 1 ml of water and dry over phosphorus pentoxide at 80° at a pressure of 2 kPa for 4 hours. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with fluorouracil RS or with the reference spectrum of fluorouracil.

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum only at about 266 nm.

C. To a volume of the injection containing 50 mg of Fluorouracil add 1 ml of bromine water; the colour of bromine is discharged.

**Tests**

**pH** (2.4.24). 8.5 to 9.5.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 70 volumes of ethyl acetate, 15 volumes of methanol and 15 volumes of water.

**Test solution.** Dilute a suitable quantity of the injection with water to produce a solution containing the equivalent of 2 per cent w/v of Fluorouracil.

**Reference solution (a).** Dilute 1 volume of test solution to 400 volumes with methanol (50 per cent).

**Reference solution (b).** A 0.005 per cent w/v solution of 5-hydroxyuracil in methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a). Spray with a freshly prepared solution containing 0.5 per cent w/v of fast blue B salt and then with 0.1 M sodium hydroxide. Any spot corresponding to 5-hydroxyuracil in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b). Ignore any secondary spot on or near the line of application.

**Urea.** Carry out the method described under Related substances applying separately to the plate 20 µl of the following solutions. For the test solution dilute a suitable quantity of the injection with water to produce a solution containing the equivalent of 0.5 per cent w/v of Fluorouracil. The reference solution contains 0.02 per cent w/v of urea in water. After development, dry the plate in air, spray with a mixture of 10 volumes of a 1 per cent w/v solution of 4-dimethylaminobenzaldehyde in ethanol (95 per cent) and 1 volume of hydrochloric acid and heat at 105° until maximum intensity of the spots is obtained. Any spot corresponding to urea in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** To an accurately measured volume containing about 50 mg of Fluorouracil add 20 ml of 1 M hydrochloric acid and sufficient water to produce 250.0 ml. Dilute 5.0 ml to 100.0 ml with 0.1 M hydrochloric acid and measure the absorbance of the resulting solution at the maximum at about 266 nm (2.4.7). Calculate the content of C₄H₃FN₂O₂ taking 552 as the specific absorbance at 266 nm.

**Storage.** Store protected from light in single dose containers at a temperature not exceeding 30°. The injection should not be allowed to freeze.
**Labelling.** The label states (1) the strength in terms of the equivalent amount of Fluorouracil in a suitable dose-volume; (2) that, if separation has occurred, the injection should be heated to 60°, shaken vigorously and allowed to cool to body temperature prior to use.

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**Fluticasone Propionate**

![Chemical Structure of Fluticasone Propionate]

C_{25}H_{31}F_{3}O_{5}S  
Mol. Wt. 500.6

Fluticasone Propionate is 5-fluoromethyl-6α,9α-difluoro-11β-hydroxy-16α-methyl-17α-propionyloxy-3-oxoandrosta-1,4-diene-17β-carbothioate.

Fluticasone Propionate contains not less than 96.0 per cent and not more than 102.0 per cent of fluticasone, C_{25}H_{31}F_{3}O_{5}S, calculated on the anhydrous basis.

**Description.** A white or almost white powder.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with fluticasone propionate RS or with the reference spectrum of fluticasone propionate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

**Tests**

**Specific optical rotation** (2.4.22). +32.0° to +36.0°, determined in a 0.5 per cent w/v solution in dichloromethane.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Solvent mixture.** 50 volumes of mobile phase A and 50 volumes of mobile phase B.

**Test solution.** Dissolve 20 mg of the substance under examination in 100 ml of the solvent mixture.

**Reference solution.** A 0.02 per cent w/v solution fluticasone propionate RS in the solvent mixture.

**Chromatographic system**

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsiline bonded to porous silica (5 µm),
- column temperature 40°,
- mobile phase: A. 0.05 per cent v/v orthophosphoric acid and 3 per cent v/v methanol in acetonitrile.
- B. 0.05 per cent v/v orthophosphoric acid and 3 per cent v/v methanol in water.
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 239 nm,
- a 50 µl loop injector.

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
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<tbody>
<tr>
<td>0</td>
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<td>57</td>
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<td>57</td>
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<tr>
<td>85</td>
<td>43</td>
<td>57</td>
</tr>
</tbody>
</table>

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency is not less than 20,000 theoretical plates.

Inject the test solution. Any individual impurity is not more than 0.5 per cent and sum of all the impurities is not more than 2.0 per cent.

**Water** (2.3.43). Not more than 0.5 per cent, determined on 0.25 g, using as solvent a mixture of equal volumes of chloroform and methanol.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 40 mg of the substance under examination in 100.0 ml of the mobile phase. Dilute 10.0 ml of this solution to 100.0 ml with the mobile phase.

**Reference solution.** A 0.004 per cent w/v solution of fluticasone propionate RS in the mobile phase.

**Chromatographic system**

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 40°,
- mobile phase: a mixture of 15 volumes of acetonitrile, 35 volumes of a buffer solution prepared by dissolving 1.15 g of ammonium dihydrogen phosphate in 1000 ml of water and adjusting the pH to 3.5 with orthophosphoric acid, and 50 volumes of methanol,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 239 nm,
- a 20 µl loop injector.
Inject the reference solution. The test is not valid unless relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of \( C_{25}H_{31}F_3O_5S \).

**Storage.** Store protected from light.

### Fluticasone Propionate Inhalation

Fluticasone Propionate Inhalation is a suspension of microfine Fluticasone Propionate in a suitable liquid filled in a suitable pressurized container. It may contain suitable pharmaceutical aids such as surfactants, stabilizing agents.

Fluticasone Propionate Inhalation delivers not less than 80.0 per cent and not more than 120.0 per cent of the stated amount of fluticasone propionate, \( C_{25}H_{31}F_3O_5S \), per inhalation by actuation of the valve.

### Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with reference solution (b).

### Tests

**Other tests.** Complies with the tests stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

Follow the procedure described under Assay with suitable dilution of the reference solution wherever the amount of active substance is to be determined in any test.

**Assay.** Carry out the test for Content of active ingredient delivered per actuation stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

Determine by liquid chromatography (2.4.14).

**Test solution.** Prepare using the mobile phase as described under the test for Content of active ingredient delivered per actuation stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

**Reference solution (a).** A 0.05 per cent w/v solution of fluticasone propionate RS in acetonitrile.

**Reference solution (b).** Dilute reference solution (a) with the mobile phase to obtain a solution containing 25 \( \mu \)g of fluticasone propionate per ml

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 mm),
- column temperature 40º,
- mobile phase: a mixture of 40 volumes of a buffer solution prepared by dissolving 1.3 g of diammonium hydrogen orthophosphate in 1000 ml of water and adjusting the pH to 7.0 with orthophosphoric acid, and 60 volumes of acetonitrile.
- flow rate, 2 ml per minute,
- spectrophotometer set at 238 nm,
- inject 200 \( \mu \)l.

Inject the reference solution (b). The test is not valid unless the column efficiency is not less than 1500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution (b).

Calculate the content of \( C_{25}H_{31}F_3O_5S \) in the solution and the content of \( C_{25}H_{31}F_3O_5S \) delivered per actuation of the valve.

Determine the content of active ingredient a second and third time by repeating the procedure on the middle ten and on the last ten successive combined actuations of the valve. For each of the three determinations the average content of \( C_{25}H_{31}F_3O_5S \) delivered per actuation of the valve meets the requirements.

**Storage.** Store protected from moisture at a temperature not exceeding 30º.

### Fluticasone Propionate Powder for Inhalation

Fluticasone Propionate Powder for Inhalation consists of Fluticasone propionate in microfine powder either alone or admixed with Lactose in a pre-metered unit for use in a suitable powder inhaler.

Fluticasone Propionate Powder for Inhalation contains not less than 90.0 per cent and not more than 125.0 per cent of the stated amount of fluticasone propionate, \( C_{25}H_{31}F_3O_5S \) per unit dose.

### Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

### Tests

**Other tests.** Complies with the tests stated under Inhalation Preparations (Powders for Inhalation).

Follow the procedure described under Assay with suitable dilution of the reference solution wherever the amount of active substance is to be determined in any test.
**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** To 10 intact capsules add 10 ml of water and mix with the aid of ultrasound till the shells get dispersed. Add 60 ml of the mobile phase and continue mixing with the aid of ultrasound for 10 minutes with intermittent shaking. Add sufficient of the mobile phase to produce 100.0 ml. Dilute suitably, if required, to get a solution containing 25 µg of Fluticasone Propionate per ml.

**Reference solution.** A solution containing 0.5 mg of fluticasone propionate per ml prepared by dissolving 10 mg of fluticasone propionate RS in 10 ml acetonitrile and adding sufficient of the mobile phase to produce 20 ml and further dilute with mobile phase to obtain a solution containing 25 µg of Fluticasone Propionate per ml.

**Chromatographic system**
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm),
- column temperature 40º,
- mobile phase: a mixture of 40 volumes of a buffer solution prepared by dissolving 1.3 g of diammonium hydrogen orthophosphate in 1000 ml of water and adjusting the pH to 7.0 with orthophosphoric acid and 60 volumes of acetonitrile,
- flow rate. 2 ml per minute,
- spectrophotometer set at 238 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1500 theoretical plates and the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of C$_{25}$H$_{31}$F$_{3}$O$_{5}$S per unit.

**Storage.** Store protected from moisture, at temperature not exceeding 30º.

**Labelling.** The label states the quantity of active ingredient per pre-metered unit.

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**Fluphenazine Decanoate**

Fluphenazine Decanoate Ester

![Fluphenazine Decanoate Ester](image)

Fluphenazine Decanoate is 2-{4-[3-(2-trifluoromethylphenothiazin-10-yl)propyl]piperazin-1-yl}ethyl decanoate.

Fluphenazine Decanoate contains not less than 98.5 per cent and not more than 101.5 per cent of C$_{32}$H$_{44}$F$_{3}$N$_{3}$O$_{2}$S, calculated on the dried basis.

**Description.** A pale yellow, viscous liquid or yellow, crystalline, oily solid; odour, faint and ester-like.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with fluphenazine decanoate RS or with the reference spectrum of fluphenazine decanoate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in ethanol shows an absorption maximum at about 261 nm and a less well-defined maximum at about 310 nm; absorbance at about 261 nm, about 0.60.

C. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254. Impregnate the dry plate by placing it in a tank containing a 5 per cent v/v solution of n-tetradecane in n-hexane, allowing the impregnating solvent to ascend to the top and allowing to dry.

**Mobile phase.** Methanol (90 per cent).

**Test solution.** Dissolve 0.2 g of the substance under examination in 10 ml of ethanol (95 per cent).

**Reference solution.** A 2.0 per cent w/v solution of fluphenazine decanoate RS in ethanol (95 per cent).

Apply to the plate 1 µl of each solution. After development, dry the plate dry in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

D. Dissolve 5 mg in 2 ml of sulphuric acid and allow to stand for 5 minutes; a reddish-brown colour is produced.

**Tests**

**Related substances.** Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with silica gel F254.

**Mobile phase.** A mixture of 80 volumes of acetone, 30 volumes of cyclohexane and 5 volumes of strong ammonia solution.

**Test solution.** Dissolve 0.25 g of the substance under examination in 10 ml of methanol.

**Reference solution.** A 0.025 per cent w/v solution of the substance under examination in methanol.
Apply to the plate 20 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Spray the plate with sulphuric acid (50 per cent v/v) and examine in daylight. By both methods of visualisation, any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. Weigh accurately about 0.6 g and dissolve in 50 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, using crystal violet solution as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02959 g of C₃₂H₄₄F₃N₃O₂S.

Storage. Store protected from light.

Fluphenazine Decanoate Injection

Fluphenazine Decanoate Injection is a sterile solution of Fluphenazine Decanoate in Sesame Oil.

Fluphenazine Decanoate Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluphenazine decanoate, C₃₂H₄₄F₃N₃O₂S.

Identification

A.Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. Chloroform for the first development and a mixture of 80 volumes of acetone, 30 volumes of cyclohexane and 5 volumes of strong ammonia solution for the second development.

Test solution. Dilute a suitable volume of the injection with ethanol (95 per cent) to produce a solution containing 2.5 mg of Fluphenazine Decanoate per ml.

Reference solution. A 0.1 per cent w/v solution of fluphenazine hydrochloride RS in methanol.

Apply to the bottom right-hand corner of the plate 10 µl of the test solution. After development, dry the plate in air and turn through 90° in a clockwise direction. Apply to the bottom right-hand corner of the plate, to the right of the solvent front of the first development, 1 µl of the reference solution. After the second development, dry the plate in air and examine in ultraviolet light at 254 nm. Spray the plate with sulphuric acid (50 per cent v/v). By both methods of visualisation, any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Related substances. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. Chloroform for the first development and a mixture of 80 volumes of acetone, 30 volumes of cyclohexane and 5 volumes of strong ammonia solution for the second development.

Test solution. Dilute a suitable volume of the injection with ethanol (95 per cent) to produce a solution containing 2.5 mg of Fluphenazine Decanoate per ml.

Reference solution. A 0.1 per cent w/v solution of fluphenazine hydrochloride RS in methanol.

Applying the bottom right-hand corner of the plate 10 µl of the test solution. After development, dry the plate in air, turn it through 90° in a clockwise direction. Apply to the bottom right-hand corner of the plate, to the right of the solvent front of the first development, 1 µl of the reference solution. After the second development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Carry out the following procedure protected from light.

Measure accurately a volume of the injection containing about 0.25 g of Fluphenazine Decanoate and dilute with 75 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, using crystal violet solution as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02959 g of C₃₂H₄₄F₃N₃O₂S.

Storage. Store protected from light.

Labelling. The label states that the injection is for intramuscular injection only.
Fluphenazine Hydrochloride

\[
\begin{align*}
\text{C}_{22}\text{H}_{26}\text{F}_3\text{N}_3\text{OS},2\text{HCl} & \quad \text{Mol. Wt. 510.5} \\
\end{align*}
\]

Fluphenazine Hydrochloride is 2-\{4-[3-(2-trifluoromethylphenothenazin-10-yl)propyl]piperazin-1-yl\}ethanol dihydrochloride.

Fluphenazine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent w/v of C\textsubscript{22}H\textsubscript{26}F\textsubscript{3}N\textsubscript{3}OS,2HCl, calculated on the dried basis.

**Description.** A white or almost white, crystalline powder.

**Identification**

*Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.*

A. Dissolve 0.1 g in 10 ml of water, make alkaline with 1 M sodium hydroxide, extract with 5 ml of chloroform, filter through anhydrous sodium sulphate and evaporate the solvent in a current of nitrogen. The oily residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with fluphenazine hydrochloride RS treated in the same manner or with the reference spectrum of fluphenazine.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in methanol shows an absorption maximum at about 258 nm and a less well-defined maximum at about 310 nm; absorbance at about 258 nm, between 0.63 and 0.70.

C. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with kieselguhr G. Impregnate the dry plate by placing in a tank containing a shallow layer of a mixture of 36 volumes of acetone, 3 volumes of formamide and 1 volume of 2-phenoxyethanol. Allow the impregnating solvent to ascend to the top, remove the plate from the tank and use it immediately.

*Mobile phase.* A mixture of 100 volumes light petroleum (40° to 60°) saturated with 2-phenoxyethanol and 2 volumes of diethylamine.

*Test solution.* Dissolve 0.2 g of the substance under examination in 100 ml of methanol.

**Tests**

**pH** (2.4.24). 1.9 to 2.3, determined in a 5.0 per cent w/v solution in carbon dioxide-free water.

**Related substances.** Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

*Mobile phase.* A mixture of 80 volumes of acetone, 30 volumes of cyclohexane and 5 volumes of strong ammonia solution.

*Test solution.* Dissolve 0.1 g of the substance under examination in 10 ml of 0.1 M methanolic sodium hydroxide.

*Reference solution (a).* Dilute 1 ml of the test solution to 100 ml with 0.1 M methanolic sodium hydroxide.

*Reference solution (b).* Dilute 5 ml of reference solution (a) to 10 ml with 0.1 M methanolic sodium hydroxide.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, examine in ultraviolet light at 254 nm. Apply to the plate 2 µl of each solution. After development, dry the plate in air, examine in ultraviolet light at 365 nm and observe the fluorescence produced after about 2 minutes. Heat the plate at 120° for 20 minutes, cool, spray with ethanolic sulphuric acid (20 per cent) and observe the colour produced. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

D. Mix about 5 mg with 45 mg of heavy magnesium oxide and ignite in a crucible until an almost white residue is obtained (usually less than 5 minutes). Allow to cool, add 1 ml of water, 0.05 ml of dilute phenolphthalein solution and about 1 ml of 2 M hydrochloric acid to render the solution colourless and filter. To a freshly prepared mixture of 0.1 ml of alizarin red S solution and 0.1 ml of zirconyl nitrate solution, add 1 ml of the filtrate, mix, allow to stand for 5 minutes and examine the colour of the solution as well as of a blank prepared in the same manner. The colour of the test solution is yellow and that of the blank is red.

E. Gives the reaction of chlorides (2.3.1).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.
Assay. Weigh accurately about 0.25 g and dissolve in a mixture of 10 ml of anhydrous formic acid and 40 ml of acetic anhydride. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02552 g of C22H26F3N3OS,2HCl.

Storage. Store protected from light.

Fluphenazine Hydrochloride Injection

Fluphenazine Hydrochloride Injection is a sterile solution of Fluphenazine Hydrochloride in Water for Injection.

Fluphenazine Hydrochloride Injection contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of fluphenazine hydrochloride, C22H26F3N3OS,2HCl.

Description. A clear, colourless solution.

Identification

A. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with kieselguhr G. Impregnate the dry plate by placing in a tank containing a shallow layer of a mixture of 36 volumes of acetone, 3 volumes of formamide and 1 volume of 2-phenoxyethanol. Allow the impregnating solvent to ascend to the top, remove the plate from the tank and use it immediately.

Mobile phase. A mixture of 100 volumes light petroleum (40° to 60°) saturated with 2-phenoxyethanol and 2 volumes of diethylamine.

Test solution. Use a quantity of the injection containing 2 mg of Fluphenazine Hydrochloride and dilute it to 1 ml with methanol.

Reference solution. A 0.2 per cent w/v solution of fluphenazine hydrochloride RS in methanol.

After development, dry the plate in air, examine in ultraviolet light at 365 nm and observe the fluorescence produced after about 2 minutes. Heat the plate at 120° for 20 minutes, cool, spray with ethanolic sulphuric acid (20 per cent) and observe the colour produced. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. To a quantity of the injection containing 5 mg of Fluphenazine Hydrochloride add 2 ml of sulphuric acid and allow to stand for 5 minutes; an orange colour is produced.

Tests

pH (2.4.24). 4.8 to 5.2.

Related substances. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 80 volumes of acetone, 30 volumes of cyclohexane and 5 volumes of strong ammonia solution.

Test solution. Dilute a quantity of the injection containing 20 mg of Fluphenazine Hydrochloride with sufficient 0.1 M methanolic sodium hydroxide to make 10 ml.

Reference solution (a). Dilute 1 volume of the test solution to 50 volumes with 0.1 M methanolic sodium hydroxide.

Reference solution (b). Dilute 1 volume of the test solution to 100 volumes with 0.1 M methanolic sodium hydroxide.

Apply to the plate 50 µl of the test solution and 25 µl of reference solutions (a) and (b). After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (b). Ignore any spot remaining on the line of application.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Carry out the following procedure protected from light.

To an accurately measured quantity of the injection containing about 5 mg of Fluphenazine Hydrochloride add a mixture of 1 volume of 1 M hydrochloric acid and 99 volumes of ethanol (90 per cent) to produce 50.0 ml. Dilute 10.0 ml of this solution to 100.0 ml with the acid-ethanol mixture and measure the absorbance of the resulting solution at the maximum at about 258 nm (2.4.7). Calculate the content of C22H26F3N3OS,2HCl taking 620 as the specific absorbance at 258 nm.

Storage. Store protected from light.

Fluphenazine Tablets

Fluphenazine Hydrochloride Tablets

Fluphenazine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluphenazine hydrochloride, C22H26F3N3OS,2HCl. The tablets are coated.

Identification

A. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with kieselguhr G. Impregnate the dry plate by placing in a tank containing a shallow layer of a mixture of 36 volumes of acetone, 3 volumes of formamide
and 1 volume of 2-phenoxethanol. Allow the impregnating solvent to ascend to the top, remove the plate from the tank and use it immediately.

**Mobile phase.** A mixture of 100 volumes light petroleum (40° to 60°) saturated with 2-phenoxethanol and 2 volumes of diethylamine.

**Test solution.** Shake a quantity of the powdered tablets with sufficient methanol to produce a solution containing 0.2 per cent w/v of Fluphenazine Hydrochloride, centrifuge and use the supernatant liquid.

**Reference solution.** A 0.2 per cent w/v solution of fluphenazine hydrochloride RS in methanol.

After development, dry the plate in air, examine in ultraviolet light at 365 nm and observe the fluorescence produced after about 2 minutes. Heat the plate at 120° for 20 minutes, cool, spray with ethanolic sulphuric acid (20 per cent) and observe the colour produced. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Extract a quantity of the powdered tablets containing 5 mg of Fluphenazine Hydrochloride with 5 ml of acetone, filter and evaporate the filtrate to dryness. Add 2 ml of sulphuric acid to the residue and allow to stand for 5 minutes; an orange colour is produced.

C. Extract a quantity of the powdered tablets containing 10 mg of Fluphenazine Hydrochloride with 10 ml of ethanol containing 0.2 per cent v/v of strong ammonia solution and evaporate the extract to dryness. Heat 0.5 ml of chromic-sulphuric acid mixture in a small test-tube in a water-bath for 5 minutes; the solution wets the sides of the tube readily and there is no greasiness. Add 2 or 3 mg of the residue and again heat in a water-bath for 5 minutes; the solution does not wet the sides of the tube and does not pour easily from the tube.

**Tests**

**Related substances.** Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 80 volumes of acetone, 30 volumes of cyclohexane and 5 volumes of strong ammonia solution.

**Test solution.** Remove the coating from a suitable quantity of tablets; shake a quantity of the powdered tablet cores containing 20 mg of Fluphenazine Hydrochloride with 10 ml of 0.1 M methanolic sodium hydroxide for 5 minutes, centrifuge and use the supernatant liquid.

**Reference solution (a).** Dilute 1 volume of the test solution to 50 volumes with 0.1 M methanolic sodium hydroxide.

**Reference solution (b).** Dilute 1 volume of the test solution to 100 volumes with 0.1 M methanolic sodium hydroxide.

Apply to the plate 50 µl of the test solution and 25 µl of reference solutions (a) and (b). After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than two such spots is more intense than the spot in the chromatogram obtained with reference solution (b). Ignore any spot remaining on the line of application.

**Uniformity of content.** Comply with the test stated under Tablets.

**Carry out the procedure protected from light.**

Powder 1 tablet and dissolve the powder as completely as possible in a mixture of 99 volumes of ethanol (80 per cent) and 1 volume of 1 M hydrochloric acid. Add sufficient of the acid-ethanol mixture to produce 100.0 ml and filter. Dilute suitably, if necessary with the acid-ethanol mixture and measure the absorbance of the resulting solution at the maximum at about 258 nm (2.4.7). Calculate the content of C$_{15}$H$_{13}$FO$_2$ taking 620 as the specific absorbance at 258 nm.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Carry out the following procedure protected from light.

Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 5 mg of Fluphenazine Hydrochloride, dissolve as completely as possible in a mixture of 99 volumes of ethanol (80 per cent) and 1 volume of 1 M hydrochloric acid, add sufficient of the acid-ethanol mixture to produce 100.0 ml and filter. Dilute 10.0 ml of the filtrate to 50.0 ml with the acid-ethanol mixture and measure the absorbance of the resulting solution at the maximum at about 258 nm (2.4.7). Calculate the content of C$_{15}$H$_{13}$FO$_2$ taking 620 as the specific absorbance at 258 nm.

**Storage.** Store protected from light.

**Flurbiprofen**

\[
\text{C}_{15}\text{H}_{13}\text{FO}_2
\]

Mol. Wt. 244.3

Flurbiprofen is (RS)-2-(2-fluorobiphenyl-4-yl)propionic acid.

Flurbiprofen contains not less than 99.0 per cent and not more than 100.5 per cent of C$_{15}$H$_{13}$FO$_2$, calculated on the dried basis.
**Description.** A white or almost white, crystalline powder.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *flurbiprofen RS* or with the reference spectrum of flurbiprofen.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in 0.1 M sodium hydroxide shows an absorption maximum only at about 247 nm; absorbance at about 247 nm, about 0.8.

C. Heat 0.5 ml of *chromic-sulphuric acid mixture* in a small test-tube in a water-bath for 5 minutes; the solution wets the sides of the tube readily and there is no greasiness. Add 2 or 3 mg of the substance under examination and heat in a water-bath for 5 minutes; the solution does not wet the sides of the tube and does not pour easily from the tube.

**Tests**

**Related substances.** Determine by liquid chromatography (2.4.17).

**Solvent mixture.** A mixture of 45 volumes of *acetonitrile* and 55 volumes of *water*.

**Test solution (a).** Dissolve 0.2 g of the substance under examination in 100 ml with solvent mixture.

**Test solution (b).** Dissolve 0.2 g of the substance under examination and 1 mg of 2-(biphenyl-4-yl) propionic acid RS in 100 ml of the same solvent mixture.

**Reference solution.** A 0.001 per cent w/v solution of 2-(biphenyl-4-yl)propionic acid RS in the same solvent mixture.

**Chromatographic system**

- a stainless steel column 15 cm x 3.9 mm, packed with octadeclisilyl silica gel (5 µm),
- mobile phase: a mixture of 60 volumes of *water*, 35 volumes of *acetonitrile* and 5 volumes of *glacial acetic acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Adjust the sensitivity of the instrument so that with the reference solution the height of the peak due to 2-(biphenyl-4-yl)propionic acid is about 40 per cent of the full-scale deflection on the recorder.

In the chromatogram obtained with test solution (a) the area of any secondary peak is not greater than the area of the peak in the chromatogram obtained with the reference solution and the sum of the areas of any such peaks is not greater than twice the area of the peak in the chromatogram obtained with the reference solution.

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying.** Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa.

**Assay.** Weigh accurately about 0.5 g, dissolve in 100 ml of *ethanol (95 per cent)* previously neutralised to phenolphthalein solution and titrate with 0.1 M sodium hydroxide using phenolphthalein solution as indicator.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.02443 g of C₁₅H₁₃FO₂.

**Flurbiprofen Tablets**

Flurbiprofen Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of flurbiprofen, C₁₅H₁₃FO₂. The tablets are coated.

**Identification**

Extract a quantity of the powdered tablets containing 0.5 g of Flurbiprofen with 25 ml of acetone, filter, evaporate the filtrate to dryness with the aid of a current of air without heating and dry at 60° at a pressure of 2 kPa. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *flurbiprofen RS* or with the reference spectrum of flurbiprofen.

B. Heat 0.5 ml of *chromic-sulphuric acid mixture* in a small test-tube in a water-bath for 5 minutes; the solution wets the sides of the tube readily and there is no greasiness. Add 2 or 3 mg of the substance under examination and heat in a water-bath for 5 minutes; the solution does not wet the sides of the tube and does not pour easily from the tube.

**Tests**

**Related substances.** Determine by liquid chromatography (2.4.17).

**Test solution.** Disperse a quantity of the powdered tablets containing 0.5 g of Flurbiprofen in 50 ml of water, add 200 ml of *acetonitrile*, mix and centrifuge. Use the supernatant liquid.

**Reference solution.** A 0.001 per cent w/v solution of 2-(biphenyl-4-yl)propionic acid RS in the test solution.
Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with octadecysilyl silica gel (5 µm),
- mobile phase: a mixture of 60 volumes of water, 35 volumes of acetonitrile and 5 volumes of glacial acetic acid,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Adjust the sensitivity of the instrument so that with the reference solution the height of the peak due to 2-(biphenyl-4-y1) propionic acid is about 40 per cent of the full-scale deflection on the recorder.

In the chromatogram obtained with the test solution the area of any secondary peak is not greater than the area of the peak in the chromatogram obtained with the reference solution and the sum of the areas of any such peaks is not greater than twice the area of the peak in the chromatogram obtained with the reference solution.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Flurbiprofen, shake with 60 ml of 0.1 M sodium hydroxide for 5 minutes, dilute to 100.0 ml with 0.1 M sodium hydroxide, filter if necessary and dilute 10.0 ml of the filtrate to 100.0 ml with the same solvent. Further dilute 10.0 ml to 100.0 ml with the same solvent and measure the absorbance of the resulting solution at the maximum at about 247 nm (2.4.7). Calculate the content of C₁₅H₁₃FO₂ taking 802 as the specific absorbance at 247 nm.

Folic Acid

Pteroylglutamic Acid

\[
\begin{array}{c}
\text{H}_2\text{N} \\
\text{N} \\
\text{N} \\
\text{O} \\
\text{COOH}
\end{array}
\]

\[\text{C}_{19}\text{H}_{19}\text{N}_7\text{O}_6\quad \text{Mol. Wt. 441.4}\]

Folic Acid is (2S)-[4-[(2-amino-4-hydroroxypetidin-6-yl) methylamino]benzamido]glutamic acid.

Folic acid contains not less than 95.0 per cent and not more than 102.0 per cent of C₁₉H₁₉N₇O₆, calculated on the anhydrous basis.

Description. A yellow to yellowish-orange, crystalline powder; odourless or almost odourless.

Identification
A. When examined in the range 230 nm to 380 nm (2.3.13), a 0.001 per cent w/v solution in 0.1 M sodium hydroxide shows three absorption maxima, at about 256 nm, 283 nm and 365 nm; absorbance at about 256 nm, about 0.59, at about 283 nm, about 0.575 and at about 365 nm, about 0.206.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 60 volumes of ethanol (95 per cent), 20 volumes of strong ammonia solution and 20 volumes of 1-propanol.

Test solution. Dissolve 50 mg of the substance under examination in 100 ml of a mixture of 9 volumes of methanol and 2 volumes of strong ammonia solution.

Reference solution. A 0.05 per cent w/v solution of folic acid RS in a mixture of 9 volumes of methanol and 2 volumes of strong ammonia solution.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests
Specific optical rotation (2.4.22). About +20°, determined in a 1.0 per cent w/v solution in 0.1 M sodium hydroxide.

Free amines. The absorbance (2.4.7) of the unreduced solution as determined in the Assay is not more than one-sixth of the absorbance of the reduced solution.

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Water (2.3.43). 5.0 to 8.5 per cent, determined on 0.15 g.

Assay. Weigh accurately about 50 mg, dissolve in 50 ml of 0.1 M sodium hydroxide and add sufficient 0.1 M sodium hydroxide to produce 100.0 ml (solution A). To 3.0 ml of solution A add 20 ml of 2 M hydrochloric acid and dilute to 100.0 ml with water. To 50 ml add 0.5 g of zinc powder; allow to stand protected from light for 20 minutes with frequent shaking and filter. Dilute 10.0 ml of the filtrate to 25 ml with water; add 5 ml of 2 M hydrochloric acid and 5 ml of a 0.1 per cent w/v solution of sodium nitrite, mix and allow to stand for 2 minutes. Add ml of a 0.5 per cent w/v solution of ammonium sulphamate, mix and allow to stand for 2 minutes. Add 5 ml of a 0.1 per cent w/v solution of N-(1-naphthyl)ethylenediamine dihydrochloride, mix and allow to stand for 10 minutes. Add sufficient water to produce 50.0 ml and measure the
absorbance of the resulting solution at about 550 nm (2.4.7), using as the blank a solution prepared in a similar manner but using 25 ml of water and beginning at the words “add 5 ml of 2 M hydrochloric acid...”. To a further 30.0 ml of solution A add 20 ml of 2 M hydrochloric acid and sufficient water to produce 100.0 ml. Mix 10.0 ml of this solution with 15 ml of water and carry out the operations described above beginning at the words “add 5 ml of 2 M hydrochloric acid...”. Subtract one-tenth of the absorbance of the unreduced solution from that of the reduced solution and from the result calculate the amount of C_{19}H_{19}N_{7}O_{6}, using the result obtained by repeating the operation using folic acid RS instead of the substance under examination.

Storage. Store protected from light.

Folic Acid Tablets

Folic Acid Tablets contain not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of folic acid, C_{19}H_{19}N_{7}O_{6}.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 60 volumes of ethanol (95 per cent), 20 volumes of strong ammonia solution and 20 volumes of 1-propanol.

Test solution. Extract a quantity of the powdered tablets containing 0.5 mg of Folic Acid with 1 ml of a mixture of 1 volume of strong ammonia solution and 9 volumes of methanol, centrifuge and use the supernatant liquid.

Reference solution. A 0.05 per cent w/v solution of folic acid RS in a mixture of 9 volumes of methanol and 2 volumes of strong ammonia solution.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Dissolve a quantity of the powdered tablets containing 5 mg of Folic Acid as completely as possible in 5 ml of 0.1 M sodium hydroxide and filter. To the filtrate add 45 ml of 0.5 M hydrochloric acid and 5 g of zinc powder and shake for 30 minutes. To 5 ml of the reduced solution, add 2 ml of a 0.1 per cent w/v solution of sodium nitrite, allow to stand for 2 minutes, add 2 ml of a 0.5 per cent w/v solution of ammonium sulphamate, mix, allow to stand for 3 minutes and add 2 ml of a 0.1 per cent w/v solution of N-(1-naphthyl)ethylenediamine dihydrochloride; a deep magenta colour is produced.

Tests

Hydrolysis products. Determine by liquid chromatography (2.4.7).

Protect the solutions from light.

Test solution. Shake a quantity of the powdered tablets containing 5.0 mg of Folic Acid with 50 ml of the mobile phase, centrifuge and use the supernatant liquid.

Reference solution. A solution containing 0.00005 per cent w/w of 4-aminobenzoic acid and 0.0002 per cent w/w of N-(4-aminobenzoyl)-L-glutamic acid in the mobile phase.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm) (such as Spherisorb ODS 1),
- mobile phase: 0.05 M potassium dihydrogen phosphate, adjusted to pH 5.5 with 5 M sodium hydroxide,
- flow rate. 2 ml per minute,
- spectrophotometer set at 269 nm,
- a 20 µl loop injector.

In the chromatogram obtained with the reference solution two peaks due to N-(4-aminobenzoyl)-L-glutamic acid and 4-aminobenzoic acid are obtained In the order of their emergence. The areas of the peaks due to 4-aminobenzoic acid and N-(4-aminobenzoyl)-L-glutamic acid in the chromatogram obtained with the reference solution are greater than the areas of any corresponding peaks in the chromatogram obtained with the test solution. The test is not valid unless the resolution between the two peaks in the chromatogram obtained with the reference solution is greater than 3.

Uniformity of content. Comply with the test stated under Tablets.

Test solution. Shake 1 tablet with 5.0 ml of 0.1 M sodium hydroxide, add sufficient mobile phase to produce a solution containing 0.001 per cent w/v of Folic Acid, centrifuge and use the supernatant liquid.

Reference solution. Add 1.0 ml of 0.5 M hydrochloric acid to 5.0 ml of a 0.002 per cent w/v solution of folic acid RS in 0.1 M sodium hydroxide and dilute to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm) (such as Spherisorb ODS 1),
- mobile phase: 0.05 M potassium dihydrogen phosphate, adjusted to pH 5.5 with 5 M sodium hydroxide,
- flow rate. 2 ml per minute,
- spectrophotometer set at 269 nm,
- a 20 µl loop injector.

The chromatographic procedure described under Assay may be carried out. Calculate the content of C_{19}H_{19}N_{7}O_{6} in the tablet.
**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing about 20 mg of Folic Acid with 50 ml of 0.1 M sodium hydroxide, dilute to 100.0 ml with the same solvent, centrifuge and dilute 5.0 ml of the supernatant liquid to 100.0 ml with the mobile phase.

**Reference solution.** Dilute 5.0 ml of a 0.02 per cent w/v solution of folic acid RS in 0.1 M sodium hydroxide to 100.0 ml with the mobile phase.

**Chromatographic system**
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilyl silica gel (10 µm),
- mobile phase: a mixture of 93 volumes of 0.05 M potassium dihydrogen phosphate and 7 volumes of acetonitrile adjusted to pH 6.0 with 5 M sodium hydroxide,
- flow rate. 2 ml per minute,
- spectrophotometer set at 283 nm,
- a 20 µl loop injector.

**NOTE -** For tablets containing 2 mg or less of Folic Acid the average of the 10 individual results obtained in the test for Uniformity of content may be taken in lieu of the Assay.

**Storage.** Store protected from light.

---

**Formoterol Fumarate Dihydrate**

![Chemical Structure](formoterol.png)

\((C_{19}H_{24}N_{2}O_{4})_{2}, C_{4}H_{4}O_{4}, 2H_{2}O\)  
Mol. Wt. 840.91

Formoterol Fumarate Dihydrate is dihydrate salt of fumaric acid with \((RS)\)-2'-hydroxy-5'-(\(RS\))-1-hydroxy-2-[[\(RS\)]-p-methoxy-\(\alpha\)-methyl[phenethyl][amino]ethyl]formanilide.

Formoterol Fumarate Dihydrate contains not less than 98.5 per cent and not more than 101.5 per cent of formoterol fumarate, \(C_{42}H_{52}N_{4}O_{12}\), calculated on the anhydrous basis.

**Description.** A white or almost white or slightly yellow powder.

**Identification**

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with formoterol fumarate dihydrate RS or with the reference spectrum of formoterol fumarate dihydrate.

**Tests**

**pH** (2.4.24). 5.5 to 6.5, determined in a 0.1 per cent w/v solution in carbon dioxide-free water.

**Optical rotation** (2.4.22). \(-0.10^\circ\) to \(+0.10^\circ\), determined in a 1.0 per cent w/v solution in methanol.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Solvent mixture.** 84 volumes of a buffer solution prepared by dissolving 6.10 g of sodium dihydrogen phosphate monohydrate and 1.03 g of disodium hydrogen phosphate dihydrate in 1000 ml water, and 16 volumes of acetonitrile.

**Test solution.** Dissolve 20 mg of the substance under examination in 100 ml of the solvent mixture.

**Reference solution.** A 0.00004 per cent w/v solution of formoterol fumarate RS in the solvent mixture.

**Chromatographic system**
- a stainless steel column 15 cm x 4.6 mm, packed with spherical octylsilyl silica gel (5 µm)
- mobile phase: A. acetonitrile,  
  B. a buffer solution prepared by dissolving 3.73 g of sodium dihydrogen phosphate monohydrate and 0.35 g of orthophosphoric acid in 1000 ml of water,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 214 nm,
- a 20 µl loop injector.

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>16</td>
<td>84</td>
</tr>
<tr>
<td>10</td>
<td>16</td>
<td>84</td>
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<tr>
<td>37</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>40</td>
<td>16</td>
<td>84</td>
</tr>
<tr>
<td>55</td>
<td>16</td>
<td>84</td>
</tr>
</tbody>
</table>

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 4000 theoretical plates.

Inject the test solution. Any individual impurity is not more than 0.5 per cent and the sum of all the impurities is not more than 1.0 per cent.

**Water** (2.3.43). Not more than 5.0 per cent, determined on 0.1 g.

**Assay.** Weigh accurately about 0.35 g and dissolve in 50 ml of anhydrous acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.
1 ml of 0.1 M perchloric acid is equivalent to 0.04024 g of C_{42}H_{52}N_{4}O_{12}.

Storage. Store protected from light and moisture.

Formoterol Fumarate and Budesonide Powder for Inhalation

Formoterol Fumarate and Budesonide Powder for Inhalation consists of Formoterol Fumarate and Budesonide in microfine powder either alone or admixed with Lactose in a pre-metered unit for use in a suitable powder inhaler.

Formoterol Fumarate and Budesonide Powder for Inhalation contains not less than 90.0 per cent and not more than 125.0 per cent of the stated amounts of formoterol fumarate C_{42}H_{52}N_{4}O_{12} and budesonide C_{25}H_{34}O_{6} per pre-metered unit.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with reference solution (c).

Tests

Other tests. Complies with the tests stated under the Inhalation Preparations (Powders for Inhalation).

Follow the procedure described under Assay with suitable dilution of the reference solution wherever the amount of active substance is to be determined in any test.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. To a suitable number of intact capsules add 10 ml of water and disperse with the aid of ultrasound till the shells get disintegrated. Add 60 ml of the mobile phase and mix further with the aid of ultrasound for 10 minutes with intermittent shaking. Add sufficient of the mobile phase to produce 100.0 ml. Dilute suitably with the mobile phase, if required, to get a final concentration of 0.6 µg per ml of Formoterol Fumarate in the mobile phase.

Reference solution (a). A 0.06 mg per ml solution of formoterol fumarate RS prepared by initially dissolving in 5 ml acetonitrile and then making up to volume with the mobile phase.

Reference solution (b). A 0.2 mg per ml solution of budesonide RS prepared by initially dissolving in 5 ml acetonitrile and then making up to volume with the mobile phase.

Reference solution (c). Dilute suitable volumes of reference solution (a) and reference solution (b) with the mobile phase to obtain a solution containing 0.6 µg of Formoterol Fumarate and 40 µg per ml of Budesonide per ml.

Framycetin Sulphate

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 65 volumes of a buffer solution prepared by dissolving 0.138 g sodium dihydrogen orthophosphate monohydrate and 0.122 g of decane sulphonic acid sodium salt in 100 ml of water and adjusting the pH to 3.0 with orthophosphoric acid, and 35 volumes of acetonitrile,
- flow rate. 2 ml per minute,
- spectrophotometer set at 220 nm,
- inject 200 µl.

Inject reference solution (c). The order of elution is formoterol fumarate, budesonide epimer B and epimer A. The test is not valid unless the column efficiency determined from the formoterol fumarate and both the epimer peaks of Budesonide is not less than 1800 and 4000 theoretical plates respectively, the resolution between budesonide epimer peaks is not less than 1.5 and the relative standard deviation for formoterol fumarate and sum of peaks of budesonide epimer A and epimer B in replicate injections is not more than 2.0 per cent. Inject the test solution and reference solution (c).

Calculate the contents of C_{42}H_{52}N_{4}O_{12} and C_{25}H_{34}O_{6} per unit.

Storage. Store protected from moisture, at temperature not exceeding 30º.

Labelling. The label states the quantities of active ingredients per pre-metered unit.

C_{21}H_{46}N_{6}O_{13}.3H_{2}SO_{4}  Mol. Wt. 908.9
Framycetin Sulphate is 2-deoxy-4-O-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-5-O-[3-O-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]streptamine (neomycin B) sulphate. The base is produced by the growth of selected strains of *Streptomyces fradiae* or *Streptomyces decaris* or by any other means.

Framycetin Sulphate contains an amount of framycetin sulphate equivalent to not less than 630 µg of neomycin B per mg, calculated on the dried basis.

**Description.** A white or yellowish-white powder; odourless or almost odourless; hygroscopic.

**Identification**

A. Determine by thin-layer chromatography (2.4.17), coating the plate in the following manner. Mix 0.3 g of *carbomer* with 240 ml of *water*, allow to stand for 1 hour with moderate shaking, adjust the pH to 7 by the gradual addition, with shaking, of 2 M sodium hydroxide and add 30 g of *silica gel* H. Spread a uniform layer of the suspension 0.75 mm thick, heat at 110° for 1 hour and allow to cool. Use the plate immediately.

**Mobile phase.** A 10 per cent w/v solution of potassium dihydrogen phosphate.

**Test solution.** A 0.1 per cent w/v solution of the substance under examination.

**Reference solution (a).** A 0.1 per cent w/v solution of framycetin sulphate *RS*.

**Reference solution (b).** A solution containing 0.1 per cent w/v each of framycetin sulphate *RS*, kanamycin sulphate *RS* and streptomycin sulphate *RS*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air, spray with a mixture of equal volumes of a 46 per cent w/v solution of sulphuric acid and of a 0.2 per cent w/v solution of 1,3-napthalenediol in *ethanol* (95 per cent) and heat at 150° for about 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

B. Dissolve 10 mg in 5 ml of *water*, add 0.1 ml of *pyridine* and 2 ml of a 0.1 per cent w/v solution of *ninhydrin* and heat in a water-bath at 65° to 70° for 10 minutes; an intense violet colour is produced.

C. A 5 per cent w/v solution gives the reactions of sulphates (2.3.1).

**Tests**

**pH** (2.4.24). 6.0 to 7.0, determined in a 1.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +52.0° to +55.5°, determined at 20° in a 10.0 per cent w/v solution.

**Neamine.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel* H.

**Mobile phase.** A mixture of 30 volumes of *methanol*, 20 volumes of *strong ammonia solution* and 10 volumes of dichloromethane.

**Test solution.** Dissolve 0.25 g of the substance under examination in *water* and dilute to 10 ml with *water*.

**Reference solution.** Dissolve 0.5 mg of *neamine RS* in 2 ml of *water*.

Apply to the plate as 5-mm bands 5 µl of each solution. Dry the bands and develop over a path of at least 8 cm. Dry the plate at 105° for 10 minutes. Spray it with *ninhydrin and stannous chloride reagent* and heat at 110° for 15 minutes. Spray the plate again with the same reagent and heat at 110° for 15 minutes. Any band corresponding to neamine in the chromatogram obtained with the test solution is not more intense than the band in the chromatogram obtained with the reference solution (1 per cent).

**Neomycin C.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel* of a suitable grade.

**Mobile phase.** A mixture of 80 volumes of a 20 per cent w/v solution of *sodium chloride* and 20 volumes of *methanol*.

**Test solution.** Dissolve 40 mg of the substance under examination in *water* and dilute to 5 ml with *water*.

**Reference solution (a).** Dissolve 40 mg of framycetin sulphate *RS* in *water* and dilute to 5 ml with *water*.

**Reference solution (b).** Dissolve 30 mg of framycetin sulphate *RS* in *water* and dilute to 25 ml with *water*. Dilute 5 ml of this solution to 25 ml with *water*.

**Reference solution (c).** Dissolve 40 mg of neomycin sulphate *RS* in *water* and dilute to 5 ml with *water*.

Apply to the plate as 5-mm bands 10 µl of each solution. Dry the bands and develop over a path of at least 12 cm. Dry the plate at 100° to 105° for 10 minutes. In the chromatogram obtained with the test solution, the principal band corresponds to the principal band in the chromatogram obtained with reference solution (a) and the band for neomycin C with Rf value slightly less than that of the principal band is not more intense than the band in the chromatogram obtained with reference solution (b) (3 per cent).

The test is not valid unless in the chromatogram obtained with reference solution (c) a band appears with Rf value slightly less than that of the principal band.

**Sulphate.** 27.0 to 31.0 per cent of SO₄, calculated on the dried basis, determined by the following method. Weigh accurately about 0.25 g, dissolve in 100 ml of *water*, adjust the pH to 11.
with strong ammonia solution and add 10.0 ml of 0.1 M barium chloride. Titrate with 0.1 M disodium edetate using 0.5 mg of metalphthalein as indicator; add 50 ml of ethanol (95 per cent) when the colour of the solution begins to change and continue the titration until the violet-blue colour disappears. 1 ml of 0.1 M barium chloride is equivalent to 0.009606 g of SO₄₂⁻.

**Alcohols.** Not more than 2 per cent w/w, calculated as methanol, CH₄O, determined by the following method. Dissolve 0.2 g in 5 ml of water and add 0.05 ml of 0.05 M sulphuric acid. Distil the mixture and collect about 2.5 ml of the distillate in a flask. Add 25.0 ml of a 0.0167 M potassium dichromate in a mixture of 30 volumes of water and 20 volumes of sulphuric acid. Heat on a water-bath for 30 minutes, cool and add sufficient water to produce 500.0 ml. Add 10 ml of potassium iodide solution, allow to stand for 5 minutes and titrate with 0.1 M sodium thiosulphate using starch solution, added towards the end of the titration, as indicator, until the solution becomes pale green. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of potassium dichromate equivalent to the alcohols present.

1 ml of 0.0167 M potassium dichromate is equivalent to 0.000534 g of CH₄O.

**Sulphated ash** (2.3.18). Not more than 1.0 per cent.

**Loss on drying** (2.4.19). Not more than 8.0 per cent, determined on 1.0 g by drying over phosphorus pentoxide at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

**Assay.** Determine by the microbiological assay of antibiotics, Method A (2.2.10), and express the results in μg of neomycin B per mg.

**Framycetin Sulphate intended for administration into internal body cavities without a further appropriate procedure for removal of bacterial endotoxins complies with the following additional requirement.**

**Bacterial endotoxins** (2.2.3). Not more than 1.3 Endotoxin Units per mg.

**Framycetin Sulphate intended for administration into internal body cavities without a further appropriate sterilisation procedure complies with the following additional requirement.**

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage.** Store protected from light and moisture at a temperature not exceeding 30°. If the material is sterile, the container should be tamper-evident and sealed so as to exclude micro-organisms.

**Labelling.** The label states (1) the strength in terms of μg of neomycin B per mg; (b) where applicable, that the material is free from bacterial endotoxins; (c) where applicable, that the material is sterile.

### Fructose

**d-Fructose**

```
\[
\text{C}_6\text{H}_{12}\text{O}_6 \quad \text{Mol. Wt. 180.2}
\]
```

Fructose is d(-)-fructopyranose.

**Description.** A white, crystalline powder with a very sweet taste.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with fructose RS or with the reference spectrum of fructose.

B. Dissolve 0.1 g in 10 ml of water, add 3 ml of potassium cupri-tartrate solution; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

C. Dissolve 5 g in water and dilute to 10 ml with the same solvent. To 0.5 ml of this solution add 0.2 g of resorcinol and 9 ml of dilute hydrochloric acid and heat on a water-bath for 2 minutes; a red colour is produced.

**Tests**

**Appearance of solution.** Dissolve 5.0 g in water and dilute to 10 ml with the same solvent. The solution is clear (2.4.1). Add 10 ml of water. The solution is colourless (2.4.1).

**Acidity or alkalinity.** Dissolve 6.0 g in 25 ml of carbon dioxide-free water and add 0.3 ml of phenolphthalein solution. The solution is colourless. Not more than 0.15 ml of 0.1 M sodium hydroxide is required to change the colour of the solution to pink.

**Specific optical rotation** (2.4.22). -91.0° to -93.5°, calculated on the anhydrous basis and determined on a solution prepared by dissolving 10.0 g in 80 ml of water, adding 0.2 ml of 5 M ammonia, mixing well, allowing to stand for 30 minutes and diluting to 100.0 ml with water.

**5-Hydroxymethylfurfural and related compounds.** To 5 ml of solution A add 5 ml of water and measure the absorbance (2.4.7) of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.32).
**Arsenic** (2.3.10). Dissolve 10.0 g in 50 ml of water and add 10 ml of stannated hydrochloric acid. The resulting solution complies with the limit test for arsenic (1 ppm).

**Heavy metals** (2.3.13). A solution of 4.0 g in 10 ml of water, 2 ml of dilute acetic acid and sufficient water to produce 25.0 ml complies with the limit test for heavy metals, Method A (5 ppm).

**Chlorides** (2.3.12). 20 ml of a 10 per cent w/v solution (solution A) complies with the limit test for chlorides (125 ppm).

**Sulphates** (2.3.17). 7.5 ml of solution A diluted to 15 ml with water complies with the limit test for sulphates (200 ppm).

**Foreign sugars.** Dissolve 5.0 g in water and dilute to 10 ml with the same solvent. To 1 ml of the solution add 9 ml of ethanol (95 per cent). Any opalescence in the solution is not more intense than that in a mixture of 1 ml of the initial solution and 9 ml of water.

**Barium.** To 10 ml of solution A add 1 ml of 1 M sulphuric acid. Examine exactly after 1 hour; any opalescence in the solution is not more intense than that in a mixture of 10 ml of solution A and 1 ml of water.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

Fructose intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

**Bacterial endotoxins** (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** To an accurately measured volume containing about 5.0 g of Fructose, add 0.2 ml of 5 M ammonia and sufficient water to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.5427 represents the weight, in g, of fructose, C6H12O6, in the volume taken for Assay.

**Storage.** Store in single dose containers at a temperature not exceeding 30°.

**Labelling.** The label states (1) the strength as the percentage w/v of fructose, C6H12O6; (2) that the injection should not be used if it contains visible particles.

**Fructose Injection**

Fructose Intravenous Infusion

Fructose Injection is a sterile solution of Fructose in Water for Injections.

Fructose Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of fructose, C6H12O6. It contains no antimicrobial agent.

**Description.** A clear, colourless solution.

**Identification**

A. The solution prepared as directed in the Assay is laevo-rotatory.

B. To 1 ml add 0.05 ml of potassium cupri-tartrate solution; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

**Tests**

**pH** (2.4.24). 3.0 to 6.0, determined in a solution diluted, if necessary, with water for injections to contain not more than 5.0 per cent w/v of Fructose and to which 0.3 ml of a saturated solution of potassium chloride has been added for each 100 ml of solution.

**5-Hydroxymethylfurfural and Related substances.** Dilute a volume containing 1.0 g of Fructose to 500.0 ml with water and measure the absorbance (2.4.7) of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.50.

**Heavy metals** (2.3.13). A volume of the injection containing 4.0 g of fructose that has been evaporated to a volume of about 10 ml, cooled and diluted to 25 ml with water complies with the limit test for heavy metals, Method A (5 ppm).

**Bacterial endotoxins** (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

**Frusemide**

Furosemide

\[
\text{C}_{12}\text{H}_{11}\text{ClN}_{2}\text{O}_{5}\text{S} \quad \text{Mol. Wt. 330.7}
\]

Frusemide is 4-chloro-N-furfuryl-5-sulphamoylanthranilic acid.
Frusemide contains not less than 98.5 per cent and not more than 101.0 per cent of C\textsubscript{12}H\textsubscript{11}ClN\textsubscript{2}O\textsubscript{5}S, calculated on the dried basis.

**Description.** A white or almost white, crystalline powder.

**Identification**

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with frusemide RS or with the reference spectrum of frusemide.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.0005 per cent w/v solution in 0.1 M sodium hydroxide shows three absorption maxima at about 228 nm, 271 nm and 333 nm. The ratio of the absorbance at the maximum at about 271 nm to that at the maximum at about 228 nm is 0.52 to 0.57.

C. Dissolve about 5 mg in 10 ml of methanol. Transfer 1 ml of this solution to a flask, add 10 ml of dilute hydrochloric acid and boil under a reflux condenser on a water-bath for 15 minutes. Cool, add 15 ml of 1 M sodium hydroxide and 5 ml of a 0.1 per cent w/v solution of sodium nitrite. Allow to stand for 3 minutes, add 5 ml of a 0.5 per cent w/v solution of ammonium sulphamate, mix and add 5 ml of a 0.1 per cent w/v solution of N-(1-naphthyl)ethylenediamine dihydrochloride; a red-violet colour is produced.

**Tests**

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Chlorides** (2.3.12). Shake 1 g with 40 ml of water for 5 minutes and filter. The filtrate complies with the limit test for chlorides (250 ppm).

**Sulphates** (2.3.17). Shake 1.0 g with a mixture of 30 ml of distilled water and 0.2 ml of 5 M acetic acid for 5 minutes and filter. 15 ml of the filtrate complies with the limit test for sulphates (300 ppm).

**Free amines.** Dissolve 0.1 g in 25 ml of methanol. To 1 ml of the solution add 3 ml of dimethylformamide, 12 ml of water and 1 ml of 1 M hydrochloric acid. Cool, add 1 ml of a 0.5 per cent w/v solution of sodium nitrite, shake and allow to stand for 5 minutes. Add 1 ml of a 2.5 per cent w/v solution of sulphamic acid, shake and allow to stand for 3 minutes. Add 1 ml of a 0.5 per cent w/v solution of N-(1-naphthyl)ethylenediamine dihydrochloride and dilute to 25 ml with water. Measure the absorbance of the resulting solution at the maximum at about 530 nm (2.4.7), using as the blank a solution obtained by treating 1 ml of methanol and 3 ml of dimethylformamide in a similar manner; absorbance is not more than 0.12.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105\(^\circ\)C.

**Assay.** Weigh accurately about 0.5 g, dissolve in 40 ml of dimethylformamide and titrate with 0.1 M sodium hydroxide using bromothymol blue solution as indicator. Carry out a blank titration.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.03307 g of C\textsubscript{12}H\textsubscript{11}ClN\textsubscript{2}O\textsubscript{5}S.

**Storage.** Store protected from light.

**Frusemide Injection**

Frusemide Injection is a sterile solution of Frusemide in Water for Injections prepared with the aid of Sodium Hydroxide.

Frusemide Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of frusemide, C\textsubscript{12}H\textsubscript{11}ClN\textsubscript{2}O\textsubscript{5}S.

**Description.** A clear, colourless or almost colourless solution.

**Identification**

A. When examined in the range 220 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows three absorption maxima at about 228 nm, 271 nm and 333 nm.

B. To a volume of the injection containing 5 mg of Frusemide add 10 ml of dilute hydrochloric acid and boil under a reflux condenser on a water-bath for 15 minutes. Cool, add 15 ml of 1 M sodium hydroxide and 5 ml of a 0.1 per cent w/v solution of sodium nitrite. Allow to stand for 3 minutes, add 5 ml of a 0.5 per cent w/v solution of ammonium sulphamate, mix and add 5 ml of a 0.1 per cent w/v solution of N-(1-naphthyl)ethylenediamine dihydrochloride; a red-violet colour is produced.

**Tests**

**pH** (2.4.24). 8.0 to 9.3.

**Free amines.** To a volume of the injection containing 0.1 g of Frusemide add sufficient methanol to produce 25 ml, shake and filter. To 1 ml of the filtrate add 3 ml of dimethylformamide, 12 ml of water and 1 ml of 1 M hydrochloric acid. Cool, add 1 ml of a 0.5 per cent w/v solution of sodium nitrite, shake and allow to stand for 5 minutes. Add 1 ml of a 2.5 per cent w/v solution of sulphamic acid, shake and allow to stand for 3 minutes. Add 1 ml of a 0.5 per cent w/v solution of N-(1-naphthyl)ethylenediamine dihydrochloride and dilute to 25 ml with water. Measure the absorbance of the resulting solution at the maximum at about 530 nm (2.4.7), using as the blank a
solution obtained by treating 1 ml of methanol and 3 ml of dimethylformamide in a similar manner; absorbance is not more than 0.20.

**Bacterial endotoxins** (2.2.3). Not more than 3.5 Endotoxin Units per mg of frusemide.

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** Dilute an accurately measured volume of the injection containing about 20 mg of Frusemide with water to produce 100.0 ml. Dilute 5.0 ml to 100.0 ml with 0.1 M sodium hydroxide. Measure the absorbance of the resulting solution at the maximum at about 271 nm (2.4.7). Calculate the content of C12H11ClN2O5S taking 580 as the specific absorbance at 271 nm.

**Storage.** Store protected from light.

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**Frusemide Tablets**

Frusemide Tablets

Frusemide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of frusemide, C12H11ClN2O5S.

**Identification**

A. When examined in the range 220 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows three absorption maxima at about 228 nm, 271 nm and 333 nm.

B. Shake a quantity of the powdered tablets containing 80 mg of Frusemide with 10 ml of ethanol (95 per cent), filter and evaporate the filtrate to dryness. Dissolve 25 mg of the residue obtained in 2.5 ml of ethanol (95 per cent) and add 2 ml of 4-dimethylaminobenzaldehyde solution; a green colour is produced which changes to deep red.

**Tests**

**Free amines.** To a quantity of the powdered tablets containing 0.1 g of Frusemide add 25 ml of methanol, shake and filter. To 1 ml of the filtrate add 3 ml of dimethylformamide, 12 ml of water and 1 ml of 1 M hydrochloric acid. Cool, add 1 ml of a 0.5 per cent w/v solution of sodium nitrite, shake and allow to stand for 3 minutes. Add 1 ml of a 2.5 per cent w/v solution of sulphamic acid, shake and allow to stand for 3 minutes. Add 1 ml of a 0.5 per cent w/v solution of N-(1-naphthyl) ethylenediamine dihydrochloride and dilute to 25 ml with water. Measure the absorbance of the resulting solution at the maximum at about 530 nm (2.4.7), using as the blank a solution obtained by treating 1 ml of methanol and 3 ml of dimethylformamide in a similar manner; the absorbance is not more than 0.20.

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**Furazolidone**

C6H5N3O5 \( \text{Mol. Wt. 225.2} \)

Furazolidone is 3-(5-nitrofurfurylideneamino)oxazolidin-2-one

Furazolidone contains not less than 97.0 per cent and not more than 103.0 per cent of C6H5N3O5, calculated on the dried basis.

**Description.** A yellow, crystalline powder; odourless or almost odourless.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with furazolidone RS or with the reference spectrum of furazolidone.

B. Dissolve 1 mg in 1 ml of dimethylformamide and add 0.05 ml of 1 M ethanolic potassium hydroxide; a deep blue colour is produced.

**Tests**

**pH** (2.4.24). 4.5 to 7.0, determined in a solution prepared by shaking 1.0 g for 15 minutes with 100 ml of carbon dioxide-free water and filtering.

**Nitrofurfural diacetate.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 95 volumes of toluene and 5 volumes of dioxan.

**Test solution.** Dissolve 50 mg of the substance under examination in 5 ml of dimethylformamide by heating on a
water-bath for a few minutes, allow to cool and dilute to 10 ml with acetone.

Reference solution. A solution containing 0.01 per cent w/v of nitrofurfural diacetate RS in a mixture of equal volumes of dimethylformamide and acetone.

Apply to the plate 20 µl of the test solution and 10 µl of the reference solution. After development, dry the plate in air and heat it at 105° for 5 minutes. Spray with a solution prepared by dissolving 0.75 g of phenylhydrazine hydrochloride in 10 ml of ethanol (95 per cent), diluting to 50 ml with water, adding activated charcoal, filtering and then adding 25 ml of hydrochloric acid and sufficient water to produce 200 ml. Any spot corresponding to nitrofurfural diacetate in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Carry out the following procedure protected from light.

Weigh accurately about 80 mg, add 150 ml of dimethylformamide, swirl to dissolve and add sufficient water to produce 500.0 ml. Dilute 5.0 ml to 100.0 ml with water and mix. Measure the absorbance of the resulting solution at the maximum at about 367 nm (2.4.7). Calculate the content of C₈H₇N₃O₅ taking 750 as the specific absorbance at 367 nm.

Storage. Store protected from light.

**Furazolidone Oral Suspension**

Furazolidone Oral Suspension is a suspension of Furazolidone in a suitable aqueous flavoured vehicle.

Furazolidone Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of furazolidone, C₈H₇N₃O₅.

Identification

Add a quantity of the suspension containing 50 mg of Furazolidone to 10 ml of a freshly prepared mixture of 9 volumes of dimethylformamide and 1 volume of 1 M ethanolic potassium hydroxide. The solution turns purple, immediately changes to deep blue and on standing for about 10 minutes, again turns purple.

Tests

pH (2.4.24). 6.0 to 8.5.

Other tests. Complies with the tests stated under Oral Liquids.

Assay. Carry out the following procedure protected from light.

To an accurately measured volume of the suspension containing about 50 mg of Furazolidone add 5 ml of water and mix. Transfer this mixture to a 250-ml volumetric flask with the aid of dimethylformamide. Add about 150 ml of dimethylformamide, shake by mechanical means for 10 minutes, dilute to volume with dimethylformamide and mix. Dilute 5.0 ml of this solution to 100.0 ml with water and mix well. Measure the absorbance of the resulting solution at the maximum at about 367 nm (2.4.7), using dimethylformamide solution (5 per cent v/v) as the blank. Calculate the content of C₈H₇N₃O₅ taking 750 as the specific absorbance at 367 nm.

Storage. Store protected from light at a temperature not exceeding 30°.

**Furazolidone Tablets**

Furazolidone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of furazolidone, C₈H₇N₃O₅.

Identification

To a quantity of the powdered tablets containing 50 mg of Furazolidone add 10 ml of a freshly prepared mixture of 9 volumes of dimethylformamide and 1 volume of 1 M ethanolic potassium hydroxide. The solution turns purple, immediately changes to deep blue and on standing for 10 minutes, again turns purple.

Tests

Other tests. Comply with the tests stated under Tablets.

Assay. Carry out the following procedure protected from light.

Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 80 mg of Furazolidone into a 200-ml volumetric flask, add 100 ml of dimethylformamide, warm to about 50° and shake well. Cool, dilute to volume with dimethylformamide, mix and centrifuge a small quantity of the mixture. Dilute 5.0 ml of the clear, supernatant liquid to 250.0 ml with water and mix. Measure the absorbance of the resulting solution at the maximum at about 367 nm (2.4.7), using dimethylformamide diluted 50 times with water as the blank. Calculate the content of C₈H₇N₃O₅ taking 750 as the specific absorbance at 367 nm.

Storage. Store protected from light at a temperature not exceeding 30°.
Fusidic Acid

Fusidic Acid is ent-16α-acetoxy-3β,11β-dihydroxy-4β,8β,14β-trimethyl-18-nor-5β,10α-cholesta(17Z)-17(20),24-dien-21-oic acid hemihydrate, an antimicrobial substance produced by the growth of certain strains of Fusidium coccineum or by any other means.

Fusidic Acid contains not less than 97.5 per cent and not more than 101.0 per cent of C_{31}H_{48}O_{6}, calculated on the anhydrous basis.

Description. A white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with fusidic acid RS or with the reference spectrum of fusidic acid.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 80 volumes of chloroform, 10 volumes of glacial acetic acid, 10 volumes of cyclohexane and 2.5 volumes of methanol.

Test solution. Dissolve 0.2 g of the substance under examination in 100 ml of ethanol (95 per cent).

Reference solution. A 0.24 per cent w/v solution of diethanolamine fusidate RS in ethanol (95 per cent).

Apply to the plate 5 µl of each solution. After development, dry the plate at 105°C for 10 minutes and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.17).

Test solution. Dissolve 0.5 g of the substance under examination in 100 ml of the mobile phase.

Reference solution (a). Dissolve 5 mg of 3-ketofusidic acid RS in 5 ml of the mobile phase. To 1 ml of this solution add 0.2 ml of the test solution and dilute to 20 ml with the mobile phase.

Reference solution (b). Dilute 2.0 ml of the test solution to 10 ml with the mobile phase.

Chromatographic system

- a stainless steel column 12.5 cm x 4 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 50 volumes of acetonitrile, 20 volumes of water, 20 volumes of a 1 per cent w/v solution of phosphoric acid and 10 volumes of methanol,
- flow rate. 2 ml per minute,
- spectrophotometer set at 235 nm,
- a 20 µl loop injector.

Continue the chromatography for at least 3.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution the sum of the areas of any secondary peaks is not greater than 4 times the area of the peak corresponding to fusidic acid in the chromatogram obtained with reference solution (a). Ignore any peak with an area less than that of the principal peak in the chromatogram obtained with reference solution (b). The test is not valid unless the resolution factor between the peaks corresponding to 3-ketofusidic acid and fusidic acid in the chromatogram obtained with reference solution (a) is not less than 2.5 and unless the principal peak in the chromatogram obtained with reference solution (b) has a signal-to-noise ratio of not less than 3.

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). 1.4 to 2.0 per cent, determined on 0.5 g.

Assay. Weigh accurately about 0.5 g and dissolve in 10 ml of ethanol (95 per cent). Titrate with 0.1 M sodium hydroxide using phenolphthalein solution as indicator.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.05167 g of C_{31}H_{48}O_{6}.

Storage. Store protected from light.

Fusidic Acid Oral Suspension

Fusidic Acid Mixture

Fusidic Acid Oral Suspension is a suspension of Fusidic Acid in powder of suitable fineness in a suitable flavoured vehicle. It should not be diluted.

Fusidic Acid Oral Suspension contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of anhydrous fusidic acid, C_{31}H_{48}O_{6}.
Identification

A. To a quantity of the oral suspension containing 0.1 g of anhydrous fusidic acid add 5 ml of water and extract with three quantities, each of 10 ml, of chloroform. Wash the combined extracts with two quantities, each of 10 ml, of water, dry with anhydrous sodium sulphate, evaporate to dryness and dissolve the residue in 1 ml of chloroform IR. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum obtained with that obtained with fusidic acid RS or with the reference spectrum of fusidic acid.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to fusidic acid in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 4.8 to 5.2.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 160 volumes of chloroform, 40 volumes of cyclohexane, 40 volumes of glacial acetic acid and 5 volumes of methanol.

Test solution. Extract a quantity of the oral suspension containing 0.1 g of anhydrous fusidic acid with two quantities, each of 20 ml, of chloroform, evaporate the combined extracts to dryness and dissolve the residue in 10 ml of ethanol (95 per cent).

Reference solution (a). A solution containing 0.04 per cent w/v of diethanolamine fusidate RS in ethanol (95 per cent).

Reference solution (b). A solution containing 0.04 per cent w/v of 3-ketofusidic acid RS in ethanol (95 per cent).

Apply to the plate 5 µl of each solution. After development, dry the plate at 110° for 10 minutes, spray with ethanolic sulphuric acid (10 per cent v/v), dry at 110° for 10 minutes and examine in ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the principal spot in the chromatogram obtained with reference solution (a). Any yellow spot in the chromatogram obtained with the test solution is not more intense than the principal spot in the chromatogram obtained with reference solution (b).

Other tests. Complies with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Shake thoroughly an accurately weighed quantity of the oral suspension containing about 30 mg of anhydrous fusidic acid with 25 ml of the mobile phase, dilute to 50.0 ml with the mobile phase, filter and use the clear filtrate.

Reference solution. A solution containing 0.07 per cent w/v of diethanolamine fusidate RS in the mobile phase.

Chromatographic system

– a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm) (such as LiChrosorb RP-18),
– mobile phase: a mixture of 60 volumes of acetonitrile, 30 volumes of a 1 per cent v/v solution of glacial acetic acid and 10 volumes of methanol,
– flow rate. 1.5 ml per minute,
– spectrophotometer set at 235 nm,
– a 20 µl loop injector.

The assay is not valid unless the column efficiency, determined using the principal peak in the chromatogram obtained with the reference solution, is not less than 14,000 theoretical plates per metre.

Determine the weight per ml of the oral suspension (2.4.29), and calculate the content of C₃₁H₄₈O₆, weight in volume.

Storage. Store protected from light at a temperature not exceeding 30°.

Labelling. The label states the quantity of active ingredient in terms of the equivalent amount of anhydrous fusidic acid.
G

Gallamine Triethiodide ....
Gallamine Injection ....
Gatifloxacin ....
Gatifloxacin Infusion ....
Gatifloxacin Tablets ....
Gelatin ....
Hard Gelatin Capsule Shells ....
Gentamicin Sulphate ....
Gentamicin Eye Drops ....
Gentamicin Injection ....
Glibenclamide ....
Glibenclamide Tablets ....
Glipizide ....
Glipizide Tablets ....
2-Deoxy-D-Glucose ....
Glycerin ....
Glyceryl Monostearate ....
Concentrated Glyceryl Trinitrate Solution ....
Glyceryl Trinitrate Tablets ....
Glycine ....
Glycine Irrigation Solution ....
Griseofulvin ....
Griseofulvin Tablets ....
Guaiphenesin ....
Gallamine Triethiodide

\[
\begin{align*}
\text{C}_{90}\text{H}_{60}\text{I}_3\text{N}_3\text{O}_3 & \quad \text{Mol. Wt. 891.5} \\
\text{Gallamine Triethiodide is 2,2',2''-(benzene-1,2,3-triyltrioxy)tris(tetraethylammonium) triiodide.} & \\
\text{Gallamine Triethiodide contains not less than 98.0 per cent and not more than 101.0 per cent of C}_{90}\text{H}_{60}\text{I}_3\text{N}_3\text{O}_3, \text{calculated on the dried basis.} & \\
\text{Description. A white or almost white powder; odourless or almost odourless; hygroscopic.} & \\
\end{align*}
\]

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with gallamine triethiodide RS.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in 0.01 M hydrochloric acid shows an absorption maximum at about 225 nm; absorbance at about 225 nm, 0.50 to 0.55.

C. To 5 ml of a 1 per cent w/v solution add 1 ml of potassium mercuri-iodide solution; a yellow precipitate is produced.

D. Acidify 2 ml of a 0.5 per cent w/v solution with 0.2 ml of 2 M nitric acid; the resulting solution gives reaction A of iodides (2.3.1).

Tests

Appearance of solution. A 2.0 per cent w/v solution is clear (2.4.1), and, when examined immediately after preparation, not more intensely coloured than reference solution YS7 (2.4.1).

Acidity or alkalinity. To 50 ml of water add 0.2 ml of methyl red solution and adjust the pH to 6 by adding either 0.01 M sulphuric acid or 0.02 M sodium hydroxide until the colour is orange-yellow. Add 1 g of the substance under examination and shake to dissolve. Not more than 0.2 ml of 0.01 M sulphuric acid or 0.02 M sodium hydroxide is required to restore the orange-yellow colour.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with microcrystalline cellulose.

Mobile phase. A mixture of 66 volumes of 1-butanol, 17 volumes of glacial acetic acid and 17 volumes of water.

Test solution. Dissolve 0.5 g of the substance under examination in 100 ml of ethanol (95 per cent).

Reference solution. Dilute 1 volume of the test solution to 100 volumes with ethanol (95 per cent).

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air and spray with iodoplatinate reagent. In the chromatogram obtained with the test solution an elongated blue spot, which may appear to be double, is produced. Any secondary spot in the chromatogram obtained with the test solution of higher Rf value than the principal spot is not more intense than the principal spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.27 g and dissolve in a mixture of 40 ml of acetone and 15 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration. 1 ml of 0.1 M perchloric acid is equivalent to 0.02972 g of C90H60I3N3O3.

Storage. Store protected from light.

Gallamine Injection

Gallamine Triethiodide Injection

Gallamine Injection is a sterile solution of Gallamine Triethiodide in Water for Injections.

Gallamine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of gallamine triethiodide, C90H60I3N3O3.

Description. A clear, colourless or almost colourless solution.

Identification

A. When examined in the range 220 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum only at about 225 nm.

B. To 1 ml add 1 ml of iodinated potassium iodide solution; a brown precipitate is produced.

C. To 1 ml add 1 ml of potassium mercuri-iodide solution; a yellow precipitate is produced.

Tests

pH (2.4.24). 5.5 to 7.5.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).
**Gatifloxacin**

C_{19}H_{22}FN_{3}O_{4} \hspace{1cm} \text{Wt. 402.4}

Gatifloxacin is (RS)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-3-quinolinecarboxylic acid sesquihydrate

Gatifloxacin contains not less than 98.0 per cent and not more than 102.0 per cent of C_{19}H_{22}FN_{3}O_{4}, calculated on the anhydrous basis.

**Description.** A white to light yellow crystalline powder.

**Identification**

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with gatifloxacin RS.

**Tests**

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 30 mg of the substance under examination in 100 ml of the mobile phase.

**Reference solution (a).** A 0.03 per cent w/v solution of gatifloxacin RS in the mobile phase.

**Reference solution (b).** Dilute 1 ml of reference solution (a) to 100 ml with mobile phase.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm packed with octadecysilane bonded to porous silica (5µm),
- column temperature 50º,
- mobile phase: a mixture of 85 volumes of buffer solution prepared by dissolving 4.1 g of sodium acetate anhydrous in 1000 ml with water; add 4 ml triethylamine. Adjust the pH to 4.0 with acetic acid and 15 volumes of acetonitrile,
- flow rate. 1 ml per minute,
- spectrophotometer set at 295 nm,
- a 20 µl loop injector.

Inject reference solution (a). Test is not valid unless the column efficiency is not less than 4000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Water** (2.3.43). 4.0 to 8.0 per cent, determined on 0.1 g.

**Assay.** Weigh accurately about 0.3 g, dissolve in 20 ml of N,N’-dimethylformamide, add 50 ml of methanol. Titrate with 0.1 M hydrochloric acid. Determine the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml 0.1 M hydrochloric acid is equivalent of 0.03754 g of C_{19}H_{22}FN_{3}O_{4}1.5H_{2}O.

**Storage.** Store protected from light.

**Gatifloxacin Infusion**

Gatifloxacin Infusion contains Gatifloxacin.

Gatifloxacin Infusion contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of gatifloxacin C_{19}H_{22}FN_{3}O_{4}.

**Identification**

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

**Tests**

**pH** (2.4.24). 3.5 to 5.5.

**Related substances.** Determine by liquid chromatography (2.4.14).
NOTE — Protect the solutions from light.

Test solution. Dilute the Infusion to obtain 0.1 per cent w/v solution in mobile phase.

Reference solution (a). A 0.1 per cent w/v solution of gatifloxacin RS in the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of buffer solution prepared by dissolving 1.6 ml of orthophosphoric acid in 1000 ml of water. Adjust the pH 3.0 with triethylamine,
- flow rate. 1 ml per minute,
- spectrophotometer set at 240 nm,
- a 20 µl loop injector.

Inject reference solution (a). Test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

Other tests. Comply with the tests stated under Infusion.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Test solution. Dilute the Infusion to obtain 0.1 per cent w/v solution in mobile phase.

Reference solution. A 0.1 per cent w/v solution of gatifloxacin RS in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of buffer solution prepared by dissolving 1.6 ml of orthophosphoric acid in 1000 ml of water. Adjust to pH 3.0 with triethylamine, and 20 volumes of acetonitrile,
- flow rate. 1 ml per minute,
- spectrophotometer set at 240 nm,
- a 50 µl loop injector.

D. Not less than 70 per cent of the stated amount of C_{19}H_{22}FN_{3}O_{4}.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Test solution. Dilute the Infusion to obtain 0.1 per cent w/v solution in mobile phase.

Reference solution (a). A 0.1 per cent w/v solution of gatifloxacin RS in the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with mobile phase.

Inject the test solution and the reference solution. Calculate the content of C_{19}H_{22}FN_{3}O_{4}.

Storage. Store protected from light and moisture.

Gatifloxacin Tablets

Gatifloxacin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of gatifloxacin, C_{19}H_{22}FN_{3}O_{4}.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus. No 1

Medium. 900 ml of acetate buffer pH 4.0.

Speed and time. 50 rpm for 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. The filtrate obtained as given above.

Reference solution. A 0.1 per cent w/v solution of gatifloxacin RS in dissolution medium.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of 1.6 per cent v/v solution of phosphoric acid adjusted to pH 3.0 with triethylamine and 20 volumes of acetonitrile,
- flow rate. 1 ml per minute,
- spectrophotometer set at 240 nm,
- a 50 µl loop injector.

D. Not less than 70 per cent of the stated amount of C_{19}H_{22}FN_{3}O_{4}.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Test solution. Dilute the Infusion to obtain 0.1 per cent w/v solution in mobile phase.

Reference solution (a). A 0.1 per cent w/v solution of gatifloxacin RS in the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with mobile phase.
Chromatographic system as described under Assay.

Inject reference solution (a). Test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 3 times the area of the peak in the chromatogram obtained with the reference solution (b) (3.0 per cent).

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 100 mg of Gatifloxacin, dissolve in 50 ml of 0.1 per cent phosphoric acid solution and dilute to 100 ml with same solvent and filter. Dilute 5 ml of the solution to 10 ml with mobile phase.

**Reference solution.** A 0.05 per cent of gatifloxacin RS in mobile phase.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 μm),
- mobile phase: a mixture of 80 volumes of 1.6 per cent phosphoric acid solution and 20 volumes of acetonitrile,
- flow rate. 1 ml per minute,
- spectrophotometer set at 240 nm,
- a 50 μl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 3.0. The column efficiency is not less than 2500 theoretical plates. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of C₁₉H₂₂FN₃O₄.

**Storage.** Store protected from light.

**Gelatin**

Gelatin is a purified protein obtained by partial hydrolysis of animal collagen. Gelatin used in the manufacture of capsule shells or as a pharmaceutical aid in the manufacture of tablets may contain suitable antimicrobial agents.

**Description.** Light amber to faintly yellow, translucent flakes, sheets, shreds, powder or granules; odour, slight. Stable in air but is subject to microbial decomposition when moist or in solution.

**Identification**

A. Dissolve 1 g in sufficient carbon dioxide-free water at about 55° to produce 100 ml and maintain the solution at this temperature until required for use (solution A). To 2 ml of solution A add 0.05 ml of copper sulphate solution, mix and add 0.5 ml of 2 M sodium hydroxide; a violet colour is produced.

B. Add 10 ml of water to 0.5 g in a test-tube, allow to stand for 10 minutes, heat at 60° for 15 minutes, allow to stand upright at 0° for 6 hours and invert the test-tube; the contents do not immediately flow out.

**Tests**

**Appearance of solution.** Solution A is not more opalescent than opalescence standard OS4 (2.4.1), and not more intensely coloured than reference solution YS4 (2.4.1).

**pH (2.4.24).** 3.8 to 7.6, determined in solution A.

**Arsenic (2.3.10).** To 5.0 g add 10 ml of water and allow to stand for 1 hour. Warm to dissolve and add 10 ml of hydrochloric acid and a slight excess of bromine solution. Add 2 ml of stannated hydrochloric acid, heat under a reflux condenser for 1 hour, cool and add 10 ml of water and 10 ml of hydrochloric acid. The resulting solution complies with the limit test for arsenic (2 ppm).

**Heavy metals (2.3.13).** To the residue obtained in the test for Ash add 2 ml of hydrochloric acid and 0.5 ml of nitric acid, and evaporate to dryness. To the residue add 1 ml of 1 M hydrochloric acid and 15 ml of water and warm for a few minutes. Filter, wash with water and dilute the filtrate to 100 ml with water. Dilute 8 ml of this solution to 25 ml with water. The resulting solution complies with the limit test for heavy metals, Method A (50 ppm).

**Sulphur dioxide.** Not more than 200 ppm, determined by the following method. Add 150 ml of water to a 500-ml three-necked, round-bottomed flask fitted with a water-cooled reflux condenser 200 mm long the upper end of which is connected to an absorption tube. The flask is fitted with a 100-ml dropping funnel and a gas inlet tube that reaches nearly to the bottom of the flask. Pass a stream of carbon dioxide through the flask at a rate of 100 ml per minute for 15 minutes. Connect an absorption tube containing 10 ml of hydrogen peroxide solution (10 vol) previously neutralised to a 0.1 per cent w/v solution of bromophenol blue in ethanol (20 per cent) and without interrupting the flow of carbon dioxide, introduce through the funnel 25 g of the substance under examination and 80 ml of 2 M hydrochloric acid. Boil for 1 hour, disconnect the absorption tube and stop the flow of carbon dioxide. Wash the contents of the absorption tube into a 250-ml conical flask, heat on a water-bath for 15 minutes and allow to cool. Titrate with 0.1 M sodium hydroxide using a 0.1 per cent w/v solution
of bromophenol blue in ethanol (20 per cent) as indicator, until the colour changes from yellow to violet-blue.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.003203 g of sulphur dioxide.

**Microbial contamination** (2.2.9). Total microbial count, not more than 1000 per g; 1 g is free from *Escherichia coli*; 10 g is free from *Salmonellae*.

**Ash.** Not more than 3.25 per cent, determined by the following method. Weigh accurately 5.0 g, add about 2 g of liquid paraffin (to avoid loss due to swelling) and incinerate at a temperature not exceeding 500° until free from carbon. Cool and weigh.

**Loss on drying** (2.4.19). Not more than 16.0 per cent, determined by the following method. Weigh accurately about 1.0 g in a stainless steel dish weighing about 25 g and with a diameter of 70 mm and a height of 15 mm, fitted with a cover. Add 10 ml of water and allow to soak. Heat on a water-bath to form a homogenous solution and continue heating until most of the water has evaporated. Dry for 2 hours at 105° and for further periods of 30 minutes until two successive weighings do not differ by more than 1 mg (Do not powder sheet gelatin while preparing for this test).

Gelatin intended for use in the preparation of pessaries and suppositories complies with the following additional requirement.

**Jelly strength** (2.4.18). Between 150 and 250 g. (The exact jelly strength may be negotiated between the manufacturer and user on the basis of end use of the gelatin).

**Storage.** Store protected from moisture.

**Labelling.** The label states, where applicable, that the material is suitable for the preparation of pessaries and suppositories and, if so, the jelly strength.

**Hard Gelatin Capsule Shells**

Hard Gelatin Capsule Shells are soluble containers for incorporation of drugs, usually in the form of powders, pellets or granules, and are commonly intended for oral administration. The shells are acted upon by digestive fluids and the filled contents are released. They are composed of gelatin, water and additives such as plasticizers, humectants, surfactants, dispersing agents, flavouring agents, antimicrobial agents, sweetening agents, opacifying agents and one or more colouring agents permitted under the Drugs and Cosmetics Rules, 1945. Ingredients other than colouring agents and opacifying agents comply with the standards of this Pharmacopoeia.

**Description.** Hard Gelatin Capsule Shells (shells or cases) consist of two cylindrical, telescoping pieces (cap and body), one end of which is rounded and closed and the other, open. Shapes other than cylindrical can also be formed as per requirements. The two pieces are uncoloured or coloured; if coloured, of identical or different colours; transparent or opaque, partially or completely and printed or unprinted or bear other surface markings. The cap overlaps the body and maintains a tight friction closure. The closure may be strengthened by suitable means.

The shells are of various sizes, usually designated by different numbers, 5 being the smallest and 000 the biggest. Shells of sizes 0 to 4 are commonly used. Shells of special shapes, sizes, lengths and designations are also available. The shells are smooth and uniform in size, shape and colour. Guidelines on dimensions in respect of different sizes of commonly used capsules are given in chapter 5.8.

**Identification**

Boil one capsule shell with 20 ml of water, allow to cool and centrifuge. To 5 ml of the supernatant liquid add 1 ml of picric acid solution and to another 5 ml add 1 ml of tannic acid solution; a precipitate is produced in each case.

**Tests**

**Odour.** Keep 100 capsule shells in a well-closed bottle for 24 hours at a temperature between 30° and 40°; the shells do not develop any foreign odour.

*NOTE - In order to ensure that the quality of the shells is not affected by temperature and humidity, the capsule shells should be conditioned at a temperature of 25° ± 2° and a relative humidity of 50 ± 5 per cent for not less than 12 hours before conducting the test for Average weight.*

**Average weight.** Weigh 100 capsule shells and determine the average weight of a capsule. The average weight is within ± 10 per cent of the target weight shown in Table 1. (As sizes 0 to 4 are commonly used, detailed requirements are included for these sizes only. Requirements for other sizes may be decided upon mutually between the manufacturer of the Hard Gelatin Capsule Shells and the user).

<table>
<thead>
<tr>
<th>Size</th>
<th>Target weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>96</td>
</tr>
<tr>
<td>1</td>
<td>76</td>
</tr>
<tr>
<td>2</td>
<td>63</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
</tr>
</tbody>
</table>

**Disintegration** (2.5.1). 15 minutes, using discs.

**Microbial contamination** (2.2.9). Total microbial count, not more than 1000 per g. 1 g is free from *Escherichia coli* and *Salmonellae*. 

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*IP 2007 HARD GELATIN CAPSULE SHELLS*
Loss on drying (2.4.19). 12.5 to 16.0 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours or to constant weight.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states (1) the size of the capsule shells; (2) that only permitted colours, if any, have been used; (3) the storage conditions.

Gentamicin Sulphate

![Chemical structure of Gentamicin Sulphate]

Gentamicin Sulphate is a mixture of the sulphates of antimicrobial substances produced by *Micromonospora purpurea*.

Gentamicin Sulphate has a potency of not less than 590 µg of gentamicin per mg, calculated on the anhydrous basis.

Description. A white or almost white powder; hygroscopic.

Identification. A white or almost white powder; hygroscopic.

Tests

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. The lower layer obtained by shaking together equal volumes of *strong ammonia solution*, *chloroform* and *methanol* and allowing to separate.

Test solution. Dissolve 0.5 g of the substance under examination in 100 ml of *water*.

Reference solution. A 0.5 per cent w/v solution of *gentamicin sulphate RS*.

B. Dissolve 10 mg in 1 ml of *water* and add 5 ml of a 40 per cent w/v solution of *sulphuric acid*. Heat on a water-bath for 10 minutes, cool and dilute to 25 ml with *water*. When examined in the range 240 nm to 330 nm (2.4.7), the resulting solution shows no absorption maximum.

C. In the test for Composition of gentamicin sulphate, the four principal peaks in the chromatogram obtained with the test solution correspond to the four peaks in the chromatogram obtained with the reference solution.

D. Gives reaction A of sulphates (2.3.1).

Tests

Appearance of solution. A 4.0 per cent w/v solution in *carbon dioxide-free water* (solution A) is clear (2.4.1), and not more intensely coloured than degree 6 of the appropriate range of reference solutions (2.4.1).

pH (2.4.24). 3.5 to 5.5, determined in solution A.

Specific optical rotation (2.4.22). +107° to +121°, determined in a 10.0 per cent w/v solution.

Composition of gentamicin sulphate. Determine by liquid chromatography (2.4.14).

Test solution. Add 5 ml of *methanol* and 4 ml of *phthalaldehyde reagent* to 10 ml of a 0.1 per cent w/v solution of the substance under examination in *water*, mix, add sufficient *methanol* to produce 25 ml, heat in a water-bath at 60° for 15 minutes and cool. If the solution is not used immediately, cool to 0° and use within 4 hours.

Reference solution. Prepare in the same manner as the test solution but using 10 ml of a 0.1 per cent w/v solution of *gentamicin sulphate RS* in place of the solution of the substance under examination.

Chromatographic system

- a stainless steel column 10 to 12.5 cm x 4.6 to 5 mm, packed with octadecylsilica silica gel (5 µm),
- mobile phase: a solution containing 0.55 per cent w/v of *sodium heptanesulphonate monohydrate* in a mixture of 70 volumes of *methanol*, 25 volumes of *water* and 5 volumes of *glacial acetic acid*,

<table>
<thead>
<tr>
<th>Gentamycin</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
</tr>
</thead>
<tbody>
<tr>
<td>C¹</td>
<td>CH₃</td>
<td>NHCH₃</td>
<td>H</td>
</tr>
<tr>
<td>C²</td>
<td>CH₃</td>
<td>NH₂</td>
<td>H</td>
</tr>
<tr>
<td>C¹a</td>
<td>H</td>
<td>NH₂</td>
<td>H</td>
</tr>
<tr>
<td>C²a</td>
<td>H</td>
<td>NH₂</td>
<td>CH₃</td>
</tr>
</tbody>
</table>
– flow rate. 1.5 ml per minute,
– spectrophotometer set at 330 nm,
– a 5 µl loop injector.

If necessary, adjust the methanol content of the mobile phase so that in the chromatogram obtained with the reference solution, the retention time of component C₂ is 10 to 20 minutes and the peaks are well separated with relative retention times of about 0.13 (reagent), 0.27 (component C₁), 0.65 (component C₁ₐ), 0.85 (component C₂ₐ) and 1.00 (component C₂).

Adjust the sensitivity and the volume of the reference solution injected so that the height of the peak due to component C₁ is about 75 per cent of the full-scale deflection on the chart paper. Plot a horizontal baseline on the chromatogram from the level portion of the curve immediately prior to the reagent peak. Measure the peak height above this baseline for each component. Repeat the procedure with the test solution. The test is not valid unless the resolution between the peaks due to components C₂ₐ and C₂ is not less than 1.3.

From the peak heights in the chromatogram obtained with the reference solution and the proportions of the components declared for gentamicin sulphate RS, calculate the response factors for components C₁, C₁ₐ, C₂ and C₂. From these response factors and peak heights in the chromatogram obtained with the test solution, calculate the proportions of components C₁, C₁ₐ, C₂ₐ and C₂ in the substance under examination. The proportions are within the following limits. C₁, 25.0 to 50.0 per cent; C₁ₐ, 10.0 to 35.0 per cent; C₂ + C₂ₐ, 25.0 to 55.0 per cent.

Sulphate. 32.0 to 35.0 per cent of SO₄, calculated on the anhydrous basis, determined by the following method. Dissolve 0.25 g in 100 ml of distilled water, adjust the pH to 11 with strong ammonia solution and add 10 ml of 0.1 M barium chloride. Titrate with 0.1 M disodium edetate using 0.5 mg of metalphthalein as indicator; when the colour of the solution begins to change add 50 ml of ethanol (95 per cent) and continue the titration until the violet-blue colour disappears. Perform a blank determination and make any necessary correction.

1 ml of 0.1 M barium chloride is equivalent to 0.009606 g of sulphate, SO₄.

Methanol. Not more than 1.0 per cent w/w, determined by gas chromatography (2.4.13).

Test solution (a). A 25 per cent w/v solution of the substance under examination.

Test solution (b). A solution containing 25 per cent w/v of the substance under examination and 0.25 per cent v/v of 1-propanol (internal standard).

Reference solution. A solution containing 0.25 per cent v/v of the substance under examination and 0.25 per cent v/v of the internal standard.

Chromatographic system
– a column 1.5 m x 4 mm, packed with porous polymer beads (80 to 100 mesh)(such as Porapak Q),
– temperature:
  column. constant at a point between 120° and 140°,
  inlet port and detector. 50° higher than column temperature,
– flow rate. constant at 30 to 40 ml per minute of the carrier gas.

Calculate the percentage w/w of methanol taking 0.792 g as its weight per ml (2.4.29) at 20°.

Sulphated ash (2.3.18). Not more than 1.0 per cent, determined on 0.5 g.

Water (2.3.43). Not more than 15.0 per cent, determined on 0.3 g.

Assay. Determine by the microbiological assay of antibiotics, Method A (2.2.10), and express the result in µg of gentamicin per mg.

Gentamicin Sulphate intended for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 1.67 Endotoxin Units per mg of gentamicin.

Sterility. Complies with the test for sterility (2.2.11).

Storage. Store protected from moisture. If it is intended for use in the manufacture of parenteral or ophthalmic preparations, the container should be sterile and sealed so as to exclude micro-organisms.

Labelling. The label states (1) the potency in terms of µg of gentamicin per mg; (2) whether or not the contents are intended for use in the manufacture of parenteral or ophthalmic preparations.

Gentamicin Eye Drops

Gentamicin Sulphate Eye Drops

Gentamicin Eye Drops are a sterile solution of Gentamicin Sulphate in Purified Water.

Gentamicin Eye Drops contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of gentamicin.

Identification

A. Determine by thin-layer chromatography (2.4.6), coating the plate with silica gel G.
Mobile phase. The lower layer obtained by shaking together equal volumes of strong ammonia solution, chloroform and methanol and allowing to separate.

Test solution. A volume of the eye drops containing 60 µg of gentamicin.

Reference solution. Dissolve 0.1 mg of gentamicin sulphate RS in a volume of water equivalent to the volume of the eye drops used.

Apply to the plate the specified volumes of each solution. After development, dry the plate in air, spray with ethanolic ninhydrin solution and heat at 110° for 5 minutes. The three principal spots in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

B. In the test for Composition of gentamicin sulphate, the four principal peaks in the chromatogram obtained with the test solution correspond to the four peaks in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 6.5 to 7.5.

Composition of gentamicin sulphate. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a suitable volume of the eye drops with water to contain 0.045 per cent w/v of gentamicin. To 10 ml of the resulting solution add 5 ml of methanol, swirl and add 4 ml of phthalaldehyde reagent, mix, add sufficient methanol to produce 25 ml, heat on a water-bath at 60° for 15 minutes and cool.

Reference solution. Prepare in the same manner as the test solution but using 10 ml of a 0.065 per cent w/v solution of gentamicin sulphate RS in place of the preparation under examination.

Chromatographic system

- a stainless steel column 10 to 12.5 cm x 4.6 to 5 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: 0.025 M sodium heptanesulphonate monohydrate in a mixture of 70 volumes of methanol, 25 volumes of water and 5 volumes of glacial acetic acid,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 330 nm,
- a 5 µl loop injector.

If necessary, adjust the methanol content of the mobile phase so that in the chromatogram obtained with the reference solution the retention time of component C1 is about 75 per cent of the full-scale deflection on the recorder. Adjust the sensitivity and the volume of reference solution injected so that the height of the peak due to component C1 is about 75 per cent of the full-scale deflection on the recorder.

Plot a horizontal baseline on the chromatogram from the level portion of the curve immediately prior to the reagent peak. Measure the peak height above this baseline for each component. Repeat the procedure with the test solution. The test is not valid unless the resolution between the peaks due to components C2a and C2 is not less than 1.3.

From the peak heights in the chromatogram obtained with the reference solution and the proportions of the components declared for gentamicin sulphate RS, calculate the response factors and peak heights in the chromatogram obtained with the test solution, calculate the proportions of components C1, C1a, C2a and C2 in the eye drops. The proportion are within the following limits. C1, 25.0 to 50.0 per cent; C1a, 10.0 to 35.0 per cent; C2a+C2a, 25.0 to 55.0 per cent.

Other tests. Comply with the tests stated under Eye Drops.

Assay. Determine by the microbiological assay of antibiotics (2.2.10) on a solution prepared in the following manner.

Dilute a volume of the eye drops containing about 15 mg of gentamicin to 50.0 ml with sterile phosphate buffer pH 8.0 and dilute 10.0 ml of the resulting solution to 50.0 ml with the same solvent.

Calculate the content of gentamicin in the eye drops, taking each 1000 Units found to be equivalent to 1 mg of gentamicin.

Labelling. The label states the quantity of active ingredient in terms of the equivalent amount of gentamicin.

Gentamicin Injection

Gentamicin Sulphate Injection

Gentamicin Injection is a sterile solution of Gentamicin Sulphate in Water for Injection.

Gentamicin Injection contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of gentamicin.

Description. A clear, colourless to pale-yellow solution with a faint odour.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. The lower layer obtained by shaking together equal volumes of strong ammonia solution, chloroform and methanol and allowing to separate.
Test solution. A volume of the injection containing 60 µg of gentamicin.

Reference solution. Dissolve 0.1 mg of gentamicin sulphate RS in a volume of water equivalent to the volume of the injection used.

Apply to the plate the specified volumes of each solution. After development, dry the plate in air, spray with ethanolic ninhydrin solution and heat at 110° for 5 minutes. The three principal spots in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

B. In the test for Composition of gentamicin sulphate, the four principal peaks in the chromatogram obtained with the test solution correspond to the four peaks in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 3.0 to 5.0.

Composition of gentamicin sulphate. Determine by liquid chromatography (2.4.14).

Test solution. Add 5 ml of methanol to 10 ml of a solution prepared by diluting a suitable volume of the injection with water to contain the equivalent of 0.045 per cent w/v of gentamicin, swirl and add 4 ml of phthalaldehyde reagent, mix, add sufficient methanol to produce 25 ml, heat in a water-bath at 60° and cool. If the solution is not used immediately, cool at 0° and use within 4 hours.

Reference solution. Prepare in the same manner as the test solution but using 10 ml of a 0.065 per cent w/v solution of gentamicin sulphate RS in place of the solution of the injection under examination.

Chromatographic system

- a stainless steel column 10 to 12.5 cm x 4.6 to 5 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 0.025 M sodium heptanesulphonate monohydrate in a mixture of 70 volumes of methanol, 25 volumes of water and 5 volumes of glacial acetic acid,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 330 nm,
- a 5 µl loop injector.

If necessary, adjust the methanol content of the mobile phase so that in the chromatogram obtained with the reference solution the retention time of component C₂ is 10 to 20 minutes and the peaks are well separated with relative retention times of about 0.13 (reagent), 0.27 (component C₁), 0.65 (component C₁ₐ), 0.85 (component C₂ₐ) and 1.00 (component C₂).

Adjust the sensitivity and the volume of reference solution injected so that the height of the peak due to component C₁ is about 75 per cent of full-scale deflection on the recorder. Plot a horizontal baseline on the chromatogram from the level portion of the curve immediately prior to the reagent peak. Measure the peak height above this baseline for each component. Repeat the procedure with the test solution. The test is not valid unless the resolution factor between the peaks due to components C₂ₐ and C₂ is not less than 1.3.

From the peak heights in the chromatogram obtained with the reference solution and the proportions of the components declared for gentamicin sulphate RS, calculate the response factors for components C₁, C₁ₐ, C₂ₐ and C₂. From these response factors and peak heights in the chromatogram obtained with the test solution, calculate the proportions of components C₁, C₁ₐ, C₂ₐ and C₂ in the eye drops. The proportion are within the following limits. C₁, 25.0 to 50.0 per cent; C₁ₐ, 10.0 to 35.0 per cent; C₂ + C₂ₐ, 25.0 to 55.0 per cent.

Bacterial endotoxins (2.2.3). Not more than 1.67 Endotoxin Units per mg of gentamicin.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by the microbiological assay of antibiotics, Method A (2.2.10), and express the result in mg of gentamicin per ml.

Calculate the content of gentamicin in the injection, taking each 1000 Units found to be equivalent to 1 mg of gentamicin.

Labelling. The label states the strength in terms of the equivalent amount of gentamicin in a suitable dose-volume.

Glibenclamide

Glyburide

Glibenclamide is 1-{4-[2-(5-chloro-2-methoxybenzamido)ethyl]benzenesulphonyl}-3-cyclohexylurea.

Glibenclamide contains not less than 99.0 per cent and not more than 101.0 per cent of C₂₃H₂₅ClN₃O₅S, calculated on the dried basis.

Description. A white or almost white, crystalline powder.
Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with glibenclamide RS or with the reference spectrum of glibenclamide.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.01 per cent w/v solution in 0.01 M methanolic hydrochloric acid shows an absorption maximum at about 300 nm and a less intense maximum at about 275 nm; absorbance at about 300 nm, about 0.63 and at about 275 nm, about 0.29.

C. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

D. Dissolve 20 mg in 2 ml of sulphuric acid (96 per cent w/w); the solution is colourless and exhibits a blue fluorescence in ultraviolet light at 365 nm. Dissolve about 0.1 g of chloral hydrate in the solution; within 5 minutes the colour changes to deep yellow and after about 20 minutes a brownish tinge is produced.

Tests

Appearance of solution. A 1.0 per cent w/v solution in ethanol (95 per cent), prepared with the aid of heat, is clear (2.4.1), and colourless (2.4.1).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 45 volumes of chloroform, 45 volumes of cyclohexane, 5 volumes of glacial acetic acid and 5 volumes of ethanol (95 per cent).

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of a mixture of equal volumes of dichloromethane and methanol.

Reference solution (a). A 2 per cent w/v solution of glibenclamide RS in the same solvent mixture.

Reference solution (b). Dilute 1 volume of reference solution (a) to 200 volumes with the same solvent mixture.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°C.

Assay. Weigh accurately about 0.4 g and dissolve in 100 ml of ethanol (95 per cent) with the aid of heat; titrate with 0.1 M sodium hydroxide using 1 ml of dilute phenolphthalein solution as indicator until a red colour is obtained.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.04940 g of C$_{23}$H$_{28}$ClN$_3$O$_5$S.

Glibenclamide Tablets

Glibenclamide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of glibenclamide, C$_{23}$H$_{28}$ClN$_3$O$_5$S.

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows an absorption maximum at about 300 nm and a less intense maximum at about 275 nm.

B. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 45 volumes of chloroform, 45 volumes of cyclohexane, 5 volumes of glacial acetic acid and 5 volumes of ethanol (95 per cent).

Test solution. Extract a quantity of the powdered tablets containing 20 mg of Glibenclamide with four quantities, each of 5 ml, of a mixture of 20 volumes of dichloromethane and 10 volumes of acetone, evaporate the combined extracts to dryness at a pressure of 2 kPa and at a temperature not exceeding 40°C and dissolve the residue in 4 ml of a mixture of equal volumes of chloroform and methanol.

Reference solution (a). A 0.5 per cent w/v solution of glibenclamide RS in the same solvent mixture.

Reference solution (b). Dilute 2 ml of reference solution (a) to 100 ml with the same solvent mixture.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

Uniformity of content. Comply with the test stated under Tablets.

Powder one tablet, warm with 10 ml of 0.1 M methanolic hydrochloric acid and centrifuge. Repeat the extraction with
three further quantities, each of 10 ml, of \(0.1\) \(M\) methanolic hydrochloric acid. Cool the combined extracts, add sufficient \(0.1\) \(M\) methanolic hydrochloric acid to produce 50.0 ml and measure the absorbance of the resulting solution at the maximum at about 300 nm (2.4.7). Calculate the content of \(C_{23}H_{32}ClN_3O_5S\) taking 63 as the specific absorbance at 300 nm.

**Other Tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 20 mg of Glibenclamide and shake with 40 ml of \(0.1\) \(M\) methanolic hydrochloric acid, heat gently and centrifuge. Repeat the extraction with three further quantities, each of 20 ml, of \(0.1\) \(M\) methanolic hydrochloric acid. To the combined extracts add sufficient \(0.1\) \(M\) methanolic hydrochloric acid to produce 200.0 ml and measure the absorbance of the resulting solution at the maximum at about 300 nm (2.4.7), using \(0.1\) \(M\) methanolic hydrochloric acid heated to the same degree as the blank. Calculate the content of \(C_{23}H_{32}ClN_3O_5S\) taking 63 as the specific absorbance at 300 nm.

**Glipizide**

\[
\begin{align*}
\text{C}_21\text{H}_{27}\text{N}_5\text{O}_4\text{S} & \quad \text{Mol. Wt. 445.5} \\
\text{Glipizide is 1-cyclohexyl-3-[[4-[[5-} & \\
& \text{methylpyrazine-2-yi} & \\
& \text{carbonyl]amino}[ethy]} & \\
& \text{phenyl]sulphonyl]urea} & \\
\text{Glipizide contains not less than 98.0 per cent} & \\
& \text{and not more than 102.0 per cent of} & \\
& \text{C}_21\text{H}_{27}\text{N}_5\text{O}_4\text{S, calculated on the} & \\
& \text{dried basis.} & \\
\text{Description.} & \text{A white or almost white, crystalline powder.} & \\
\text{Identification} & \\
\text{Test A may be omitted if tests B and C are carried out. Tests B} & \\
& \text{and C may be omitted if test A is carried out.} & \\
& \text{A. Determine by infrared absorption spectrophotometry (2.4.6).} & \\
& \text{Compare the spectrum with that obtained with glipizide RS.} & \\
& \text{B. When examined in the range 220 to 350 nm (2.4.7), a 0.002} & \\
& \text{per cent solution in methanol, shows two maxima, at about} & \\
& \text{226 nm and 274 nm. The ratio of the absorbance at 226 nm to} & \\
& \text{that at about 274 nm, 2.0 to 2.4.} & \\
\end{align*}
\]

C. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Solvent mixture.** Equal volumes of methanol and methylene chloride.

**Mobile phase.** A mixture of 25 volumes of anhydrous formic acid, 25 volumes of ethyl acetate and 50 volumes of methylene chloride.

**Test solution.** Dissolve 0.10 g of the substance under examination in 100 ml of solvent mixture.

**Reference solution.** A 0.10 per cent w/v solution of glipizide RS in solvent mixture.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in the ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with the reference solution.

**Tests**

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 25 mg of the substance under examination in 100 ml of the mobile phase.

**Reference solution (a).** A 0.025 per cent w/v solution of glipizide RS in mobile phase.

**Reference solution (b).** Dilute 1 ml of reference solution (a) to 100 ml with mobile phase.

**Chromatographic system**

- a stainless steel column 15 cm x 4.6 mm packed with octadecysilyl silica (5 µm),
- mobile phase: a mixture of 17 volumes of acetonitrile and 83 volumes of a 0.35 per cent w/v of dipotassium hydrogen phosphate, adjusted to pH 8.0 with orthophosphoric acid,
- flow rate. 1 ml per minute,
- spectrophotometer set at 274 nm,
- a 50 µl loop injector.

Inject reference solution (a). Test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.
Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1 g by drying in an oven at 105º.

Assay. Weigh accurately about 0.4 g, dissolve in 50 ml of dimethylformamide, add 0.2 ml of quinaldine red solution. Titrate with 0.1 M lithium methoxide until the colour changes from red to colourless.

1 ml of 0.1 M lithium methoxide is equivalent to 0.04455 g of C₂₁H₂₇N₅O₄S.

Storage. Store protected from moisture.

Glipizide Tablets

Glipizide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of glipizide, C₂₁H₂₇N₅O₄S.

Identification

A. Shake a quantity of the powdered tablets containing 25 mg of Glipizide with 10 ml of dichloromethane for 5 minutes, filter, dry the filtrate with anhydrous sodium sulphate, filter again and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with glipizide RS.

B. When examined in the range 210 nm to 320 nm (2.4.7), a final solution obtained in the assay shown are absorption maximum at about 226 nm and 274 nm.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17).

Mobile phase. A mixture of 20 volumes of ethyl acetate, 20 volumes of anhydrous formic acid and 40 volumes of dichloromethane.

Test solution. Extract a quantity of powdered tablet containing 0.1 g of Glipizide with four 10 ml quantities of acetone, evaporate the combined extracts to dryness under reduced pressure at a temperature not exceeding 30º and dissolve the residue in sufficient of a mixture of equal volumes of dichloromethane and methanol to produce 5 ml.

Reference solution (a). Dilute 1 volume of test solution to 200 volumes with a mixture of equal volumes of dichloromethane and methanol.

Reference solution (b). Dilute 1 volume of test solution to 500 volumes with the same solvent mixture.

Reference solution (c). A 0.010 per cent w/v solution of glipizide impurity A RS (4-[2-(5-methylpyrazine-2-carboxamido)ethyl]benzenesulphonamide) in a mixture of equal volumes of dichloromethane and methanol.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and examine in the ultraviolet light at 254 nm. Any spot corresponding to glipizide impurity A in the chromatogram obtained with test solution is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.5 per cent). Any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent) and not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent).

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 15 mg of Glipizide, dissolve in 30 ml of methanol with gentle heating on a water bath, cool and add sufficient methanol to produce 50.0 ml. Filter and dilute 5.0 ml of the filtrate to 50.0 ml with methanol. Measure the absorbance of the resulting solution at 274 nm (2.4.7). Calculate the content of C₂₁H₂₇N₅O₄S taking 237 as the specific absorbance at 274 nm.

2-Deoxy-D-Glucose

C₆H₁₂O₅ Mol. Wt. 164.2

2-Deoxy-d-Glucose is 2-deoxyGlucose

2-Deoxy-D-Glucose contains not less than 97.0 per cent and not more than 103.0 per cent of C₆H₁₂O₅, calculated on the dried basis.

Description. A white to off white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare with spectrum with that obtained with 2-deoxy-D-glucose RS.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). +44.0º to +48.0º, determined in a 1.0 per cent w/v solution in water.
**Test solution.** Dissolve 0.5 g of the substance under examination in 5 ml of dimethyl sulfoxide in a 20-ml headspace vial.

**Reference stock solution.** Weigh about 44.5 mg of toluene, dilute to 50 ml with dimethyl sulfoxide.

**Reference solution.** Weigh about 25 mg of isopropyl alcohol and 15 mg of methanol in to the 50 ml volumetric flask, add 5 ml of reference stock solution and make up to the volume with dimethyl sulphoxide. Take 5 ml in a 20-ml headspace vial.

Chromatographic system
- a capillary column 30 m × 0.53 mm, packed with 6 per cent cyanopropylphenyl and 94 per cent dimethylpolysiloxane,
- temperature column 42° to 200° @ 30° per minute,
  inlet port 180° and detector 240°
- flow rate. 3.6 ml per minute of the helium carrier gas.
Headspace conditions
- vial pressure 10 psi, sqample oven 85°, pressurisation time 0.2 minute.

Inject 1 ml of the reference solution. The test is not valid unless the resolution between the peaks due to isopropyl alcohol, the peak due to methanol and toluene is not less than 2 and the tailing factor is not more than 1.5 for each component.

Inject 1 ml of the test solution and the reference solution. In the chromatogram obtained with the test solution, the area of the peaks due to isopropyl alcohol, methanol and toluene is not more than the area of peaks in the chromatogram obtained with the reference solution.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 1 g of the substance under examination in 100 ml of the mobile phase.

**Reference solution (a).** A 1.0 per cent w/v solution of 2-deoxy-D-glucose RS in the mobile phase.

**Reference solution (b).** Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

The chromatographic system described under Assay.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 2000 theoretical plates.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with reference solution (b) (2.0 per cent).

**Water** (2.3.43). Not more than 1 per cent, determined on 0.2 g.

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 0.2 g of the substance under examination in 100.0 ml of the mobile phase.

**Reference solution.** A 0.2 per cent w/v solution of 2-deoxy-D-glucose RS in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with rigid spherical etyrene-divinyl benzene copolymer (5 to 10µm),
- mobile phase: a mixture of 55 volumes of water and 45 volumes of methanol,
- flow rate. 0.5 ml per minute,
- detector set at refractive index,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.
Calculated the content of C₆H₁₂O₅.

**Storage.** Store protected from moisture.

**Glycerin**

\[
\text{H}_3\text{C}(-\text{O}H)\text{CH}(-\text{O}H)\text{CH}(-\text{O}H)
\]

\[
\text{C}_3\text{H}_8\text{O}_3 \quad \text{Mol. Wt. 92.1}
\]

Glycerin is propane-1,2,3-triol.

Glycerin contains not less than 98.0 per cent and not more than 101.0 per cent of C₃H₈O₃, calculated on the anhydrous basis.

**Description.** A clear, colourless or almost colourless, syrupy liquid; odourless; very hygroscopic.

**Identification**

**Test A** may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. To 5 ml add 1 ml of water and mix carefully. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with glycerin (85 per cent) RS or with the reference spectrum of glycerin (85 per cent).
B. Mix 1 ml with 0.5 ml of nitric acid and superimpose 0.5 ml of potassium dichromate solution; a blue ring develops at the interface of the two liquids. Allow to stand for 10 minutes; the blue colour does not diffuse into the lower layer.

C. Heat 1 ml with 2 g of potassium hydrogen sulphate in an evaporating dish. Irritant vapours are evolved which blacken filter paper moistened with alkaline potassium mercuri-iodide solution.

D. Refractive index (2.4.27). 1.470 to 1.475, determined at 20°.

Tests

Appearance of solution. Dissolve 50 g of the substance under examination in sufficient carbon dioxide-free water to produce 100 ml (solution A). Solution A is clear (2.4.1). Dilute 10 ml of solution A to 25 ml with water. The solution is colourless (2.4.1).

Acidity or alkalinity. To 50 ml of solution A add 0.5 ml of phenolphthalein solution. The solution is colourless and not more than 0.2 ml of 0.1 M sodium hydroxide is required to produce a pink colour. Reserve the final solution for the test for Ester.

Heavy metals (2.3.13). Dissolve 4.0 g in 2 ml of 0.1 M hydrochloric acid and sufficient water to produce 25 ml. The resulting solution complies with the limit test for heavy metals, Method A (5 ppm).

Iron (2.3.14). 10.0 g complies with the limit test for iron (4 ppm).

Chlorides (2.3.12). 20.0 ml of solution A complies with the limit test for chlorides (25 ppm).

Sulphates (2.3.17). 10.0 ml of solution A complies with the limit test for sulphates (30 ppm).

Aldehydes and reducing substances. To 7.5 ml of solution A in a glass-stoppered flask add 7.5 ml of water and 1 ml of decolourised pararosaniline solution, close the flask and allow to stand for 1 hour. Any colour produced is not more intense than that obtained in a standard prepared at the same time and in the same manner but using 7.5 ml of formaldehyde standard solution (5 ppm CH₂O) in place of solution A. The test is not valid unless the standard solution is pink.

Ester. Add 0.1 M sodium hydroxide to the solution reserved in the test for Acidity or alkalinity until a total of 10.0 ml has been added and boil under a reflux condenser for 5 minutes. Cool, add 0.5 ml of phenolphthalein solution and titrate with 0.1 M hydrochloric acid. Not less than 8.0 ml of 0.1 M hydrochloric acid is required to decolorise the solution.

Ethylene glycol, diethylene glycol and related substances. Determine by gas chromatography (2.4.13).

Test solution. A 10.0 per cent w/v solution of the substance under examination.

Reference solution. A solution containing 0.05 per cent w/v of the substance under examination, 0.05 per cent w/v of ethylene glycol and 0.05 per cent w/v of diethylene glycol.

Chromatographic system

- a glass column 2 m x 3 mm, packed with 10 per cent diethylene glycol succinate on acid-washed and silanised, flux-calcinated siliceous earth (such as Chromosorb WHP 80-100 mesh),
- temperature: column 200°, inlet port and detector 280°,
- flame ionisation detector,
- flow rate: 30 ml per minute of the carrier gas.

Inject 3 µl or other suitable volume of the test solution. Record the chromatogram adjusting the sensitivity so that the height of the peak due to glycerin is more than 50 per cent of full-scale deflection. Inject the same volume of the reference solution and record the chromatogram. The order of elution is ethylene glycol, diethylene glycol and glycerin.

The test is not valid unless in the chromatogram obtained with the reference solution the resolution factor between the peaks corresponding to diethylene glycol and glycerin is not less than 3.0 and the area of any secondary peak in the chromatogram obtained with the test solution is less than the area of the peak corresponding to diethylene glycol in the chromatogram obtained with the reference solution.

Sugars. Heat 10 ml of solution A with 1 ml of 1 M sulphuric acid on a water-bath for 5 minutes. Add 3 ml of 2 M sodium hydroxide (carbonate-free), mix and add dropwise 1 ml of freshly prepared copper sulphate solution; a clear blue solution is produced. Continue heating on the water-bath for 5 minutes; the solution remains blue and no precipitate is produced.

Sulphated ash (2.3.18). Not more than 0.01 per cent, determined on 5.0 g.

Water (2.3.43). Not more than 2.0 per cent, determined on 1.5 g.

Assay. Weigh accurately about 0.1 g, mix thoroughly with 45 ml of water, add 25.0 ml of a 2.14 per cent w/v solution of sodium periodate and 1.0 ml of 1 M sulphuric acid. Allow the mixture to stand protected from light for 15 minutes. Add 5 ml of a 50 per cent w/v solution of ethylene glycol, allow to stand protected from light for 20 minutes and titrate with 0.1 M sodium hydroxide using 0.5 ml of phenolphthalein solution as indicator. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of sodium hydroxide required by the test substance.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.00921 g of C₃H₈O₃.

Storage. Store protected from moisture.
Glyceryl Monostearate

Monostearin

Glyceryl Monostearate is a mixture of monoglycerides of stearic and palmitic acids, together with variable quantities of di- and triglycerides.

Glyceryl Monostearate contains not less than 35.0 per cent of monoglycerides, calculated as glyceryl monostearopalmitate, C₃₀H₄₀O₄, and not more than 7.0 per cent of free glycerin C₃H₈O₃, both calculated on the anhydrous basis.

**Description.** A white or almost white, hard, waxy mass or flakes; almost odourless.

**Identification**

A. Heat 1 g with 2 g of potassium bisulphate in an evaporating dish. Irritant, lachrymatory fumes are evolved which darken filter paper impregnated with alkaline potassium mercuri-iiodide solution.

B. Heat 2.5 g with 40 ml of ethanolic potassium hydroxide solution for 30 minutes on a water-bath under a reflux condenser. Add 30 ml of water, evaporate the ethanol, acidify the hot mixture with 15 ml of dilute hydrochloric acid, cool and extract with 50 ml of ether. Wash the ether layer with two quantities, each of 10 ml, of a 20 per cent w/v solution of sodium chloride, dry the ether extract over anhydrous sodium sulphate and filter. Evaporate the solvent and dry the residue under reduced pressure. Melt the residue and fill one or two capillary tubes (for the determination of melting range) and allow to stand for 24 hours in a desiccator. Carry out the determination of melting range by Method II (2.4.21); the residue melts at 54° to 64°.

**Tests**

**Acid value** (2.3.23). Not more than 5.0, determined on 0.5 g dissolved in 50 ml of a mixture of equal volumes of ethanol (95 per cent) and ether.

**Saponification value** (2.3.37). 155 to 170.

**Iodine value** (2.3.28). Not more than 5.0 (iodine bromide method).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 2.0 per cent, determined on 0.5 g dissolved in a mixture of 10 ml of anhydrous methanol and 10 ml of anhydrous chloroform.

**Assay.** Weigh accurately about 0.4 g and dissolve in 50 ml of dichloromethane in a glass-stoppered separating funnel. Add 25 ml of water and shake vigorously for 1 minute. Allow the layers to separate (if an emulsion is formed, add a few drops of glacial acetic acid). Repeat the extraction with three further quantities, of 25, 20 and 20 ml, of water and reserve the dichloromethane solution (solution A). Filter the combined aqueous extracts through a filter paper moistened with water, wash the filter with two quantities, each of 5 ml, of water and dilute the combined filtrate and washings to 100.0 ml with water (solution B).

For monoglycerides — Filter solution A through a cotton wool plug. Wash the separating funnel and the filter with three quantities, each of 5 ml, of dichloromethane. Dilute the combined filtrate and washings to 100.0 ml with dichloromethane. To 25.0 ml of this solution add 25.0 ml of periodic-acetic acid solution, shake cautiously, allow to stand at 25° to 30° for 30 minutes, add 100 ml of water and 12 ml of potassium iodide solution. Titrate the liberated iodine with 0.1 M sodium thiosulphate using 1 ml of starch solution as indicator. Repeat the determination using 25 ml of dichloromethane instead of 25.0 ml of the solution under examination. The difference between the titrations represents the amount of sodium thiosulphate required.

1 ml of 0.1 M sodium thiosulphate is equivalent to 0.00172 g of monoglycerides, calculated as glyceryl monostearopalmitate, C₃₀H₄₀O₄.

The quantity of 0.1 M sodium thiosulphate used in the assay is not less than 85 per cent of the quantity of sodium thiosulphate used in the blank assay.

For free glycerin — To 50.0 ml of solution B in a 400-ml conical flask fitted with a ground-glass stopper add 25.0 ml of periodic-acetic acid solution, shake cautiously, allow to stand at 25° to 30° for 30 minutes, add 100 ml of water and 12 ml of potassium iodide solution. Titrate the liberated iodine with 0.1 M sodium thiosulphate using 1 ml of starch solution as indicator. Repeat the determination using 50 ml of water instead of 50 ml of the solution under examination. The difference between the titrations represents the amount of sodium thiosulphate required.

1 ml of 0.1 M sodium thiosulphate is equivalent to 0.0023 g of glycerin, calculated as C₃H₈O₃.

**Storage.** Store protected from light.

## Concentrated Glyceryl Trinitrate Solution

**Concentrated Nitroglycerin Solution**

Concentrated Glyceryl Trinitrate Solution is a solution of propane-1,2,3-triol trinitrate in Ethanol (95 per cent).

Concentrated Glyceryl Trinitrate Solution contains not less than 9.0 per cent w/v and not more than 11.0 per cent w/v of C₃H₇N₃O₅.
GLYCERYL TRINITRATE TABLETS

**CAUTION** — Undiluted glyceryl trinitrate can be exploded by percussion or excessive heat. Proper precautions should be exercised in handling it and only exceedingly small amounts should be isolated.

**Description.** A clear, colourless to pale yellow solution.

**Identification**
Carry out the procedure described under Related substances but using the following solutions.

**Mobile phase.** Toluene.
**Test solution.** Dilute the substance under examination with acetone to contain 0.05 per cent w/v of glyceryl trinitrate.

**Reference solution.** Extract one powdered glyceryl trinitrate tablet 0.5 mg RS with 1 ml of acetone and centrifuge.

The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. To 1 ml add 200 ml of ether, evaporate 6 ml of the resulting solution to dryness and dissolve the residue in 0.2 ml of sulphuric acid containing a trace of diphenylamine; an intense blue colour is produced.

**Tests**

**Weight per ml** (2.4.29). 0.830 g to 0.850 g.

**Inorganic nitrates.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel H.

**Mobile phase.** A mixture of 60 volumes of toluene, 30 volumes of acetone and 15 volumes of glacial acetic acid

**Test solution.** The substance under examination.

**Reference solution.** A freshly prepared 0.1 per cent w/v solution of potassium nitrate in ethanol (90 per cent).

Apply to the plate 5 µl of each solution. After development, dry the plate in a stream of air and spray with diphenylamine solution. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Assay.** Dilute 1.0 ml to 50.0 ml with a 90 per cent v/v solution of glacial acetic acid and dilute 10.0 ml of this solution to 100.0 ml with the same solvent. To 1.0 ml of the resulting solution add 2 ml of phenoldisulphonic acid solution, mix and allow to stand for 15 minutes. Add 8 ml of water; mix well, allow to cool and add slowly, with swirling, 10 ml of strong ammonia solution. Cool and dilute to 20.0 ml with water. Measure the absorbance of the resulting solution at about 405 nm (2.4.7), using as the blank 1 ml of a 90 per cent v/v solution of glacial acetic acid treated in the same manner, beginning at the words “add 2 ml of phenoldisulphonic acid solution,” Dissolve 0.1335 g of potassium nitrate previously dried at 105° in water to produce 50.0 ml; to 10.0 ml add sufficient glacial acetic acid to produce 100.0 ml. Using 1.0 ml of this solution, repeat the procedure beginning at the words “add 2 ml of phenoldisulphonic acid solution,”.

Calculate the content of C₃H₅N₃O₉ from the values of the absorbances so obtained.

1 ml of the potassium nitrate solution is equivalent to 0.0002 g of C₃H₅N₃O₉.

**Storage.** Store protected from light at a temperature between 8° and 15°.

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**Glyceryl Trinitrate Tablets**

Nitroglycerin Tablets; Trinitrin Tablets

Glyceryl Trinitrate Tablets contain not less than 85.0 per cent and not more than 115.0 per cent of the stated amount of glyceryl trinitrate, C₃H₅N₃O₉.

**Identification**
A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** Toluene.

**Test solution.** Extract a quantity of the powdered tablets containing 0.5 mg of glyceryl trinitrate with 1 ml of acetone and centrifuge.

**Reference solution.** Extract one powdered glyceryl trinitrate tablet 0.5 mg RS with 1 ml of acetone and centrifuge.

Apply to the plate 20 µl of each solution. After development, dry the plate in a stream of air, spray with diphenylamine solution and irradiate for 15 minutes with ultraviolet light at 365 nm. Examine the plate in daylight. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.
B. Extract a quantity of the powdered tablets containing 3 mg of glyceryl trinitrate with 5 ml of ether and filter. Evaporate the ether and dissolve the residue in 0.2 ml of sulphuric acid containing a trace of diphenylamine; an intense blue colour is produced.

Tests

Uniformity of content. Comply with the test stated under Tablets.

Place one tablet in a centrifuge tube containing a few glass beads, add 5 ml of a 90 per cent v/v solution of glacial acetic acid, shake for 1 hour and centrifuge. To 1.0 ml of the resulting solution add 2 ml of phenoldisulphonic acid solution, mix and allow to stand for 15 minutes. Add 8 ml of water, mix well, allow to cool and add slowly, with swirling, 10 ml of strong ammonia solution. Cool and dilute to 20.0 ml with water. Measure the absorbance of the resulting solution at about 405 nm (2.4.7), using as the blank 1 ml of a 90 per cent v/v solution of glacial acetic acid treated in the same manner, beginning at the words “add 2 ml of phenoldisulphonic acid solution,”. Dissolve 0.1335 g of potassium nitrate previously dried at 105° in water to produce 50.0 ml; to 10.0 ml add sufficient glacial acetic acid to produce 100.0 ml. Using 1.0 ml of this solution, repeat the procedure beginning at the words “add 2 ml of phenoldisulphonic acid solution,”.

Calculate the content of C₃H₅N₃O₉ from the values of the absorbances so obtained.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 1 mg of glyceryl trinitrate, add 5 ml of a 90 per cent v/v solution of glacial acetic acid, shake for 1 hour and centrifuge. To 1.0 ml of the resulting solution add 2 ml of phenoldisulphonic acid solution, mix and allow to stand for 15 minutes. Add 8 ml of water, mix well, allow to cool and add slowly, with swirling, 10 ml of strong ammonia solution. Cool and dilute to 20.0 ml with water. Measure the absorbance of the resulting solution at about 405 nm (2.4.7), using as the blank 1 ml of a 90 per cent v/v solution of glacial acetic acid treated in the same manner, beginning at the words “add 2 ml of phenoldisulphonic acid solution,”. Dissolve 0.1335 g of potassium nitrate previously dried at 105° in water to produce 50.0 ml; to 10.0 ml add sufficient glacial acetic acid to produce 100.0 ml. Using 1.0 ml of this solution, repeat the procedure beginning at the words “add 2 ml of phenoldisulphonic acid solution,”.

Calculate the content of C₃H₅N₃O₉ from the values of the absorbances so obtained.

1 ml of the potassium nitrate solution is equivalent to 0.0002 g of C₃H₅N₃O₉.

Storage. Store protected from light and moisture in glass containers of not more than 100 tablets, at a temperature not exceeding 30°. The container should be closed by means of a screw cap lined with aluminium or tin foil. Cotton wool wadding or other additional packing that absorbs glyceryl trinitrate should be avoided.

Labelling. The label states that the tablets should be allowed to dissolve slowly in the mouth.

Glycine

Aminoacetic acid

\[ \text{H}_2\text{N} - \text{COOH} \]

\[ \text{C}_2\text{H}_5\text{NO}_2 \]

Mol. Wt. 75.1

Glycine is 2-aminoethanoic acid.

Glycine contains not less than 98.5 per cent and not more than 101.5 per cent of C₂H₅NO₂, calculated on the dried basis.

Description. A white, crystalline powder; odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with glycine RS.

Examine the substances as discs prepared using about 1 mg for 0.4 g of potassium bromide IR.

B. Dissolve 50 mg in 5 ml of water, add 1 ml of sodium hypochlorite solution (3 per cent Cl), boil for 2 minutes, add 1 ml of hydrochloric acid and boil for 4 to 5 minutes. To the resulting solution add 2 ml of hydrochloric acid and 1 ml of a
2 per cent w/v solution of *resorcinol*, boil for 1 minute, cool, add 10 ml of *water* and mix. To 5 ml of this solution add 6 ml of *2 M sodium hydroxide*. The resulting solution is violet with a greenish yellow fluorescence. After a few minutes the solution becomes orange and then yellow and the intense fluorescence remains.

**Tests**

**Appearance of solution.** A 10.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

**pH** (2.4.24). 5.9 to 6.3, determined in a 5.0 per cent w/v solution.

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

**Chlorides** (2.3.12). 2.5 g dissolved in 20 ml of *water* complies with the limit test for chlorides (100 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

**Assay.** Weigh accurately about 0.15 g and dissolve in 100 ml of *anhydrous glacial acetic acid*. Immediately after dissolution titrate 0.1 M *perchloric acid*, using 0.05 ml of *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of 0.1 M *perchloric acid* is equivalent to 0.00751 g of C$_2$H$_5$NO$_2$.

**Glycine Irrigation Solution**

Glycine Irrigation Solution is a sterile solution of Glycine in *Water for Injections*.

Glycine Irrigation Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of C$_2$H$_5$NO$_2$. It contains no antimicrobial agent.

**Description.** A clear, colourless solution.

**Identification**

A. Evaporate 5 ml to dryness on a water-bath and dry at 105° for one hour. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with glycine *RS*.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

**Mobile phase.** A mixture of 70 volumes of *1-propanol* and 30 volumes of *strong ammonia solution*.

**Test solution.** Dilute a suitable volume of the preparation under examination with *water* so that the resulting solution contains 0.25 per cent w/v of Glycine.

**Reference solution.** A 0.25 per cent w/v solution of glycine *RS*.

Apply to the plate 2 µl of each solution. After development, dry the plate at 105° for 10 minutes, spray with ninhydrin solution and heat at 105° for 2 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

**Tests**

**pH** (2.4.24). 4.5 to 6.5.

**Bacterial endotoxins** (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

**Other tests.** Complies with the tests stated under parenteral Preparations (Injections).

**Assay.** Dilute an accurately measured volume of the preparation under examination containing about 0.15 g of Glycine to 25 ml with *water*. Add 10 ml of *formaldehyde solution*, previously adjusted to a pH of 9.0, and 0.25 ml of a mixed indicator solution prepared by dissolving 75 mg of *phenolphthalein* and 25 mg of thymol blue in 100 ml of ethanol (50 per cent). Titrate with 0.1 M *sodium hydroxide* until the yellow colour disappears and a faint violet colour appears.

1 ml of 0.1 M *sodium hydroxide* is equivalent to 0.007507 g of C$_2$H$_5$NO$_2$.

**Storage.** Store in single dose containers at a temperature not exceeding 30°.

**Labelling.** The label states (1) Not for Injection; (2) that the solution should not be used if it contains visible particles.

**Griseofulvin**

Griseofulvin is (1S,6'R)-7-chloro-2',6'-methylbenzofuran-2-spiro-1-cyclohex-2'-ene-3,4'-dione produced by the growth of certain strains of *Penicillium griseofulvum* or obtained by any other means.

Griseofulvin contains not less than 97.0 per cent and not more than 102.0 per cent of C$_{17}$H$_{17}$ClO$_6$, calculated on the dried basis.

**Description.** A white to yellowish white powder, the particles of which are generally up to 5 µm in maximum dimension,
although larger particles, which may occasionally exceed 30 µm may be present; almost odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with griseofulvin RS.

B. Dissolve about 5 mg in 1 ml of sulphuric acid and add 5 mg of powdered potassium dichromate; a wine-red colour is produced.

C. Melting range (2.4.21). 217° to 224°.

Tests

Appearance of solution. A 7.5 per cent w/v solution in dimethylformamide is clear (2.4.1), and not more intensely coloured than reference solution YS4 (2.4.1).

Acidity. Suspend 0.25 g in 20 ml of ethanol (95 per cent) and titrate with 0.2 M sodium hydroxide using phenolphthalein solution as indicator; not more than 1.0 ml is required to change the colour of the solution.

Specific optical rotation (2.4.22). +352° to +364°, determined at 20° in a 1.0 per cent w/v solution in dimethylformamide.

Related substances. Determine by gas chromatography (2.4.13).

Test solution (a). Dissolve 1.0 g of the substance under examination in 100 ml of acetone.

Test solution (b). Dissolve 1.0 g of the substance under examination and 20 mg of 9,10-diphenylanthracene (internal standard) in 100 ml of acetone.

Reference solution. A solution containing 0.050 per cent w/v of griseofulvin RS and 0.020 per cent w/v of the internal standard in acetone.

Chromatographic system

– a glass column 1.0 m x 4 mm, packed with acid-washed diatomaceous support (100 to 200 mesh) coated with 1 per cent w/w of cyanopropylmethyl phenyl silicone fluid,
– temperature: column 250°, inlet port and detector 270°,
– flow rate. 60 ml per minute of the carrier gas.

Continue the chromatography for three times the retention time of griseofulvin.

The chromatogram obtained with test solution (a) shows a peak due to griseofulvin (retention time about 11 minutes) and may show a peak due to dechlorogriseofulvin (retention time about 0.6 times that of griseofulvin) and a peak due to dehydrogriseofulvin (retention time about 1.4 times that of griseofulvin).

Calculate the ratio (r) of the area of the peak due to griseofulvin to that of the peak due to the internal standard in the chromatogram obtained with the reference solution. The ratio of the area of any peak corresponding to dechlorogriseofulvin to that of the peak due to the internal standard in the chromatogram obtained with test solution (b) is less than 0.6r.

The ratio of the area of any peak corresponding to dehydrogriseofulvin to that of the peak due to the internal standard in the chromatogram obtained with test solution (b) is less than 0.15r.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Matter soluble in light petroleum. Not more than 0.2 per cent, determined by the following method. Extract 1 g with 20 ml of light petroleum (40° to 60°) by boiling under a reflux condenser for 10 minutes; cool, filter, wash the filter with three quantities, each of 15 ml, of the light petroleum, evaporate the combined filtrate and washings to dryness, dry the residue at 105° for 1 hour and weigh.

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 80 mg and dissolve in sufficient ethanol to produce 200.0 ml. Dilute 2.0 ml to 100.0 ml with ethanol and measure the absorbance of the resulting solution at the maximum at about 291 nm (2.4.7). Calculate the content of C17H17ClO6 taking 686 as the specific absorbance at 291 nm.

Griseofulvin Tablets

Griseofulvin Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of griseofulvin, C17H17ClO6.

Identification

A. Extract a quantity of the powdered tablets containing 0.125 g of Griseofulvin with 20 ml of chloroform, add 1 g of anhydrous sodium sulphate, shake and filter. Evaporate the filtrate to dryness and dry at a pressure not exceeding 0.7 kPa for 1 hour. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with griseofulvin RS.

B. Shake a quantity of the powdered tablets containing 80 mg of Griseofulvin with 150 ml of ethanol (95 per cent) for 20 minutes. Dilute to 200 ml with ethanol (95 per cent) and filter. Dilute 2 ml of the filtrate to 100 ml with ethanol (95 per cent). When examined in the range 230 nm to 360 nm (2.4.7),
the resulting solution shows absorption maxima at about 291 nm and 325 nm, and a shoulder at about 250 nm.

C. Dissolve about 5 mg of the powdered tablets in 1 ml of sulphuric acid and add 5 mg of powdered potassium dichromate; a wine-red colour is produced.

Tests

Related substances. Determine by gas chromatography (2.4.13).

Test solution (a). Add 60 ml of chloroform to a quantity of the powdered tablets containing 50 mg of Griseofulvin, heat at 60° with shaking for 20 minutes, cool and dilute to 100 ml with chloroform. Centrifuge and evaporate 20 ml of the clear supernatant liquid to about 1 ml.

Test solution (b). Prepare in the same manner as test solution (a) but adding 1 ml of a 0.1 per cent w/v solution of 9,10-diphenylanthracene (internal standard) in chloroform before diluting to 100 ml with chloroform.

Reference solution. Dissolve 5 mg of griseofulvin RS in chloroform and add 2 ml of the internal standard solution and sufficient chloroform to produce 200 ml. Evaporate 20 ml of the solution to about 1 ml.

Chromatographic system
– a glass column 1.0 m x 4 mm, packed with acid-washed diatomaceous support (100 to 200 mesh) coated with 1 per cent w/w of cyanopropylmethyl phenyl silicone fluid,
– temperature: column. 250°, inlet port and detector. 270°,
– flow rate. 60 ml per minute of the carrier gas.

Continue the chromatography for three times the retention time of griseofulvin.

The chromatogram obtained with test solution (a) shows a peak due to griseofulvin (retention time about 11 minutes) and may show a peak due to dechlorogriseofulvin (retention time about 0.6 times that of griseofulvin) and a peak due to dehydrogriseofulvin (retention time about 1.4 times that of griseofulvin).

Calculate the ratio (r) of the area of the peak due to griseofulvin to that of the peak due to the internal standard in the chromatogram obtained with the reference solution. The ratio of the area of any peak corresponding to dechlorogriseofulvin to that of the peak due to the internal standard in the chromatogram obtained with test solution (b) is less than 0.6r. The ratio of the area of any peak corresponding to dehydrogriseofulvin to that of the peak due to the internal standard in the chromatogram obtained with test solution (b) is less than 0.15r.

Dissolution (2.5.2).

Apparatus. No 1

Medium. 900 ml of a 4.0 per cent w/v solution of sodium lauryl sulphate

Speed and time. 100 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted with methanol (80 per cent), at the maximum at about 291 nm (2.4.7). Calculate the content of C17H17ClO6, taking 725 as the specific absorbance at the maximum at about 291 nm.

D. Not less than 70 per cent of the stated amount of C17H17ClO6.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 35 mg of Griseofulvin, add 60 ml of ethyl acetate. Centrifuge and transfer two quantities, each of 5 ml, of the clear supernatant liquid into separate 100-ml volumetric flasks. Add 5 ml of 2 M methanolic methanesulphonic acid to the first flask, allow to stand at 20° for 30 minutes and dilute to 100.0 ml with methanol (solution A). Dilute the contents of the second flask to 100.0 ml with methanol (solution B). To a third volumetric flask add 5 ml of 2 M methanolic methanesulphonic acid and dilute to 100.0 ml with methanol (solution C). Measure the absorbance of each solution at the maximum at about 266 nm (2.4.7). Calculate the content of C17H17ClO6, from the difference between the absorbance obtained with solution A and the sum of the absorbances obtained with solutions B and C and from the difference obtained by repeating the experiment using 35 mg of griseofulvin RS in place of the powdered tablets.

Guaiphenesin

Guaiphenesin is (RS)-3-(2-methoxyphenoxy)propane-1,2-diol.
Guaiphenesin contains not less than 98.0 per cent and not more than 101.5 per cent of C_{10}H_{14}O_{4}, calculated on the dried basis.

**Description.** A white or almost white, crystalline powder; odourless or with a slight characteristic odour.

**Identification.**

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with guaiphenesin RS or with the reference spectrum of guaiphenesin.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Melts at 79° to 83° (2.4.21).

**Tests.**

**Appearance of solution.** A 2.0 per cent w/v solution is clear (2.4.1), and colourless (2.4.1).

**pH** (2.4.24). 5.0 to 7.0, determined in a 1.0 per cent w/v solution.

**Related substances.** Determine by thin-layer chromatography (2.4.17), using a plate prepared in the following manner. Mix 0.3 g of carbomer with 240 ml of water, allow to stand with moderate stirring for 1 hour, adjust to pH 7 by the gradual addition, with stirring, of 2 M sodium hydroxide and add 30 g of silica gel H. Spread a uniform layer of the suspension 0.75 mm thick, allow the coated plate to dry in air for 16 hours, heat at 105° for 1 hour, allow to cool and use immediately.

**Mobile phase.** A mixture of 80 volumes of carbon tetrachloride and 20 volumes of ethanol (95 per cent).

**Test solution (a).** Dissolve 0.2 g of the substance under examination in 10 ml of dichloromethane.

**Test solution (b).** Dilute 5 ml of test solution (a) to 50 ml with dichloromethane.

**Reference solution (a).** A 0.01 per cent w/v solution of the substance under examination in dichloromethane.

**Reference solution (b).** A 0.2 per cent w/v solution of guaiphenesin RS in dichloromethane.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and spray with a mixture of equal volumes of a 1 per cent w/v solution of potassium ferricyanide, a 20 per cent w/v solution of ferric chloride hexahydrate and ethanol (95 per cent). Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Heavy metals** (2.3.13). 12 ml of a solution prepared by dissolving 2.0 g in 25 ml of a mixture of 9 volumes of ethanol (95 per cent) and 1 volume of water complies with the limit test for heavy metals, Method D (25 ppm).

**Chlorides and monochlorohydrins.** To 10 ml of a 2.0 per cent w/v solution, add 2 ml of 2 M sodium hydroxide, heat on a water-bath for 5 minutes, cool and add 3 ml of 2 M nitric acid. The resulting solution complies with the limit test for chlorides (2.3.12) using 2.0 ml of chloride standard solution (25 ppm Cl)/250 ppm).

**Guaiacol.** To 10 ml of a 2.0 per cent w/v solution add 0.1 ml of ferric chloride test solution and allow to stand for 5 minutes. The resulting solution is not more intensely coloured than a mixture of 0.5 ml of CSS, 1.5 ml of FCS, 3.5 ml of CCS and 4.5 ml of a solution of hydrochloric acid containing 1 per cent w/v of HCl (2.4.1).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 100° over phosphorus pentoxide at a pressure of 1.5 to 2.5 kPa for 3 hours.

**Assay.** Weigh accurately about 50 mg and dissolve in 10 ml of water. Add 20 ml of sodium periodate solution and allow to stand for 10 minutes. Add 25.0 ml of sodium arsenite solution and 1 ml of a 16.6 per cent w/v solution of potassium iodide, allow to stand for 10 minutes and titrate with 0.05 M iodine using 2 ml of starch solution as indicator. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of iodine required. 1 ml of 0.05 M iodine is equivalent to 0.009911 g of C_{10}H_{14}O_{4}.
H

Haloperidol
Haloperidol Injection
Haloperidol Oral Solution
Haloperidol Tablets
Heparin Sodium
Heparin Injection
Histamine Phosphate
Histamine Phosphate Injection
Homatropine Hydrobromide
Homatropine Eye Drops
Hyaluronidase
Hyaluronidase Injection
Hydralazine Hydrochloride
Hydralazine Injection
Hydrochloric Acid
Dilute Hydrochloric Acid
Hydrochlorothiazide
Hydrochlorothiazide Tablets
Hydrocortisone
Hydrocortisone Acetate
Hydrocortisone Eye Ointment
Hydrocortisone Acetate Injection
Hydrocortisone Hemisuccinate
Hydrocortisone Sodium Succinate Injection
Hydrogen Peroxide Solution (20 Vol)
Hydrogen Peroxide Solution (100 Vol)
Hydroxocobalamin
Hydroxocobalamin Injection
Hydroxyprogesterone Hexanoate
Hydroxyprogesterone Injection
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Haloperidol

\[
\begin{align*}
C_{21}H_{23}ClFNO_2 & \quad \text{Mol. Wt. 375.9}
\end{align*}
\]

Haloperidol is 4-[4-(4-chlorophenyl)-4-hydroxypiperidino]-4′-fluorobutyrophenone.

Haloperidol contains not less than 99.0 per cent and not more than 101.0 per cent of C_{21}H_{23}ClFNO_2, calculated on the dried basis.

**Description.** A white to faintly yellowish, amorphous or microcrystalline powder; odourless.

**Identification**

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry \((2.4.6)\). Compare the spectrum with that obtained with haloperidol RS or with the reference spectrum of haloperidol.

B. When examined in the range 230 nm to 360 nm \((2.4.7)\), a 0.0015 per cent w/v solution in a mixture of 90 volumes of methanol and 10 volumes of 0.1 M hydrochloric acid shows an absorption maximum at about 245 nm; absorbance at about 245 nm, about 0.49 to 0.53.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

D. Determine by the oxygen-flask method \((2.3.34)\), using 20 mg of the substance under examination and 5 ml of 1 M sodium hydroxide as the absorbing liquid. When the process is complete, dilute to 10 ml with water; the resulting solution complies with the following tests.

(a) Add 0.1 ml to a mixture of 0.1 ml of a freshly prepared alizarin red S solution and 0.1 ml of zirconyl nitrate solution; the red colour becomes clear yellow.

(b) Acidify 5 ml with 0.5 M sulphuric acid; the solution gives the reactions of chlorides \((2.3.1)\).

**Tests**

**Related substances.** Determine by thin-layer chromatography \((2.4.17)\), coating the plate with silica gel G.

**Mobile phase.** A mixture of 80 volumes of chloroform, 10 volumes of glacial acetic acid and 10 volumes of methanol.

*Test solution (a).* Dissolve 0.1 g of the substance under examination in 10 ml of chloroform.

*Test solution (b).* Dilute 5 ml of test solution (a) to 50 ml with chloroform.

*Reference solution (a).* A solution containing 0.005 per cent w/v of the substance under examination in chloroform.

*Reference solution (b).* A 0.1 per cent w/v solution of haloperidol RS in chloroform.

Sulphated ash \((2.3.18)\). Not more than 0.1 per cent.

**Loss on drying** \((2.4.19)\). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.1 kPa for 3 hours.

**Assay.** Weigh accurately about 0.2 g and dissolve in 25 ml of anhydrous glacial acetic acid. Titrate with 0.05 M perchloric acid, using 0.2 ml of 1-naphtholbenzein solution as indicator and titrating until the colour changes from orange-yellow to yellowish green. Carry out a blank titration.

1 ml of 0.05 M perchloric acid is equivalent to 0.01879 g C_{21}H_{23}ClFNO_2.

**Storage.** Store protected from light.

Haloperidol Injection

Haloperidol Injection is a sterile solution of Haloperidol in Lactic Acid diluted with Water for Injections.

Haloperidol Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of haloperidol, C_{21}H_{23}ClFNO_2.

**Identification**

A. To a volume of the injection containing 20 mg of Haloperidol add 5 ml of water and 1 ml of 1 M sodium hydroxide and extract with 10 ml of chloroform. Filter the chloroform extract through absorbent cotton, evaporate the filtrate to dryness and dry the residue at 60° at a pressure not exceeding 0.7 kPa. The residue complies with the following test.

Determine by infrared absorption spectrophotometry \((2.4.6)\). Compare the spectrum with that obtained with haloperidol RS or with the reference spectrum of haloperidol.
B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum only at about 245 nm.

**Tests**

**pH** (2.4.24). 2.8 to 3.6.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 80 volumes of chloroform, 10 volumes of glacial acetic acid and 10 volumes of methanol.

**Test solution.** The injection under examination.

**Reference solution (a).** Dilute 1 volume of the injection to 100 volumes with methanol.

**Reference solution (b).** Dilute 1 volume of the injection to 200 volumes with methanol.

Apply to the plate a volume of the injection containing 0.1 mg of Haloperidol and the same volume of the reference solutions. After development, dry the plate in air and spray with dilute potassium iodobismuthate solution. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** To an accurately measured volume of the injection containing about 10 mg of Haloperidol add 8 ml of water and 10 ml of 1 M hydrochloric acid. Extract with successive quantities of 25, 25, 10 and 10 ml of ether. Wash the combined ether extracts with 10 ml of water, combine the aqueous layers and remove the ether using a rotary evaporator. Add sufficient water to produce 100.0 ml and dilute 10.0 ml to 100.0 ml with methanol. Measure the absorbance of the resulting solution at the maximum at about 245 nm (2.4.7). Calculate the content of \( \text{C}_21\text{H}_{23}\text{ClFNO}_2 \) taking 346 as the specific absorbance at 245 nm.

**Storage.** Store protected from light.

**Haloperidol Oral Solution**

Haloperidol Oral Drops; Haloperidol Solution

Haloperidol Oral Solution is a solution of Haloperidol in Purified Water prepared with the aid of Lactic Acid.

Haloperidol Oral Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of haloperidol, \( \text{C}_21\text{H}_{23}\text{ClFNO}_2 \).

**Description.** A clear, colourless solution.

**Identification**

A. To a volume of the oral solution containing 20 mg of Haloperidol, add 1 ml of 1 M sodium hydroxide, extract with 10 ml of chloroform, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with haloperidol RS treated in the same manner or with the reference spectrum of haloperidol.

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum only at about 245 nm.

**Tests**

**pH** (2.4.24). 3.5 to 4.5.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 92 volumes of dichloromethane, 8 volumes of methanol and 1 volume of strong ammonia solution.

**Test solution.** Dilute the oral solution if necessary with methanol to contain 0.1 per cent w/v of Haloperidol.

**Reference solution (a).** Dilute 1 volume of the test solution to 100 volumes with methanol.

**Reference solution (b).** Dilute 1 volume of the test solution to 200 volumes with methanol.

Apply to the plate 50 µl of each solution. After development, dry the plate in air and spray with dilute potassium iodobismuthate solution. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Other tests.** Complies with the tests stated under Oral Liquids.

**Assay.** To an accurately measured volume of the oral solution containing about 10 mg of Haloperidol add 8 ml of water and 10 ml of 1 M hydrochloric acid. Extract with successive quantities of 25, 25, 10 and 10 ml of ether. Wash the combined ether extracts with 10 ml of water, combine the aqueous layers and remove the ether using a rotary evaporator. Add sufficient water to produce 100.0 ml and dilute 10.0 ml to 100.0 ml with methanol. Measure the absorbance of the resulting solution at the maximum at about 245 nm (2.4.7). Calculate the content of \( \text{C}_21\text{H}_{23}\text{ClFNO}_2 \) taking 346 as the specific absorbance at 245 nm.

**Storage.** Store protected from light at a temperature between 15° and 25°.
Haloperidol Tablets

Haloperidol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of haloperidol, C_{21}H_{23}ClFNO_{2}.

Identification

A. To a quantity of the powdered tablets containing 10 mg of Haloperidol add 5 ml of water and 1 ml of 1 M sodium hydroxide and extract with 10 ml of chloroform. Filter the chloroform extract through absorbent cotton, evaporate the filtrate to dryness and dry the residue at 60° at a pressure not exceeding 0.7 kPa. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with haloperidol RS or with the reference spectrum of haloperidol.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 80 volumes of chloroform, 10 volumes of glacial acetic acid and 10 volumes of methanol.

Test solution (a). Shake a quantity of the powdered tablets containing 10 mg of Haloperidol with 10 ml of chloroform, filter, evaporate the filtrate to dryness and dissolve the residue in 1 ml of chloroform.

Test solution (b). Dilute 1 volume of test solution (a) to 10 volumes with chloroform.

Reference solution (a). Dilute 1 volume of test solution (a) to 200 volumes with chloroform.

Reference solution (b). A 0.1 per cent w/v solution of haloperidol RS in chloroform.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with dilute potassium iodobismuthate solution. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (a) shows a distinct and clearly visible spot.

Uniformity of content. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

Test solution. Place one tablet in 10 ml of the mobile phase, shake in an ultrasonic bath for 2 minutes, centrifuge and use the supernatant liquid after diluting suitably with the mobile phase if necessary.

Reference solution. A solution containing 0.015 per cent w/v of haloperidol RS in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 5 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 55 volumes of a 1 per cent w/v solution of ammonium acetate and 45 volumes of acetonitrile,
- flow rate, 2 ml per minute,
- spectrophotometer set at 247 nm,
- a 20 µl loop injector.

Calculate the content of C_{21}H_{23}ClFNO_{2} in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. For tablets containing more than 2 mg — Weigh and powder 20 tablets. On the powder determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of the powder containing about 20 mg of Haloperidol, shake with 60 ml of the mobile phase, place in an ultrasonic bath for 2 minutes, add sufficient quantity of the mobile phase to produce 100.0 ml. Centrifuge and use the supernatant liquid.

Reference solution. A solution containing 0.02 per cent w/v of haloperidol RS in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 5 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 55 volumes of a 1 per cent w/v solution of ammonium acetate and 45 volumes of acetonitrile,
- flow rate, 2 ml per minute,
- spectrophotometer set at 247 nm,
- a 20 µl loop injector.

Calculate the content of C_{21}H_{23}ClFNO_{2} in the tablets.

For tablets containing 2 mg or less — Use the average of the 10 individual results obtained in the test for Uniformity of content.

Storage. Store protected from light.

Heparin Sodium

Heparin Sodium is a preparation containing the sodium salt of a complex organic acid present in mammalian tissues, and having the characteristic property of delaying the clotting of shed blood. It may be obtained from the lungs or intestinal mucosa of oxen, pigs or sheep. It is prepared in conditions...
designed to minimise or eliminate microbial contamination and substances lowering blood pressure.

Heparin Sodium intended for use in the manufacture of parenteral preparations contains not less than 150 Units per mg and Heparin Sodium not intended for use in the manufacture of parenteral preparations contains not less than 120 Units per mg, both calculated on the dried basis.

**Description.** A white or greyish-white powder; odourless; moderately hygroscopic.

**Identification**

A. It delays the clotting of freshly shed blood.

B. To 0.1 g in a test-tube add 0.2 g of sodium and heat cautiously until the reaction with sodium is complete. Heat to bright red heat and carefully plunge the tube and the contents into 5 ml of water. Filter, boil the filtrate for a few minutes with 20 mg of ferrous sulphate. Cool, acidify with hydrochloric acid and add 0.05 ml of ferric chloride test solution; a blue colour is produced (distinction from dextran sulphate).

C. The residue obtained in the test for Sulphated ash gives reaction A of sodium salts (2.3.1).

**Tests**

**Appearance of solution.** A solution containing 5000 Units per ml is clear (2.4.1), and not more intensely coloured than degree 5 of the appropriate range of reference solutions (2.4.1).

**pH** (2.4.24). 5.5 to 8.0, determined in a 1.0 per cent w/v solution.

**Heavy metals** (2.3.13). 0.5 g complies with the limit test for heavy metals, Method B (40 ppm).

**Protein and nucleotidic impurities.** Absorbance of a 0.4 per cent w/v solution at about 260 nm and about 280 nm, not greater than 0.2 and 0.15 respectively (2.4.7).

**Sulphated ash** (2.3.18). 30 to 43 per cent, determined on 0.2 g.

**Loss on drying** (2.4.19). Not more than 8.0 per cent, determined on 1.0 g by drying in an oven over phosphorus pentoxide at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

**Assay.** Determine the potency of heparin sodium by comparing the concentration necessary to prevent the clotting of sheep or goat or human plasma with the concentration of the Standard Preparation of heparin sodium necessary to give the same effect under the conditions of the following method of assay.

**Standard Preparation and Unit**

**Standard Preparation.** The freeze-dried sodium salt of the purified active principle from bovine intestinal mucous membranes or any other suitable preparation, the potency of which has been determined in relation to the International Standard. The specific activity contained in 7.7 µg of the Standard Preparation and is the same as the International Unit; 1 mg contains 130 Units.

**Special Reagents**

**Prepared Plasma.** Collect blood from sheep or goats or human volunteers directly into a vessel containing 8 per cent w/v solution of sodium citrate in the proportion of 1 volume to each 19 volumes of blood to be collected. Mix immediately by gentle agitation and inversion of the vessel. Immediately centrifugate and pool the separated plasma. To 1 ml of the pooled plasma in a clean test-tube add 0.2 ml of a 1 per cent w/v solution of calcium chloride and mix. The plasma is suitable if a solid clot forms within 5 minutes.

**Solution of standard preparation.** Determine by preliminary trial, if necessary, approximately the minimum quantity of the Standard Preparation of heparin sodium which, when added in 0.8 ml of saline solution, maintains fluidity in 1 ml of prepared plasma for 1 hour after the addition of 0.2 ml of a 1 per cent w/v solution of calcium chloride. On the day of the assay prepare a solution of the Standard Preparation such that it contains in each 0.8 ml of saline solution the above-determined quantity of the Standard Preparation.

**Test solution.** Weigh accurately about 25 mg of the preparation under examination and dissolve in sufficient saline solution to give a concentration of 1 mg per ml and dilute to a concentration estimated to correspond to that of the solution of the Standard Preparation.

**Method**

To very clean test-tubes (150 mm x 16 mm) add graded amounts of the solution of standard preparation, selecting the amounts so that the largest dose does not exceed 0.8 ml and so that they correspond roughly to a geometric series in which each step is approximately 5 per cent greater than the next lower. To each tube add sufficient saline solution to make the total volume 0.8 ml. Add 1.0 ml of prepared plasma to each tube. Then add 0.2 ml of a 1 per cent w/v solution of calcium chloride, note the time, immediately stopper each tube with a suitable stopper and mix the contents by inverting three times in such a way that the entire inner surface of the tube is wet.

In the same manner set up a series using the test solution, completing the entire process of preparing and mixing the tubes of both the solution of standard preparation and the test solution within 20 minutes after the addition of the prepared plasma. Exactly one hour after the addition of the calcium chloride solution, determine the extent of clotting in each tube, recognising three grades between zero and full clotting.

The dilution of the test solution, which contains heparin sodium in the same concentration as the dilution of the solution of standard preparation, is that contained in the series of dilutions, which show the same degree of clotting as the series...
of dilutions of the solution of standard preparation. If the degree of clotting observed in the series of dilutions of the solution of standard preparation lies between that observed in two of the series of dilutions of the sample being examined, the potency of the latter is estimated. If there is no such correspondence between the degrees of clotting produced by the solution of standard preparation and any of the dilutions of the sample being examined, new dilutions of the latter are prepared and assay is repeated.

Carry out no fewer than three independent assays. Calculate the estimated potency of the preparation being examined by combining the results of these assays by standard statistical methods.

Limits of error \((P = 0.99)\). 90 and 110 per cent, with three determinations; 92 and 108 per cent, with four determinations.

Heparin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.03 Endotoxin Unit per Unit of heparin.

Heparin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility. Complies with the test for sterility (2.2.11).

Storage. Store protected from moisture in tightly-closed containers, sealed so as to exclude micro-organisms.

Labelling. The label states (1) the number of Units in the container; (2) the number of Units per mg; (3) the name and quantity of any added substance; (4) where applicable, that it is sterile; (5) the source of the material (lung or mucosal).

Heparin Injection

Heparin Sodium Injection

Heparin Injection is a sterile solution of Heparin Sodium in Water for Injections. The pH of the solution may be adjusted by the addition of a suitable alkali.

Heparin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated potency in terms of Units per ml.

Description. A clear, colourless or straw-coloured solution, free from turbidity and matter which deposits on standing

Identification

A. It delays the clotting of freshly shed blood.
B. Gives reaction A of sodium salts (2.3.1).

Tests

pH (2.4.24). 5.5 to 8.0.

Bacterial endotoxins (2.2.3). Not more than 0.03 Endotoxin Unit per Unit of heparin.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Determine the potency of heparin sodium by comparing the concentration necessary to prevent the clotting of sheep or goat or human plasma with the concentration of the Standard Preparation of heparin sodium necessary to give the same effect.

Standard Preparation and Unit

Standard Preparation. The freeze-dried sodium salt of the purified active principle from bovine intestinal mucous membranes or any other suitable preparation, the potency of which has been determined in relation to the International Standard.

Unit. The specific activity contained in 7.7 \(\mu\)g of the Standard Preparation and is the same as the International Unit; 1 mg contains 130 Units.

Special Reagents

Prepared Plasma. Collect blood from sheep or goats or human volunteers directly into a vessel containing 8 per cent w/v solution of sodium citrate in the proportion of 1 volume to each 19 volumes of blood to be collected. Mix immediately by gentle agitation and inversion of the vessel. Immediately centrifuge and pool the separated plasma. To 1 ml of the pooled plasma in a clean test-tube add 0.2 ml of a 1 per cent w/v solution of calcium chloride and mix. The plasma is suitable if a solid clot forms within 5 minutes.

Solution of standard preparation. Determine by preliminary trial, if necessary, approximately the minimum quantity of the Standard Preparation of heparin sodium which, when added in 0.8 ml of saline solution, maintains fluidity in 1 ml of prepared plasma for 1 hour after the addition of 0.2 ml of a 1 per cent w/v solution of calcium chloride. On the day of the assay prepare a solution of the Standard Preparation such that it contains in each 0.8 ml of saline solution the above-determined quantity of the Standard Preparation.

Test solution. Dilute the injection to a concentration estimated to correspond to that of the solution of the Standard Preparation.

Method

To very clean test-tubes (150 mm x 16 mm) add graded amounts of the solution of standard preparation, selecting the amounts so that the largest dose does not exceed 0.8 ml and so that they correspond roughly to a geometric series in which each
step is approximately 5 per cent greater than the next lower. To each tube add sufficient saline solution to make the total volume 0.8 ml. Add 1.0 ml of prepared plasma to each tube. Then add 0.2 ml of a 1 per cent w/v solution of calcium chloride, note the time, immediately stopper each tube with a suitable stopper and mix the contents by inverting three times in such a way that the entire inner surface of the tube is wet.

In the same manner set up a series using the test solution, completing the entire process of preparing and mixing the tubes of both the solution of standard preparation and the test solution within 20 minutes after the addition of the prepared plasma. Exactly one hour after the addition of the calcium chloride solution, determine the extent of clotting in each tube, recognising three grades between zero and full clotting.

The dilution of the test solution, which contains heparin sodium in the same concentration as the dilution of the solution of standard preparation, is that contained in the series of dilutions, which show the same degree of clotting as the series of dilutions of the solution of standard preparation. If the degree of clotting observed in the series of dilutions of the solution of standard preparation lies between that observed in two of the series of dilutions of the sample being examined, the potency of the latter is estimated. If there is no such correspondence between the degrees of clotting produced by the solution of standard preparation and any of the dilutions of the sample being examined, new dilutions of the latter are prepared and assay is repeated.

Carry out no fewer than three independent assays. Calculate the estimated potency of the preparation being examined by combining the results of these assays by standard statistical methods. Express the result in number of Units per ml.

Limits of error (P = 0.99). 90 and 110 per cent, with three determinations; 92 and 108 per cent, with four determinations.

Storage. Store at a temperature not exceeding 30°.

Labelling. The label states (1) the strength in terms of the number of Units in a suitable dose-volume except that for multiple dose containers the strength is stated as the number of Units per ml; (2) the source of the material (lung or mucosal); (3) when no antimicrobial preservative is present that the injection contains no antimicrobial preservative; (4) that any portion of the contents not used at once should be discarded.

Histamine Phosphate
Histamine Phosphate is 2-(1H-imidazol-4-yl)ethylamine diphosphate monohydrate.

Histamine Phosphate contains not less than 98.0 per cent and not more than 101.0 per cent of C_{6}H_{10}N_{2}H_{8}O_{4}, calculated on the anhydrous basis.

Description. Colourless, long prismatic crystals; odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with histamine phosphate RS.

B. In the test for Histidine, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. Dissolve 0.1 g in 7 ml of water and add 3 ml of sodium hydroxide solution. Dissolve 50 mg of sulphanilic acid in 10 ml of water containing 0.1 ml of hydrochloric acid and 0.1 ml of a 10 per cent w/v solution of sodium nitrite. On mixing the two solutions a deep red colour is produced.

D. Gives reaction A of phosphates (2.3.1).

Tests

Appearance of solution. Solution A is clear (2.4.1), and not more intensely coloured than reference solution BYS7 (2.4.1). pH (2.4.24). 3.7 to 3.9, determined in a 5.0 per cent w/v solution in carbon dioxide-free water prepared from distilled water (solution A).

Histidine. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 75 volumes of acetonitrile, 20 volumes of water and 5 volumes of strong ammonia solution.

Test solution (a). Dissolve 0.5 g of the substance under examination in 10 ml of water.

Test solution (b). Dilute 5 ml of test solution to 25 ml with water.

Reference solution (a). A 1.0 per cent w/v solution of histamine phosphate RS.

Reference solution (b). A solution containing 0.05 per cent w/v of DL-histidine monohydrochloride.

Reference solution (c). A mixture of equal volumes of test solution (a) and reference solution (b).

Apply to the plate 1 µl of each solution. After development, dry the plate in a current of air and repeat the development in the same direction. Dry the plate in a current of air, spray with ninhydrin solution and heat at 110° for 10 minutes. Any spot
corresponding to histidine monohydrochloride in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

**Sulphates** (2.3.17). 3 ml of solution A diluted to 15 ml with distilled water complies with the limit test for sulphates (0.1 per cent).

**Water** (2.3.43). 5.0 to 6.2 per cent, determined on 0.3 g.

**Assay.** Weigh accurately about 0.14 g, dissolve in 5 ml of anhydrous formic acid and add 20 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.01536 g of C₅H₉N₃·2H₃PO₄.

**Storage.** Store protected from light.

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**Histamine Phosphate Injection**

Histamine Phosphate Injection is a sterile solution of Histamine Phosphate in Water for Injections.

Histamine Phosphate Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of histamine phosphate, C₅H₉N₃·2H₃PO₄·H₂O.

**Identification**

A. Evaporate a volume of the injection containing about 2 mg of Histamine Phosphate on a water bath to dryness, dissolve the residue in 0.5 ml of water, and add 0.5 ml of sodium hydroxide. Add 2 drops of a 10 per cent w/v solution of sodium nitrite and 1 ml of a solution prepared by mixing 50 mg of sulphanilic acid with 10 ml of water containing 2 drops of hydrochloric acid; an orange-red colour is produced.

B. To 1 ml of the injection containing not less than 1 mg of Histamine Phosphate (concentrate a larger volume by evaporation, if necessary), add ammonium molybdate solution dropwise; a yellow precipitate, which is soluble in ammonia, is formed.

**Tests**

**pH** (2.4.24). 3.0 to 6.0.

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** Measure accurately a volume of the injection containing about 10 mg of Histamine Phosphate, transfer to a tared 25 ml centrifuge tube containing a thin glass rod slightly curved at the end, add 0.5 ml of nitranilic acid solution with continuous stirring and allow to stand for 15 minutes. Add 10 ml of ethanol (95 per cent), mix and keep at 0° for 3 hours. Centrifuge for 1 minute, dislodge any particles at the surface and again centrifuge for 1 minute. Decant the supernatant liquid and stir the precipitate with 5 ml of ice-cold ethanol (95 per cent). Centrifuge for 2 minutes, decant and repeat the washing with two further quantities, each of 5 ml, of ice-cold ethanol (95 per cent) and finally with 5 ml of ether. Smear the residue over the inside of the tube by means of the glass rod and dry to constant weight at 130°.

1 g of the residue is equivalent to 0.9529 g of C₁₆H₂₁NO₃·2H₃PO₄·H₂O.

**Storage.** Store protected from light.

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**Homatropine Hydrobromide**

**Homatropine Hydrobromide** is (1R,3r,5S)-3-(RS)-mandeloxytropane hydrobromide.

Homatropine Hydrobromide contains not less than 99.0 per cent and not more than 101.0 per cent of C₁₆H₂₁NO₃·HBr, calculated on the dried basis.

**Description.** Colourless crystals or a white, crystalline powder; odourless.

**Identification**

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with homatropine hydrobromide RS or with the reference spectrum of homatropine hydrobromide.

B. Dissolve 50 mg in 1 ml of water and add 2 ml of 2 M acetic acid. Heat, add 4 ml of picric acid solution and allow to cool, shaking occasionally. The crystals, after washing with two quantities, each of 3 ml, of iced water and drying at 105° melt at 182° to 186° (2.4.21).
Homatropine Eye Drops contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of homatropine hydrobromide, \(C_{16}H_{21}NO_3\cdot HBr\).

**Identification**

A. To a volume containing 60 mg of Homatropine Hydrobromide add 3 ml of dilute ammonia solution, extract with 15 ml of chloroform, dry the chloroform extract over anhydrous sodium sulphate, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with homatropine hydrobromide RS or with the reference spectrum of homatropine hydrobromide.

B. To the residue obtained in test A, add 1.5 ml of a 2 per cent w/v solution of mercuric chloride in ethanol (60 per cent); a yellow colour is produced which becomes red on gentle warming (distinction from most other alkaloids except atropine and hyoscyamine).

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 134 volumes of ethyl acetate, 33 volumes of anhydrous formic acid and 33 volumes of water.

**Test solution.** Use the eye drops, diluted if necessary with water to contain 1 per cent w/v of Homatropine Hydrobromide.

**Reference solution.** Dilute 1 volume of the test solution to 200 volumes with methanol (90 per cent).

Apply to the plate 5 µl of each solution. After development, dry the plate at 105° until the odour of the solvent is no longer detectable, allow to cool and spray with dilute potassium iodobismuthate solution until spots appear. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent, determined on 0.5 g.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.3 g, dissolve in 20 ml of anhydrous glacial acetic acid and add 7 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.03563 g of \(C_{16}H_{21}NO_3\cdot HBr\).

**Storage.** Store protected from light.

Homatropine Eye Drops

Homatropine Hydrobromide Eye Drops

Homatropine Eye Drops are a sterile solution of Homatropine Hydrobromide in Purified Water.
quantities, each of 5 ml, of chloroform, shake the combined extracts with 1 g of anhydrous sodium sulphate, filter and evaporate the filtrate to dryness. Dissolve the residue in 10.0 ml of dichloromethane. To 1.0 ml of this solution add 0.2 ml of a mixture of 4 volumes of N,O-bis (trimethylsilyl)acetamide and 1 volume of trimethylchlorosilane, mix and allow to stand for 30 minutes.

Test solution (b). Prepare in the same manner as test solution (a) but omitting the addition of solution A.

Reference solution. Add 1 ml of solution A and 1 ml of dilute ammonia solution to 5.0 ml of a 0.4 per cent w/v solution of homatropine hydrobromide RS and complete the procedure described under test solution (a) beginning at the words “Extract with two quantities, each of 5 ml, of chloroform....”.

Chromatographic system
- a glass column 1.5 m x 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) (such as OV-17),
- temperature: column, 220°,
  inlet port and detector, 280°,
- flow rate, 30 ml per minute of the carrier gas.

Calculate the content of C₃H₂₁NO₅,HBr in the eye drops.

**Hyaluronidase**

Hyaluronidase is a material containing enzymes, which depolymerise the mucopolysaccharide, hyaluronic acid. It may be prepared from the testes and semen of mammals and purified by fractional precipitation so as to remove inert material and to which hydrolysed gelatin or a suitable non-protein stabilising agent may be added. The product is freeze-dried in single dose containers, which are sealed so as to exclude micro-organisms.

Hyaluronidase contains not less than 300 Units per mg, calculated on the dried basis. It may contain a suitable stabilizer.

**Description.** A white or yellowish-white, fluffy powder.

**Identification**

A. A solution containing the equivalent of 100 Units in 1 ml of saline solution depolymerises an equal volume of a 1 per cent w/v solution of sodium hyaluronate at 20° in 1 minute as shown by a pronounced decrease in viscosity. This action is destroyed by heating the initial solution at 100° for 30 minutes.

B. A solution containing the equivalent of 1 Unit in 0.2 ml of saline solution when injected intracutaneously into experimental animals together with a suitable indicator shows a spreading activity when compared with a control solution.

**Tests**

**Appearance of solution.** A 1.0 per cent w/v solution in carbon dioxide-free water is clear (2.4.1), and not more than faintly yellow.

**pH** (2.4.24). 4.5 to 7.5, determined in a 0.3 per cent w/v solution in carbon dioxide-free water.

**Light absorption.** Dissolve a quantity containing 1500 Units in sufficient carbon dioxide-free water to produce 5.0 ml and measure the absorbance of the resulting solution at about 260 nm and 280 nm; absorbance at about 260 nm, not more than 0.42 and at about 280 nm, not more than 0.60 (2.4.7).

**Bacterial endotoxins** (2.2.3). Not more than 0.2 Endotoxin Unit per unit of hyaluronidase.

**Sterility** (2.2.11). Complies with the test for sterility.

**Assay.** The potency of hyaluronidase is determined by comparing its effects against those of the Standard Preparation.

**Standard Preparation**

The Standard Preparation is the 1st International Standard for Hyaluronidase, bovine, established in 1955, consisting of dried bovine testicular hyaluronidase diluted with lactose (supplied in ampoules containing 10 tablets of 20 mg each; each tablet contains approximately 200 Units).

Test solution. Dissolve a suitable quantity of the preparation under examination by adding cold diluent for hyaluronidase solutions. Dilute the solution with cold diluent for hyaluronidase solutions so that the absorbances of the dilutions being assayed will fall on the upper linear part of the reference curve prepared as follows.

To each of 12 test-tubes (100 mm x 16 mm) add 0.50 ml of hyaluronate solution and, respectively and in duplicate, 0.5, 0.4, 0.3, 0.2, 0.1 and 0.0 ml of diluent for hyaluronidase solutions. If quantities of the solution of the standard solution other than those indicated below are used, change the quantities of diluent for hyaluronidase solutions accordingly. At intervals of 30 seconds add to the tubes 0.0, 0.1, 0.2, 0.3, 0.4 and 0.5 ml of the solution of the standard solution, respectively and in duplicate, making the final volume in each tube 1.0 ml, mixing the contents by shaking gently and placing each tube in a water-bath maintained at 37.0° ± 0.2°. After exactly 30 minutes, remove each tube in order from the water-bath at intervals of 30 seconds and immediately add 4.0 ml of serum solution. Shake and allow to stand at room temperature for 30 minutes. Shake again and measure the absorbance at about 640 nm (2.4.7). Repeat the operation using 0.50 ml of phosphate-buffered saline in place of the hyaluronate solution and make any necessary corrections. Prepare a reference curve by plotting the mean of the corrected absorbance for each level against the potency.
Standard solution. Dissolve one tablet of the Standard Preparation, accurately weighed, in sufficient cold diluent for hyaluronidase solutions to give a solution of known concentration containing about 1.5 Units per ml. This solution should be prepared immediately before use.

To each of 6 test-tubes (100 mm x 16 mm) add 0.50 ml of hyaluronate solution and sufficient diluent for hyaluronidase solutions so that the final volume in each tube after the addition of the solution of the preparation being examined is 1.0 ml. At intervals of 30 seconds add to each tube sufficient of the solution of the preparation being examined so that the tubes contain about 0.3, 0.5 and 0.7 Units, respectively and in duplicate, shaking each tube gently and continuing as described under test solution, beginning at the words “placing each tube in a water-bath...”

Storage. Store protected from moisture at a temperature not exceeding 15°.

Labelling. The label states (1) the total number of Units in the container; (2) the name of any added stabilising agent; (3) that the preparation is not intended for intravenous injection.

Hyaluronidase Injection

Hyaluronidase Injection is a sterile material consisting of Hyaluronidase with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Hyaluronidase Injection contains not less than 90.0 per cent and not more than 115.0 per cent of the stated number of Units of hyaluronidase activity.

Description. A white or yellowish-white powder.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. A solution containing the equivalent of 100 Units in 1 ml of saline solution depolymerises an equal volume of a 1 per cent w/v solution of sodium hyaluronate at 20° in 1 minute as shown by a pronounced decrease in viscosity. This action is destroyed by heating the initial solution at 100° for 30 minutes.

B. A solution containing the equivalent of 1 Unit in 0.2 ml of saline solution when injected intracutaneously into experimental animals together with a suitable indicator shows a spreading activity when compared with a control solution.

Tests

pH (2.4.24). 4.5 to 7.5, determined in a 0.3 per cent w/v solution in carbon dioxide-free water.

Appearance of solution. A 1.0 per cent w/v solution in carbon dioxide-free water is clear (2.4.1), and not more than faintly yellow.

Bacterial endotoxins (2.2.3). Not more than 0.2 Endotoxin Unit per unit of hyaluronidase.

Assay. The potency of hyaluronidase is determined by comparing its effects against those of the Standard Preparation.

Standard Preparation

The Standard Preparation is the 1st International Standard for Hyaluronidase, bovine, established in 1955, consisting of dried bovine testicular hyaluronidase diluted with lactose (supplied in ampoules containing 10 tablets of 20 mg each; each tablet contains approximately 200 Units).

Test solution. Dissolve the contents of a container by adding cold diluent for hyaluronidase solutions. Dilute the solution with cold diluent for hyaluronidase solutions so that the absorbances of the dilutions being assayed will fall on the upper linear part of the reference curve prepared as follows.

To each of 12 test-tubes (100 mm x 16 mm) add 0.50 ml of hyaluronate solution and, respectively and in duplicate, 0.5, 0.4, 0.3, 0.2, 0.1 and 0.0 ml of the solution of the standard solution, respectively and in duplicate, making the final volume in each tube 1.0 ml, mixing the contents by shaking gently and placing each tube in a water-bath maintained at 37.0° ± 0.2°. After exactly 30 minutes, remove each tube in order from the water-bath at intervals of 30 seconds and immediately add 4.0 ml of serum solution. Shake and allow to stand at room temperature for 30 minutes. Shake again and measure the absorbance at about 640 nm (...). Repeat the operation using 0.50 ml of phosphate-buffered saline in place of the hyaluronate solution and make any necessary corrections. Prepare a reference curve by plotting the mean of the corrected absorbance for each level against the potency.
Standard solution. Dissolve one tablet of the Standard Preparation, accurately weighed, in sufficient cold diluent for hyaluronidase solutions to give a solution of known concentration containing about 1.5 Units per ml. This solution should be prepared immediately before use.

To each of 6 test-tubes (100 mm x 16 mm) add 0.50 ml of hyaluronate solution and sufficient diluent for hyaluronidase solutions so that the final volume in each tube after the addition of the solution of the preparation being examined is 1.0 ml. At intervals of 30 seconds add to each tube sufficient of the solution of the preparation being examined so that the tubes contain about 0.3, 0.5 and 0.7 Units, respectively and in duplicate, shaking each tube gently and continuing as described under test solution, beginning at the words “placing each tube in a water-bath...”

Storage. Store protected from moisture at a temperature not exceeding 15°.

Labelling. The label states (1) the total number of Units contained in it; (2) the nature of any added stabilising agent; (4) that the injection should be used immediately after preparation; (5) that the preparation is not intended for intravenous injection.

Hydralazine Hydrochloride

\[
\text{C}_8\text{H}_8\text{N}_4\text{HCl} \quad \text{Mol. Wt. 196.6}
\]

Hydralazine Hydrobromide is phthalazin-1-ylhydrazine hydrochloride.

Hydralazine Hydrochloride contains not less 98.5 per cent and not more than 101.0 per cent of \(\text{C}_8\text{H}_8\text{N}_4\text{HCl}\), calculated on the dried basis.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with hydralazine hydrochloride RS or with the reference spectrum of hydralazine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution shows absorption maxima at about 240 nm, 260 nm, 305 nm and 315 nm and their absorbances are about 0.55, 0.55, 0.27 and 0.22 respectively.

C. Dissolve 0.5 g in a mixture of 8 ml of 2 M hydrochloric acid and 100 ml of water. Add 2 ml of sodium nitrite solution, allow to stand for 10 minutes and filter. The precipitate, after washing with water and drying at 105°, melts at 209° to 212° (2.4.21).

D. To a solution of about 10 mg in 2 ml of water add 2 ml of a 2 per cent w/v solution of 2-nitrobenzaldehyde in ethanol (95 per cent); an orange precipitate is obtained.

E. A 5 per cent w/v solution gives the reactions of chlorides (2.3.1).

Tests

Appearance of solution. A 0.4 per cent w/v solution is clear (2.4.1), and not more intensely coloured than reference solution GYS6(2.4.1).

pH (2.4.24). 3.5 to 4.2, determined in a 2.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution (a). Dissolve 25 mg of the substance under examination in sufficient quantity of the mobile phase to make 50 ml.

Test solution (b). Dilute 1 ml of test solution (a) to 100 ml with the mobile phase.

Reference solution (a). Dilute 5 ml of test solution (b) to 25 ml with the mobile phase.

Reference solution (b). Dissolve 25 mg of phthalazine in sufficient quantity of the mobile phase to make 50 ml and dilute 4 ml of this solution to 100 ml with the mobile phase.

Reference solution (c). Mix 4 ml of test solution (a) and 10 ml of reference solution (b) and dilute to 100 ml with the mobile phase.

The solutions should be used within 8 hours of preparation.

Chromatographic system

− a stainless steel column 25 cm x 4.6 mm, packed with porous spherical particles of finely-divided silica gel chemically bonded to nitrile groups (10 µm),
− mobile phase: a mixture of 22 volumes of acetonitrile and 78 volumes of a solution containing 1.44 g of sodium dodecyl sulphate and 0.75 g of tetrabutylammonium bromide per litre adjusted to pH 3.0 with 0.05 M sulphuric acid,
− flow rate. 1 ml per minute,
− spectrophotometer set at 230 nm,
− a 20 µl loop injector.
Inject test solution (b) and adjust the sensitivity of the detector so that the height of the principal peak in the chromatogram is not less than 70 per cent of the full scale of the recorder. When the chromatograms are recorded in the prescribed conditions, the retention time of hydralazine is about 10 to 12 minutes. If necessary, adjust the concentration of acetonitrile in the mobile phase.

Inject test solution (a) and continue the chromatography for 3 times the retention time of hydralazine. Inject reference solution (a). The area of any secondary peak in the chromatogram obtained with test solution (a) is not greater than the area of the peak in the chromatogram obtained with reference solution (a).

The test is not valid unless (a) the chromatogram obtained with reference solution (c) shows two principal peaks and the resolution between the peaks is not less than 2.5 and (b) the principal peak in the chromatogram obtained with reference solution (a) has a signal-to-noise ratio of at least 3.

**Hydrazine.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 90 volumes of toluene and 10 volumes of ethanol (95 per cent).

**Test solution.** Dissolve 0.12 g of the substance under examination in 4 ml of water and 4 ml of a 15 per cent w/v solution of salicylaldehyde in methanol and 0.2 ml of hydrochloric acid. Mix and let it stand at a temperature not exceeding 25° for 2 to 4 hours to allow complete sedimentation of the precipitate. Add 4 ml of toluene, shake vigorously and centrifuge. Transfer the supernatant liquid to a 100-ml separating funnel, separate the toluene layer and shake vigorously, each time for 3 minutes, with two quantities, each of 20 ml, of a 20 per cent w/v solution of sodium metabisulphite and with two quantities, each of 50 ml, of water. Separate the toluene layer and use it as the test solution.

**Reference solution.** Prepare at the same time and in the same manner as described for the test solution using 1 ml of a solution prepared by dissolving 12 mg of hydrazine sulphate in sufficient quantity of 2 M hydrochloric acid to make 100 ml and diluting 1 ml of this solution to 100 ml with the same solvent and 3 ml of water.

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air and examine in ultraviolet light at 365 nm. Any spot in the chromatogram obtained with the test solution showing a yellow fluorescence is not more intense than the spot in the chromatogram obtained with the reference solution.

**Heavy metals (2.3.13).** Moisten the residue obtained in the test for Sulphated ash with 2 ml of hydrochloric acid, evaporate to dryness and dissolve the residue in sufficient water to produce 20 ml. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (20 ppm). Use lead standard solution (2 ppm Pb) to prepare the standard Sulphated ash (2.3.18). Not more than 0.1 per cent, determined on 2.0 g.

**Loss on drying (2.4.19).** Not more than 0.5 per cent, determined on 1.0 g by drying over phosphorus pentoxide at a pressure not exceeding 0.7 kPa.

**Assay.** Weigh accurately about 0.1 g and dissolve in a mixture of 25 ml of water and 35 ml of hydrochloric acid. Titrate with 0.05 M potassium iodate, determining the end-point potentiometrically (2.4.25) and using a calomel reference electrode and a platinum indicator electrode.

1 ml of 0.05 M potassium iodate is equivalent to 0.009832 g of C8H8N4.HCl.

### Hydralazine Injection

Hydralazine Hydrochloride Injection

Hydralazine Injection is a sterile material consisting of Hydralazine Hydrochloride with or without auxiliary substances. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use. For intravenous infusion, the injection should be diluted with an appropriate volume of a suitable diluent.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage.** The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Hydralazine Injection contains not less than 98.0 per cent and not more than 114.0 per cent of the stated amount of hydralazine hydrochloride, C8H8N4.HCl.

**Description.** A white or almost white powder; very hygroscopic.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with hydralazine hydrochloride RS or with the reference spectrum of hydralazine hydrochloride.

B. Give the reactions of chlorides (2.3.1).
Tests

pH (2.4.24). 3.5 to 4.2, determined in a 2.0 per cent w/v solution.

Appearance of solution. A 2.0 per cent w/v solution is not more opalescent than opalescence standard OS2 (2.4.1). A 2.0 per cent w/v solution in 0.01 M hydrochloric acid is not more intensely coloured than reference solution GYS6 (2.4.1).

Hydrazine. Determine by thin-layer chromatography (2.4.17), using a silica gel 60-precoated plate.

Mobile phase. The upper layer obtained by shaking together 80 volumes of hexane, 20 volumes of strong ammonia solution and 20 volumes of ethyl acetate.

Test solution. Dissolve the contents of a container in sufficient 0.1 M methanolic hydrochloric acid to produce a solution containing 0.5 per cent w/v of Hydralazine Hydrochloride. To 2.0 ml add 1.0 ml of a 2 per cent w/v solution of salicyaldehyde in methanol and 0.1 ml of hydrochloric acid, centrifuge and decant the supernatant liquid.

Reference solution. Prepare in the same manner, but using 2.0 ml of a 0.00025 per cent w/v solution of hydrazine sulphate in 0.1 M methanolic hydrochloric acid in place of the solution of the substance under examination.

Apply to the plate 40 µl of each solution. After development, dry the plate in air and spray with dimethylaminobenzaldehyde reagent. In the chromatogram obtained with the test solution, any spot corresponding to hydrazine is not more intense than the spot in the chromatogram obtained with the reference solution.

Assay. Determine the weight of the contents of 10 containers. Dissolve 0.1 g of the mixed contents of the 10 containers in a mixture of 25 ml of water and 35 ml of hydrochloric acid. Titrate with 0.05 M potassium iodate, determining the endpoint potentiometrically (2.4.25) and using a calomel reference electrode and a platinum indicator electrode.

1 ml of 0.05 M potassium iodate is equivalent to 0.009832 g of C8H8N4.HCl.

Storage. Store protected from light at a temperature not exceeding 30°.

Labelling. The label states that solutions containing glucose should not be used in the preparation of an intravenous infusion.

Hydrochloric Acid

Concentrated Hydrochloric Acid

HCl Mol. Wt. 36.5

Hydrochloric Acid contains not less than 35.0 per cent w/w and not more than 38.0 percent w/w of HCl.

Description. A clear, colourless, fuming liquid; odour, pungent.

Identification

A. When added to potassium permanganate, chlorine is evolved.

B. Gives the reactions of chlorides (2.3.1).

Tests

Arsenic (2.3.10). Mix 10.0 g with 40 ml of water and 1 ml of stannous chloride solution AsT; the resulting solution complies with the limit test for arsenic (1 ppm).

Heavy metals (2.3.13). Evaporate 3.5 ml to dryness on a water-bath, add 2 ml of dilute acetic acid to the residue and add water to make 25 ml. The resulting solution complies with the limit test for heavy metals, Method A (5 ppm).

Bromide and iodide. Dilute 5 ml with 10 ml of water, add 1 ml of chloroform and, dropwise with constant shaking, chlorinated lime solution; the chloroform layer does not become brown or violet.

Free chlorine. To 60 ml add 50 ml of carbon dioxide-free water, 1 ml of a 10 per cent w/v solution of potassium iodide and 0.5 ml of starch solution and allow to stand in the dark for 2 minutes. Any blue colour produced disappears on the addition of 0.2 ml of 0.01 M sodium thiosulphate.

Sulphite (2.3.17). Dilute 1 ml with 10 ml of water and add 0.25 ml of barium chloride solution and 0.5 ml of 0.001 M iodine; the colour of the iodine is not completely discharged.

Sulphates (2.3.17). Mix 6.5 ml with 10 mg of sodium bicarbonate, evaporate to dryness on a water-bath and dissolve the residue in 15 ml of distilled water. The resulting solution complies with the limit test for sulphates (20 ppm).

Residue on evaporation. Not more than 0.01 per cent, determined on 100 g.

Assay. Weigh accurately about 2.0 g, add 30 ml of water, mix and titrate with 1 M sodium hydroxide using methyl red solution as indicator.

1 ml of 1 M sodium hydroxide is equivalent to 0.03646 g of HCl.

Storage. Store in stoppered containers of glass or any other inert material at a temperature not exceeding 30°.

Dilute Hydrochloric Acid

Dilute Hydrochloric Acid is prepared by mixing 274 g of Hydrochloric Acid and 726 g of Purified Water.

Dilute Hydrochloric Acid contains not less than 9.5 per cent and not more than 10.5 per cent w/w of HCl.
**Description.** A clear, colourless liquid.

**Identification**

A. When added to potassium permanganate, chlorine is evolved.

B. Gives the reactions of chlorides (2.3.1).

**Tests**

**Arsenic** (2.3.10). Mix 20.0 g with 20 ml of water and 1 ml of stannous chloride AsT; the resulting solution complies with the limit test for arsenic (0.5 ppm).

**Heavy metals** (2.3.13). Dissolve the residue obtained in the test for Residue on evaporation in 1 ml of 2 M hydrochloric acid, dilute to 25 ml with water; to 2.5 ml of the resulting solution add 2 ml of dilute acetic acid and add water to make 25 ml. The resulting solution complies with the limit test for heavy metals, Method A (2 ppm).

**Free chlorine.** To 60 ml add 50 ml of carbon dioxide-free water, 1 ml of a 10 per cent w/v solution of potassium iodide and 0.5 ml of starch solution and allow to stand in the dark for 2 minutes. Any blue colour produced disappears on the addition of 0.2 ml of 0.01 M sodium thiosulphate.

**Residue on evaporation.** Not more than 0.01 per cent, determined on 100 g.

**Sulphates** (2.3.17). Mix 26 ml with 10 mg of sodium bicarbonate, evaporate to dryness on a water-bath and dissolve the residue in 15 ml of distilled water. The resulting solution complies with the limit test for sulphates (5 ppm).

**Assay.** Weigh accurately about 6.0 g, add 30 ml of water, mix and titrate with 1 M sodium hydroxide using methyl red solution as indicator.

1 ml of 1 M sodium hydroxide is equivalent to 0.03646 g of HCl.

**Storage.** Store in stoppered containers of glass or any other inert material at a temperature not exceeding 30°.

**Hydrochlorothiazide**

Hydrochlorothiazide contains not less than 98.0 per cent and not more than 102.0 per cent of C₇H₈ClN₃O₄S₂, calculated on the dried basis.

**Description.** A white or almost white, crystalline powder; odourless.

**Identification**

*Test A may be omitted if tests B, C and D are carried out. Tests B and D may be omitted if tests A and C are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with hydrochlorothiazide RS or with the reference spectrum of hydrochlorothiazide.

B. When examined in the range 230 nm to 300 nm (2.4.7), a 0.001 per cent w/v solution in 0.01 M sodium hydroxide shows an absorption maximum only at about 273 nm; absorbance at about 273 nm, 0.5 to 0.54. When examined in the range 300 nm to 360 nm, a 0.005 per cent w/v solution in 0.01 M sodium hydroxide shows an absorption maximum at 323 nm; absorbance at about 323 nm, 0.45 to 0.48.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** Ethyl acetate.

**Test solution.** Dissolve 0.5 g of the substance under examination in 100 ml of acetone.

**Reference solution.** A 0.5 per cent w/v solution of hydrochlorothiazide RS in acetone.

Apply to the plate 4 µl of each solution. After development, dry the plate in a current of air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

D. Heat gently about 1 mg with 2 ml of a freshly prepared 0.05 per cent w/v solution of chromotropic acid sodium salt in a cooled mixture of 7 volumes of water and 13 volumes of sulphuric acid; a violet colour develops.

**Tests**

**Acidity or alkalinity.** Shake 0.5 g of the powdered substance under examination with 25 ml of water for 2 minutes and filter. To 10 ml of the filtrate add 0.2 ml of 0.01 M sodium hydroxide and 0.15 ml of methyl red solution. The solution is yellow and not more than 0.4 ml of 0.01 M hydrochloric acid is required to change the colour of the solution to red.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 85 volumes of ethyl acetate and 15 volumes of 2-propanol.

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Hydrochlorothiazide is 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide.
**Test solution.** Dissolve 0.2 g of the substance under examination in 10 ml of acetone.

**Reference solution.** A 0.01 per cent w/v solution of the substance under examination in acetone.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of air until the odour of the solvent is no longer detectable and spray with ethanolic sulphuric acid (10 per cent); use about 10 ml for a plate (200 mm x 200 mm) and spray in small portions, allowing the solvent to evaporate each time and avoiding excessive wetting. Heat to 105° for 30 minutes and immediately place above, but not in, 10 ml of a saturated solution of sodium nitrite in a glass tank. Carefully add 0.5 ml of sulphuric acid to the sodium nitrite solution and allow to stand in the closed tank for 15 minutes. Remove the plate, heat it in a ventilated oven at 40° for 15 minutes, spray with three quantities, each of 5 ml, of a freshly prepared 0.5 per cent w/v solution of N-(1-naphthyl)ethylenediamine dihydrochloride in ethanol (95 per cent) and examine by transmitted light. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Chlorides** (2.3.12). Dissolve 1.0 g in 25 ml of acetone and dilute to 30 ml with water. 7.5 ml of the resulting solution complies with the limit test for chlorides (250 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.12 g and dissolve in 50 ml of anhydrous pyridine. Titrate with 0.1 M tetrabutylammonium hydroxide, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M tetrabutylammonium hydroxide is equivalent to 0.01488 g of C₇H₈ClN₃O₄S₂.

**Hydrochlorothiazide Tablets**

Hydrochlorothiazide Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of hydrochlorothiazide, C₇H₈ClN₃O₄S₂.

**Identification**

Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** Ethyl acetate.

**Test solution.** Triturate a quantity of the powdered tablets containing 10 mg of Hydrochlorothiazide with 10 ml of acetone and filter.

**Reference solution.** A 0.1 per cent w/v solution of hydrochlorothiazide RS in acetone.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of air, examine in ultraviolet light at 254 nm and then spray with ethanolic sulphuric acid (20 per cent), heat at 105° for 30 minutes and immediately expose to nitrous fumes in a closed tank for 15 minutes (the nitrous fumes may be generated by adding 7 M sulphuric acid dropwise to a solution containing 10 per cent w/v of sodium nitrite and 3 per cent w/v of potassium iodide). Place the plate in a current of warm air for 15 minutes and spray with a 0.5 per cent w/v solution of N-(1-naphthyl)ethylenediamine dihydrochloride in ethanol (95 per cent). Examine the plate again. By each method of visualisation the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 85 volumes of ethyl acetate and 15 volumes of 2-propanol.

**Test solution.** Shake a quantity of the powdered tablets containing 50 mg of Hydrochlorothiazide with 50 ml of acetone, filter, evaporate the filtrate to dryness and dissolve the residue in 10 ml of acetone.

**Reference solution.** Dilute 1 volume of the test solution to 100 volumes with acetone.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of air until the odour of the solvent is no longer detectable and spray with ethanolic sulphuric acid (10 per cent); use about 10 ml for a plate (200 mm x 200 mm) and spray in small portions, allowing the solvent to evaporate each time and avoiding excessive wetting. Heat to 105° for 30 minutes and immediately place above, but not in, 10 ml of a saturated solution of sodium nitrite in a glass tank. Carefully add 0.5 ml of sulphuric acid to the sodium nitrite solution and allow to stand in the closed tank for 15 minutes. Remove the plate, heat it in a ventilated oven at 40° for 15 minutes, spray with three quantities, each of 5 ml, of a freshly prepared 0.5 per cent w/v solution of N-(1-naphthyl)ethylenediamine dihydrochloride in ethanol (95 per cent) and examine by transmitted light. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Dissolution** (2.5.2).

Apparatus. No 1

Medium. 900 ml of 0.1 M hydrochloric acid.
Speed and time. 100 rpm and 45 minutes.
Withdraw a suitable volume of the solution and filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 mm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with 0.1 M hydrochloric acid. Measure the absorbance of the resulting solution at the maximum at about 272 nm (2.4.7). Calculate the content of C₁₇H₂₄ClN₃O₄S₂ taking 644 as the specific absorbance at 272 nm.

D. Not less than 60 per cent of the stated amount of C₁₇H₂₄ClN₃O₄S₂.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 20 mg of Hydrochlorothiazide, add 50 ml of 0.1 M sodium hydroxide, shake for 20 minutes and dilute to 100.0 ml with 0.1 M sodium hydroxide. Mix, filter, dilute 5.0 ml of the filtrate to 100.0 ml with water and measure the absorbance of the resulting solution at the maximum at about 273 nm (2.4.7). Calculate the content of C₁₇H₂₄ClN₃O₄S₂ taking 520 as the specific absorbance at 273 nm.

Hydrocortisone
Cortisol

\[
\text{C}_{21}\text{H}_{30}\text{O}_5 \quad \text{Mol. Wt. 362.5}
\]

Hydrocortisone is 11β,17α,21-trihydroxypregn-4-ene-3,20-dione.

Hydrocortisone contains not less than 96.0 per cent and not more than 104.0 per cent of C₂₁H₃₀O₅, calculated on the dried basis.

Description. A white to practically white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Test C may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with hydrocortisone RS or with the reference spectrum of hydrocortisone.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase (a). A mixture of 77 volumes of dichloromethane, 15 volumes of ether, 8 volumes of methanol and 1.2 volumes of water.

Mobile phase (b). A mixture of 80 volumes of ether, 15 volumes of toluene and 5 volumes of 1-butanol saturated with water.

Solvent mixture. A mixture of 90 volumes of chloroform and 10 volumes of methanol.

Test solution. Dissolve 0.25 g of the substance under examination in 100 ml with solvent mixture.

Reference solution. A 0.25 per cent w/v solution of hydrocortisone RS in the same solvent mixture.

Apply to the plate 2 µl of each solution. Develop the chromatograms successively with each mobile phase. After both developments, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Spray the plate with ethanolic sulphuric acid (20 per cent), heat at 120° for 10 minutes or until spots appear and allow to cool. Examine the chromatograms in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution is similar in colour in daylight, fluorescence in ultraviolet light at 365 nm, position and size to the principal spot in the chromatogram obtained with the reference solution.

C. To 2 ml of a 0.1 per cent w/v solution in ethanol (95 per cent) add 2 ml of sulphuric acid; an intense yellow colour is produced with a green fluorescence, which is particularly intense in ultraviolet light at 365 nm. Add the solution to 10 ml of water and mix; the fluorescence in ultraviolet light at 365 nm does not disappear.

Tests

Specific optical rotation (2.4.22). +150° to +156°, determined in a 1.0 per cent w/v solution in dioxan.

Light absorption (2.4.7). A 0.001 per cent w/v solution in ethanol (95 per cent) exhibits a maximum at about 240 nm; absorbance at about 240 nm, between 0.42 and 0.45.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in sufficient tetrahydrofuran to produce 10 ml.

Reference solution (a). Dissolve 2 mg of hydrocortisone RS and 2 mg of prednisolone RS in the mobile phase and dilute to 100 ml with the mobile phase.
Reference solution (b). Dilute 1 ml of the test solution to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated end-capped octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 220 ml of tetrahydrofuran and 700 ml of water, allowed to equilibrate, diluted to 1000 ml with water and mixed again,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Equilibrate the column with the mobile phase for about 30 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is not less than 50 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are: prednisolone, about 14 minutes and hydrocortisone about 15.5 minutes. The test is not valid unless the resolution between the peaks corresponding to prednisolone and hydrocortisone is at least 2.2. If necessary, adjust the concentration of tetrahydrofuran in the mobile phase.

Inject separately the solvent mixture of the test solution as a blank, the test solution and reference solution (b). Continue the chromatography of the test solution for four times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak other than the principal peak, is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent): the sum of the areas of all the peaks other than the principal peak is not greater than 1.5 times that of the principal peak in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Weigh accurately about 0.1 g, dissolve in sufficient ethanol to produce 100.0 ml. Dilute 2.0 ml of this solution to 100.0 ml with ethanol and mix. Measure the absorbance of the resulting solution (2.4.7) at the maximum at 241.5 nm. Calculate the content of C$_{23}$H$_{32}$O$_6$ taking 440 as the specific absorbance at 241.5 nm.

Storage. Store protected from light.
Reference solution (b). Prepare in the same manner as test solution (b) but using 2 ml of the solution obtained in preparing reference solution (a) in place of the solution obtained in preparing test solution (a).

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in each of the chromatograms obtained with the test solutions is similar to the principal spot in the chromatogram obtained with the corresponding reference solution. Spray with ethanolic sulphuric acid (20 per cent v/v) and heat at 120°C for 10 minutes or until the spots appear. Allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in each of the chromatograms obtained with the test solutions is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm, position and size to that in the chromatogram obtained with the corresponding reference solution. The principal spots in the chromatograms obtained with test solution (b) and reference solution (b) have an Rf value distinctly lower than that of the principal spots in the chromatograms obtained with test solution (a) and reference solution (a).

D. Gives the reaction of acetyl groups (2.3.1).

Tests

Specific optical rotation (2.4.22). +158° to +167°, determined in a 1.0 per cent w/v solution in dioxan.

Light absorption (2.4.7). A 0.001 per cent w/v solution in ethanol (95 per cent) shows an absorption maximum at about 240 nm; absorbance at about 240 nm, between 0.38 and 0.40.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in sufficient methanol to produce 10 ml.

Reference solution (a). Dissolve 2 mg of hydrocortisone acetate RS and 2 mg of cortisone acetate RS in the mobile phase and dilute to 100 ml with the mobile phase.

Reference solution (b). Dilute 1 ml of the test solution to 100 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated end-capped octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 400 ml of acetonitrile and 550 ml of water, allowed to equilibrate, diluted to 1000 ml with water and mixed again,
- flow rate, 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Equilibrate the column with the mobile phase for about 30 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is not less than 50 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are: hydrocortisone acetate, about 10 minutes and cortisone acetate about 12 minutes. The test is not valid unless the resolution between the peaks corresponding to hydrocortisone acetate and cortisone acetate is at least 4.2. If necessary, adjust the concentration of acetonitrile in the mobile phase.

Inject separately the test solution and reference solution (b). Continue the chromatography of the test solution for 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution: the area of any peak other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and not more than one such peak has an area greater than half the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all the peaks other than the principal peak is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent). Ignore any peak due to the solvent and any peak with an area less than 0.05 times that of the principal peak in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 2.0 g by drying in an oven at 105°C for 3 hours.

Assay. Weigh accurately about 0.1 g, dissolve in sufficient ethanol to produce 100.0 ml. Dilute 2.0 ml of this solution to 100.0 ml with ethanol and mix. Measure the absorbance of the resulting solution (2.4.7) at the maximum at 241.5 nm. Calculate the content of C23H32O6 taking 395 as the specific absorbance at 241.5 nm.

Storage. Store protected from light.

Hydrocortisone Eye Ointment

Hydrocortisone Acetate Eye Ointment; Cortisol Acetate Eye Ointment

Hydrocortisone Eye Ointment contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of hydrocortisone acetate, C23H32O6.

Identification

Boil 2 g with 20 ml of methanol, shake, cool to 0°C for 30 minutes, filter and evaporate the filtrate to dryness. The residue complies with the following tests.
A. Determine by thin-layer chromatography (2.4.17), coating the plate with **silica gel G**.

**Solvent mixture.** A mixture of 90 volumes of **acetone** and 10 volumes of **formamide**.

**Mobile phase.** A mixture of 30 volumes of **toluene** and 10 volumes of **chloroform**.

**Test solution.** Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

**Reference solution (a).** Dissolve 25 mg of hydrocortisone RS in 10 ml of the solvent mixture.

**Reference solution (b).** Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with **ethanolic sulphuric acid (20 per cent v/v)**. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

B. Dissolve about 1 mg in 1 ml of **sulphuric acid** and mix; an orange colour is produced with a green fluorescence, which is particularly intense under ultraviolet light at 365 nm. Add the solution to 10 ml of **water** and mix; the fluorescence under ultraviolet light at 365 nm does not disappear.

C. Dissolve 10 mg in 1 ml of **methanol**, warm and add 1 ml of **potassium cupri-tartrate solution**; an orange-red precipitate is slowly formed.

**Tests**

**Other tests.** Complies with the tests stated under Eye Ointments.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh accurately a quantity of the ointment containing about 10 mg of Hydrocortisone Acetate, shake with 20.0 ml of **methanol** for 30 minutes and centrifuge. To 10.0 ml of the clear, supernatant layer add sufficient methanol to produce 50.0 ml.

**Reference solution.** A solution containing 0.01 per cent w/v of hydrocortisone acetate RS in methanol.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with porous silica particles (5 µm),
- mobile phase: a mixture of 425 volumes of butyl chloride saturated with water, 70 volumes of tetrahydrofuran, 35 volumes of methanol and 30 volumes of glacial acetic acid,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Calculate the content of C₂₃H₃₂O₆ in the ointment.

**Storage.** Store protected from light at a temperature not exceeding 30°.

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**Hydrocortisone Acetate Injection**

Cortisol Acetate Injection

Hydrocortisone Acetate Injection is a sterile suspension of a very fine powder of Hydrocortisone Acetate in Water for Injections or Sodium Chloride Injection containing suitable dispersing agents.

Hydrocortisone Acetate Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of hydrocortisone acetate, C₂₃H₃₂O₆.

**Identification**

Filter a volume containing 50 mg of Hydrocortisone Acetate through a sintered-glass filter, wash the residue with four quantities, each of 5 ml, of water, dissolve in 20 ml of chloroform, wash the chloroform solution with four quantities, each of 10 ml, of water, discard the washings, filter the chloroform solution through a plug of cotton and evaporate the filtrate to dryness. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with hydrocortisone acetate RS or with the reference spectrum of hydrocortisone acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with **silica gel G**.

**Solvent mixture.** A mixture of 90 volumes of **acetone** and 10 volumes of **formamide**.

**Mobile phase.** A mixture of 30 volumes of **toluene** and 10 volumes of **chloroform**.

**Test solution.** Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.
Reference solution (a). Dissolve 25 mg of hydrocortisone acetate RS in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with ethanolic sulphuric acid (20 per cent v/v). Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

Tests

Other tests. Complies with the tests stated under Parenteral Preparations (Injections)

Assay. Determine by liquid chromatography (2.4.14).

Test solution. To an accurately measured volume of the injection containing about 50 mg of Hydrocortisone Acetate add 70 ml of methanol, shake to produce a clear solution and dilute to 100.0 ml with methanol. Dilute 10.0 ml of the resulting solution to 20.0 ml with water.

Reference solution. Dissolve 25.0 mg of hydrocortisone acetate RS in 50 ml of methanol and add sufficient water to produce 100.0 ml.

Chromatographic system
   a stainless steel column 10 cm x 5 mm, packed with octadecylsilyl silica gel (5 µm) (such as Spherisorb ODS 1),
   mobile phase: 50 per cent v/v solution of methanol,
   flow rate: 2 ml per minute,
   spectrophotometer set at 240 nm,
   a 20 µl loop injector.

Calculate the content of C25H34O8 in the injection.

Storage. Store protected from light.

Labelling. The label states (1) that the contents are meant for local injection only; (2) that the container should be gently shaken before a dose is withdrawn; (3) the names of the dispersing agents used, if any.

Hydrocortisone Acetate

C25H34O8
Mol. Wt. 462.6
Hydrocortisone Acetate is 11β,17α-dihydroxy-3,20-dioxopregn-4-en-21-yl acetate.

Hydrocortisone Acetate contains not less than 98.0 per cent and not more than 101.0 per cent of C25H34O8, calculated on the dried basis.

Description. A white or almost white, crystalline powder; hygroscopic.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Dry the substances before use at 105° for 3 hours and examine them as discs. Compare the spectrum with that obtained with hydrocortisone hemisuccinate RS or with the reference spectrum of hydrocortisone hemisuccinate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. Add a mixture of 1.2 volumes of water and 8 volumes of methanol to a mixture of 15 volumes of ether and 77 volumes of dichloromethane.

Test solution (a). Dissolve 25 mg of the substance under examination in methanol and dilute to 5 ml with the same solvent. Use this solution to prepare test solution (b). Dilute 2 ml of the solution to 10 ml with dichloromethane.

Test solution (b). Transfer 2 ml of the solution obtained in preparing test solution (a) to a 15-ml glass tube with a glass or plastic stopper. Add 10 ml of a 0.8 g per litre solution of sodium hydroxide in methanol and immediately pass a stream of nitrogen through the solution for 5 minutes. Stopper the tube. Heat in a water-bath at 45° protected from light for 30 minutes. Allow to cool.

Reference solution (a). Prepare in the same manner as test solution (a) but using hydrocortisone hemisuccinate RS in place of the substance under examination.
**Reference solution (b).** Prepare in the same manner as test solution (b) but using 2 ml of the solution obtained in preparing reference solution (a) in place of the solution obtained in preparing test solution (a).

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in each of the chromatograms obtained with the test solutions is similar to the principal spot in the chromatogram obtained with the corresponding reference solution. Spray with ethanolic sulphuric acid (20 per cent v/v) and heat at 120° for 10 minutes or until the spots appear. Allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in each of the chromatograms obtained with the test solutions is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm, position and size to that in the chromatogram obtained with the corresponding reference solution. The principal spots in the chromatograms obtained with test solution (b) and reference solution (b) have an Rf value distinctly lower than that of the principal spots in the chromatograms obtained with test solution (a) and reference solution (a).

C. Add about 2 mg to 2 ml of sulphuric acid; a yellow to brownish red colour develops with a green fluorescence that is particularly intense when examined in ultraviolet light at 365 nm.

D. About 30 mg gives the reaction of esters (2.3.1).

**Tests**

**Appearance of solution.** A 2.0 per cent w/v solution in sodium bicarbonate solution is clear (2.4.1).

**Light absorption** (2.4.7). A 0.001 per cent w/v solution in ethanol (95 per cent) shows an absorption maximum at about 240 nm; absorbance at about 240 nm, between 0.34 and 0.36.

**Specific optical rotation** (2.4.22). +147° to +153°, determined in a 1.0 per cent w/v solution in ethanol.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 25 mg of the substance under examination in sufficient methanol to produce 10 ml.

**Reference solution (a).** Dissolve 2 mg of hydrocortisone hemisuccinate RS and 2 mg of dexamethasone RS in the mobile phase and dilute to 100 ml with the mobile phase.

**Reference solution (b).** Dilute 1 ml of the test solution to 100 ml with the mobile phase.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated end-capped octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 330 ml of acetonitrile and 600 ml of water and 1.0 ml of phosphoric acid, allowed to equilibrate, diluted to 1000 ml with water and mixed again,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Equilibrate the column with the mobile phase for about 30 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is not less than 50 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are: dexamethasone, about 12.5 minutes and hydrocortisone hemisuccinate about 15 minutes. The test is not valid unless the resolution between the peaks corresponding to dexamethasone and hydrocortisone hemisuccinate is at least 5.0. If necessary, adjust the concentration of acetonitrile in the mobile phase.

Inject separately the test solution and reference solution (b). Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with the test solution: the area of any peak other than the principal peak, is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all the peaks other than the principal peak is not greater than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.75 per cent). Ignore any peak due to the solvent and any peak with an area less than 0.05 times that of the principal peak in the chromatogram obtained with reference solution (b).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 4.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.1 g, dissolve in sufficient ethanol to produce 100.0 ml. Dilute 2.0 ml of this solution to 100.0 ml with ethanol and mix. Measure the absorbance of the resulting solution (2.4.7) at the maximum at about 241.5 nm. Calculate the content of C25H34O8 taking 353 as the specific absorbance at 241.5 nm.

**Storage.** Store protected from light and moisture.

**Hydrocortisone Sodium Succinate Injection**

Cortisol Sodium Succinate Injection

Hydrocortisone Sodium Succinate Injection is a sterile material made from Hydrocortisone Hemisuccinate with the aid of a
suitable alkali such as Sodium Hydroxide or Sodium Carbonate. It may contain suitable buffering agents. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage.** The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Hydrocortisone Sodium Succinate Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of hydrocortisone, \( \text{C}_{21}\text{H}_{30}\text{O}_{5} \).

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Injections).*

**Tests**

**pH** (2.4.24). 6.5 to 8.0, determined in a solution containing the equivalent of 5.0 per cent w/v of hydrocortisone.

**Related substances.** Determine by liquid chromatography (2.4.14).

*Solvent mixture. A mixture of equal volumes of acetonitrile and water.*

**Test solution.** Dissolve a sufficient quantity of the contents of the sealed container in a solvent mixture to produce a solution containing the equivalent of 0.25 per cent w/v of hydrocortisone.

*Reference solution (a).* Dilute 2 volumes of the test solution to 100 volumes with solvent mixture.

*Reference solution (b).* Dilute a 0.035 per cent w/v solution of hydrocortisone RS in acetonitrile with solvent mixture.

*Reference solution (c).* Dilute a solution containing 0.04 per cent w/v each of hydrocortisone hemisuccinate RS and dexamethasone RS with solvent mixture.

**Chromatographic system**

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 330 ml of acetonitrile and 600 ml of water and 1 ml of phosphoric acid, allowed to equilibrate, diluted to 1000 ml with water and mixed again,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Equilibrate the column with the mobile phase for about 30 minutes.

Inject reference solution (c). The test is not valid unless in the chromatogram obtained the resolution between the peaks corresponding to dexamethasone and hydrocortisone hemisuccinate is at least 5.0.

Inject the test solution. Allow the chromatography to proceed for twice the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any peak corresponding ro hydrocortisone is not greater than the area of the peak in the chromatogram obtained with reference solution (b) (7 per cent) and the area of any other secondary peak is not greater than the area of the peak in the chromatogram obtained with reference solution (a) (2 per cent).

**Assay.** Determine the weight of the contents of 10 containers. Dissolve the mixed contents of the 10 containers in sufficient water to produce a solution containing the equivalent of 0.001 per cent w/v of hydrocortisone. Measure the absorbance of the resulting solution at the maximum at about 248 nm (2.4.7). Calculate the content of \( \text{C}_{21}\text{H}_{30}\text{O}_{5} \) taking 449 as the specific absorbance at 248 nm.
Storage. Store protected from moisture in single dose containers at a temperature not exceeding 30°.

Labelling. The label states (1) the strength in terms of the equivalent amount of hydrocortisone; (2) that the prepared solution should be used only if it is clear; (3) that the solution should be used immediately after preparation.

**Hydrogen Peroxide Solution (20 Vol)**

H₂O₂  
Mol. Wt. 34.0

Hydrogen Peroxide Solution (6 per cent); Dilute Hydrogen Peroxide Solution

Hydrogen Peroxide Solution (20 Vol) is an aqueous solution of hydrogen peroxide. It may contain a suitable stabilising agent.

Hydrogen Peroxide Solution (20 Vol) contains not less than 5.0 per cent w/v and not more than 7.0 per cent w/v of H₂O₂, corresponding to about 20 times its volume of available oxygen.

**Description.** A clear, colourless liquid; odourless. It decomposes in contact with oxidisable organic matter and with certain metals and also if allowed to become alkaline.

**Identification**

A. To 1 ml add 0.2 ml of 1M sulphuric acid and 0.25 ml of 0.02 M potassium permanganate; the solution becomes colourless with evolution of gas.

B. Shake 0.05 ml with 2 ml of 1M sulphuric acid, 2 ml of ether and 0.05 ml of potassium chromate solution; the ether layer becomes blue.

**Tests**

**Acidity.** To 10 ml add 20 ml of water and 0.25 ml of methyl red solution. Not less than 0.2 ml and not more than 1.0 ml of 0.1 M sodium hydroxide is required to change the colour of the solution.

**Organic stabilizers.** Shake 20 ml with successive quantities of 10, 5 and 5 ml of chloroform. Evaporate the combined chloroform extracts at a temperature not exceeding 25° at a pressure of 2 kPa and dry in a desiccator. The residue weighs not more than 10 mg (500 ppm).

**Non-volatile matter.** Place 10 ml in a platinum dish and allow to stand until effervescence ceases. Evaporate the solution on a water-bath and dry the residue at 105°; the residue weighs not more than 20 mg.

**Assay.** To 1.0 ml add 20 ml of 1M sulphuric acid and titrate with 0.02 M potassium permanganate.

1 ml of 0.02 M potassium permanganate is equivalent to 0.001701 g of H₂O₂ or 0.56 ml of oxygen.

**Storage.** Store protected from light in containers resistant to hydrogen peroxide at a temperature not exceeding 30°. If the solution does not contain a stabilising agent, it should be stored in a refrigerator (2° to 8°). It should not be stored for long periods.

**Labelling.** The label states whether or not the solution contains a stabilising agent.

**Hydrogen Peroxide Solution (100 Vol)**

Hydrogen Peroxide Solution (27 per cent); Strong Hydrogen Peroxide Solution

Hydrogen Peroxide Solution (100 Vol) is an aqueous solution of hydrogen peroxide. It may contain a suitable stabilising agent.

Hydrogen Peroxide Solution (100 Vol) contains not less than 26.0 per cent w/w and not more than 28.0 per cent w/w of H₂O₂, corresponding to about 100 times its volume of available oxygen.

**Description.** A clear, colourless liquid; odourless. It decomposes vigorously in contact with oxidisable organic matter and with certain metals and also if allowed to become alkaline.

**Identification**

A. To 1 ml add 0.2 ml of 1M sulphuric acid and 0.25 ml of 0.02 M potassium permanganate; the solution becomes colourless with evolution of gas.

B. Shake 0.05 ml with 2 ml of 1M sulphuric acid, 2 ml of ether and 0.05 ml of potassium chromate solution; the ether layer becomes blue.

**Tests**

**Acidity.** Dilute 10 ml with 100 ml of water and add 0.25 ml of methyl red solution. Not less than 0.05 ml and not more than 0.5 ml of 0.1 M sodium hydroxide is required to change the colour of the solution.

**Organic stabilisers.** Shake 20 ml with successive quantities of 10, 5 and 5 ml of chloroform. Evaporate the combined chloroform extracts at a temperature not exceeding 25° at a pressure of 2 kPa and dry in a desiccator. The residue weighs not more than 10 mg (500 ppm).

**Non-volatile matter.** Place 10 ml in a platinum dish and allow to stand until effervescence ceases. Evaporate the solution on a water-bath and dry the residue at 105°; the residue weighs not more than 20 mg.

**Non-volatile matter.** Place 10 ml in a platinum dish and allow to stand until effervescence ceases. Evaporate the solution on a water-bath and dry the residue at 105°; the residue weighs not more than 20 mg.
Assay. Dilute about 1.0 g of hydroxocobalamin to 100.0 ml with water. To 10.0 ml of the resulting solution add 20 ml of 1 M sulphuric acid and titrate with 0.02 M potassium permanganate. 1 ml of 0.02 M potassium permanganate is equivalent to 0.001701 g of H₂O₂ or 0.56 ml of oxygen.

Storage. Store protected from light in containers resistant to hydrogen peroxide at a temperature not exceeding 30°C. If the solution does not contain a stabilising agent, it should be stored in a refrigerator (2°C to 8°C). It should not be stored for long periods.

Labelling. The label states whether or not the solution contains a stabilising agent.

Hydroxocobalamin

C₆₂H₈₉CoN₁₃O₁₅P  Mol. Wt. 1346.4

Hydroxocobalamin is Coα-[α-(5,6-dimethylbenzimidazolyl)-Coβ-hydroxocobamide. It occurs either as aquocobalamin chloride (Coα-[α-(5,6-dimethylbenzimidazolyl)]-ICoβ-aquocobamide chloride) or as aquocobalamin sulphate. Hydroxocobalamin contains not less than 96.0 per cent and not more than 102.0 per cent of aquocobalamin chloride, C₆₂H₈₉CoN₁₃O₁₅P·HCl, or of aquocobalamin sulphate, C₆₁H₈₀CoN₁₃O₁₄P₂·H₂SO₄, both calculated on the dried basis.

Description. Dark red crystals or a crystalline powder; very hygroscopic. Some decomposition may occur on drying.

Identification

A. Measure the absorbance of the solution used in the Assay at the maxima at about 274 nm, 351 nm and 525 nm (2.4.7); ratios of the absorbances at about 274 nm and 525 nm to that at about 351 nm, about 0.8 and about 0.3 respectively.

B. Fuse 1 mg of the substance with 50 mg of potassium hydrogen sulphate, cool, break up the mass, add 3 ml of water and boil until dissolved. Add 0.05 ml of phenolphthalein solution and sufficient 5 M sodium hydroxide to produce a faint pink colour. Add 0.5 g of sodium acetate, 0.5 ml of 1 M acetic acid and 0.5 ml of a 0.2 per cent w/v solution of nitroso R salt; a red or orange-red colour is produced immediately. Add 0.5 ml of hydrochloric acid and boil for 1 minute; the red colour persists.

C. Gives the reactions of chlorides or of sulphates, as the case may be (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Use freshly prepared solutions and protect them from bright light.

Test solution. Dissolve 10 mg of the substance under examination in the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (a). Dilute 5 ml of the test solution to 100 ml with the mobile phase.

Reference solution (b). Dilute 1 ml of the test solution to 10 ml with the mobile phase. Dilute 1 ml of this solution to 100 ml with the mobile phase.

Reference solution (c). Dissolve 25 mg of the substance under examination in 10 ml of water, warming if necessary. Allow to cool and add 1 ml of a 20 g/l solution of chloramine T and 0.5 ml of 0.05 M hydrochloric acid. Dilute to 25 ml with water. Shake and allow to stand for 5 minutes. Inject immediately.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octylsilyl silica gel (5 μm),
- mobile phase: a mixture of 19.5 volumes of methanol and 80.5 volumes of a solution containing 15 g per litre of citric acid and 8.1 g of disodium hydrogen phosphate,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 351 nm,
- a 20 μl loop injector.

Inject reference solution (c). The test is not valid unless the chromatogram obtained shows three principal peaks and the resolution between each pair of adjacent peaks is at least 3.0.
Inject reference solution (b). The chromatogram obtained shows one principal peak with a signal-to-noise ratio of at least 5.

Inject the test solution and reference solution (a). Continue the chromatography for four times the retention time of the principal peak in the chromatogram obtained with reference solution (a). In the chromatogram obtained with the test solution, the sum of the areas of any peaks apart from the principal peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (5 per cent). Ignore any peak whose area is less than that of the principal peak in the chromatogram obtained with reference solution (b).

Loss on drying (2.4.19). 8 to 12 per cent (aquocobalamin chloride) and 8 to 16 per cent (aquocobalamin sulphate), determined on 1.0 g by drying in an oven at 100° at a pressure not exceeding 0.7 kPa.

Assay. Protect the solutions from light throughout the Assay.

Weigh accurately about 25 mg and dissolve in sufficient solution containing 0.8 per cent v/v of glacial acetic acid and 1.09 per cent w/v of sodium acetate to produce 1000 ml.

Measure the absorbance of the resulting solution at the maximum at about 351 nm (2.4.7). Calculate the content of C₆₂H₈₉CoN₁₃O₁₅P·HCl, or of C₁₂₄H₁₇₈Co₂N₂₆O₃₀P₂·H₂SO₄, taking 190 or 188 respectively, as the specific absorbance at 351 nm.

Storage. Store protected from light and moisture at a temperature not exceeding 30°.

Labelling. The label states whether the contents are aquocobalamin chloride or aquocobalamin sulphate.

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**Hydroxocobalamin Injection**

Hydroxocobalamin Injection is a sterile solution of Hydroxocobalamin in Water for Injections containing sufficient Acetic Acid, Hydrochloric Acid or Sulphuric acid to adjust the pH to about 4.

Hydroxocobalamin Injection contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous hydroxocobalamin, C₆₂H₈₉CoN₁₃O₁₅P.

Identification

Measure the absorbance at about 351 nm and 361 nm; ratio of the absorbance at about 361 nm to that at about 351 nm, about 0.65 (2.4.7).

Tests

pH (2.4.24). 3.8 to 5.5.

Related substances. Determine by liquid chromatography (2.4.14).

Use freshly prepared solutions and protect them from bright light.

Test solution. Dilute the injection with the mobile phase, if necessary, to obtain a solution having a concentration of 0.1 per cent w/v of hydroxocobalamin.

Reference solution (a). Dilute 1 ml of the test solution to 20 ml with the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

Reference solution (c). Add 0.2 ml of a freshly prepared 2 per cent w/v solution of chloramine T and 0.1 ml of 0.05 M hydrochloric acid to a volume of the injection containing the equivalent of 5 mg of hydroxocobalamin, dilute to 10 ml with water, shake, allow to stand for 5 minutes and inject immediately.

Chromatographic system

- a stainless steel column 25 cm × 4 mm, packed with octylsilyl silica gel (5 µm) (such as Lichrosorb 100 CH 8/11),
- mobile phase: a mixture of 19.5 volumes of methanol and 80.5 volumes of a solution containing 1.5 per cent w/v of citric acid and 0.81 per cent w/v of disodium hydrogen orthophosphate,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 351 nm,
- a 20 µl loop injector.

Inject reference solution (c). The test is not valid unless the chromatogram obtained shows three principal peaks and the resolution between each pair of adjacent peaks is not less than 3.0.

Inject reference solution (b). The chromatogram obtained shows one principal peak with a signal-to-noise ratio of not less than 5.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution the sum of the areas of any secondary peaks is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (10 per cent). Ignore any peak the area of which is less than that of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Dilute an accurately measured volume of the injection containing about 4 mg of anhydrous hydroxocobalamin to 200.0 ml with a solution containing 0.8 per cent v/v of glacial acetic acid and 1.09 per cent w/v of sodium acetate and
measure the absorbance of the resulting solution at the maximum at about 351 nm (2.4.7). Calculate the content of C₆₂H₈₉CoN₁₃O₁₅P taking 195 as the specific absorbance at 351 nm.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of anhydrous hydroxycobalamin.

Hydroxyprogesterone Hexanoate

Hydroxyprogesterone Caproate

C₂₇H₄₀O₄  Mol. Wt. 428.6

Hydroxyprogesterone Hexanoate is 3,20-dioxopregn-4-en-17α-yl hexanoate

Hydroxyprogesterone Hexanoate contains not less than 97.0 per cent and not more than 103.0 per cent of C₂₇H₄₀O₄, calculated on the dried basis.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with hydroxyprogesterone hexanoate RS or with the reference spectrum of hydroxyprogesterone hexanoate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Solvent mixture. A mixture of 90 volumes of acetone and 10 volumes of 1,2-propane diol.

Mobile phase. A mixture of equal volumes of cyclohexane and light petroleum (40° to 60°).

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of the same solvent mixture.

Reference solution (a). Dissolve 25 mg of hydroxyprogesterone hexanoate RS in 10 ml of the same solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with ethanolic sulphuric acid (20 per cent v/v). Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. Dissolve 1 mg in 1 ml of sulphuric acid and allow to stand for 2 minutes; a faint yellow colour is produced which, on the addition of 0.5 ml of water, changes first to green, then to red and finally to reddish-violet with a blue fluorescence.

D. Heat 50 mg with 2 ml of 0.5 M ethanolic potassium hydroxide in a water-bath for 5 minutes. Add 3 ml of water, evaporate the ethanol, add 2 ml of sulphuric acid (50 per cent) and heat on a water-bath; the odour of hexanoic acid is produced.

Tests

Acidity. Dissolve 0.2 g in 25 ml of ethanol previously neutralised to bromothymol blue solution and titrate immediately with 0.01 M sodium hydroxide until a faint blue colour is produced. Not more than 1.0 ml of 0.01 M sodium hydroxide is required.

Specific optical rotation (2.4.22). +44.0° to +49.0°, determined in a 2.0 per cent w/v solution in dioxan.

Related foreign steroids. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel HF254.

Mobile phase. A mixture of equal volumes of cyclohexane and ethyl acetate.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of chloroform.

Reference solution. Dilute 1 ml of the test solution to 100 ml with chloroform.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.
Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 2.0 g by drying over phosphorus pentoxide at a pressure not exceeding 0.7 kPa for 24 hours.

Assay. Weigh accurately about 60 mg and dissolve in sufficient ethanol to produce 100.0 ml. Dilute 5.0 ml to 250.0 ml with ethanol and measure the absorbance of the resulting solution at the maximum at about 240 nm (2.4.7). Calculate the content of C$_{27}$H$_{40}$O$_4$ taking 395 as the specific absorbance at 240 nm.

Storage. Store protected from light.

**Hydroxyprogesterone Injection**

Hydroxyprogesterone Caproate Injection; Hydroxyprogesterone Hexanoate Injection

Hydroxyprogesterone Injection is a sterile solution of Hydroxyprogesterone Hexanoate in a suitable ester, in a suitable fixed oil or in any mixture of fixed oils.

Hydroxyprogesterone Hexanoate Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of hydroxyprogesterone hexanoate, C$_{27}$H$_{40}$O$_4$.

**Identification**

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel HF254.

Mobile phase. A mixture of equal volumes of cyclohexane and ethyl acetate.

Test solution. Dilute the injection with chloroform to give a solution containing 1.0 per cent w/v of Hydroxyprogesterone Hexanoate.

Reference solution. A solution containing 1 per cent w/v of hydroxyprogesterone hexanoate RS in chloroform.

Apply to the plate 1µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution. Ignore any spots due to the vehicle.

B. Dissolve a volume of the injection containing 0.1 g of Hydroxyprogesterone Hexanoate in 10 ml of light petroleum (40° to 60°) and extract with three quantities, each of 10 ml, of a mixture of 7 volumes of glacial acetic acid and 3 volumes of water. Wash the combined extracts with 10 ml of light petroleum (40° to 60°), dilute with water until the solution becomes turbid and allow to stand in ice for about 2 hours until a white precipitate is produced. The precipitate, after washing with water, melts at about 120° (2.4.21).

**Tests**

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. To a quantity of the injection containing about 0.125 g of Hydroxyprogesterone Hexanoate add sufficient chloroform to produce 100.0 ml. Dilute 5.0 ml to 100.0 ml with chloroform; to 5.0 ml add 10 ml of isoniazid solution and sufficient methanol to produce 20.0 ml. Allow to stand for 45 minutes and measure the absorbance of the resulting solution at the maximum at about 380 nm (2.4.7), using as the blank 5 ml of chloroform treated in the same manner. Calculate the content of C$_{27}$H$_{40}$O$_4$ from the absorbance obtained by repeating the operation using a 0.00625 per cent w/v solution of hydroxyprogesterone hexanoate RS in chloroform and beginning at the words “to 5.0 ml add...”

Storage. Store protected from light.

Labelling. The label states that the preparation is intended for intramuscular injection only.

**Hydroxypropyl Cellulose**

Cellulose, 2-Hydroxypropyl Ether; Hyprolase

Hydroxypropylcellulose is a cellulose having some of the hydroxyl groups in the form of the 2-hydroxypropyl ether. It may contain not more than 0.6 per cent of silica (SiO$_2$). The various grades commercially available are distinguished by a number indicative of the apparent viscosity in millipascal seconds of a 2 per cent w/w solution measured at 20°.

Description. A white or yellowish white powder; practically odourless; hygroscopic after drying.

**Identification**

A. With constant stirring add a quantity equivalent to 1 g of the dried substance into 50 ml of carbon dioxide-free water previously heated to 90°. Allow to cool, dilute to 100 ml with carbon dioxide-free water and continue stirring until solution is complete (solution A). Heat 10 ml of solution A on a water-bath with stirring. At temperatures above 40° the solution becomes cloudy or a flocculent precipitate is formed. On cooling, the solution becomes clear.

B. To 10 ml of solution A add 0.3 ml of 2 M acetic acid and 2.5 ml of a 10 per cent w/v solution of tannic acid; a yellowish white, flocculent precipitate is produced which dissolves in 6 M ammonia.

C. Without heating completely dissolve 0.2 g in 15 ml of a 70 per cent w/w solution of sulphuric acid, pour the solution with stirring into 100 ml of iced water. In a test-tube kept in ice, mix thoroughly 1 ml of the solution with 8 ml of sulphuric acid.
acid, added dropwise. Heat in a water-bath for exactly 3 minutes and cool immediately in ice. When the mixture is cool, carefully add 0.6 ml of a solution containing 3 g of ninhydrin in 100 ml of a 4.55 per cent w/v solution of sodium metabisulphite, mix well and allow to stand at 25°; a pink colour is produced immediately which becomes violet within 100 minutes.

D. Place 1 ml of solution A on a glass plate. After evaporation of the water a thin film is produced.

**Tests**

**Appearance of solution.** Solution A is not more opalescent than opalescence standard OS3 (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

**pH** (2.4.24). 5.0 to 8.5, determined in solution A.

**Apparent viscosity.** 75 to 140 per cent of the stated value, determined by the following method. Weigh accurately a quantity equivalent to 2.0 g of the dried substance and add, with constant stirring, to 50 ml of water previously heated to 90°. Allow to cool, dilute to 100 ml with water and continue stirring until solution is complete. Adjust the weight of the solution to 100 g and centrifuge the solution to expel any trapped air. Determine the viscosity, Method C, at 20° using a shear rate of 10 s⁻¹ (2.4.28).

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Chlorides** (2.3.12). Dilute 5.0 ml of solution A to 15 ml with water. The resulting solution complies with the limit test for chlorides (0.5 per cent).

**Silica.** Not more than 0.6 per cent, determined by the following method. To the residue obtained in the test for Sulphated ash add sufficient ethanol (95 per cent) to moisten the residue completely. Add 6 ml of hydrofluoric acid in small portions. Evaporate to dryness at 95° to 105° taking care to avoid loss by spurting. Cool and rinse the walls of the platinum crucible with 6 ml of hydrofluoric acid. Add 0.5 ml of sulphuric acid and evaporate to dryness. Progressively increase the temperature, ignite at 900°, allow to cool in a desiccator and weigh. The difference between the weight of the residue obtained in the test for Sulphated ash and the weight of the final residue is equal to the amount of silica in the substance under examination.

**Sulphated ash** (2.3.18). Not more than 1.6 per cent, determined on 1.0 g in a platinum crucible.

**Loss on drying** (2.4.19). Not more than 7.0 per cent, determined on 0.5 g by drying in an oven at 105°.

**Storage.** Store protected from moisture.

**Labelling.** The label states the apparent viscosity in millipascal seconds of a 2 per cent w/w solution. For products of low viscosity the label also states the concentration of the solution to be used and the apparent viscosity in millipascal seconds.

### Hydroxypropylmethylcellulose

**Hydroxypropylmethylcellulose**

Cellulose, 2-Hydroxypropylmethyl Ether; Hypermellose

Hydroxypropylmethylcellulose is a cellulose having some of the hydroxyl groups in the form of the methyl ether and some in the form of the 2-hydroxypropyl ether. The various grades commercially available are distinguished by a number indicative of the apparent viscosity in millipascal seconds of a 2 per cent w/v solution measured at 20°.

**Description.** A white or yellowish white, fibrous or granular powder; almost odourless; hygroscopic after drying.

**Identification**

A. With constant stirring add a quantity containing 1 g of the dried substance into 50 ml of carbon dioxide-free water previously heated to 90°. Allow to cool, dilute to 100 ml with carbon dioxide-free water and continue stirring until solution is complete (solution A). Heat 10 ml of solution A in a water-bath with stirring. At temperatures above 50° the solution becomes cloudy or a flocculent precipitate is formed. On cooling, the solution becomes clear or slightly opalescent.

B. To 10 ml of solution A add 10 ml of 1 M sodium hydroxide or 1 M hydrochloric acid; in either case the mixture remains stable.

C. To 10 ml of solution A add 0.3 ml of 2 M acetic acid and 2.5 ml of a 10 per cent w/v solution of tannic acid; a yellowish white, flocculent precipitate is produced which dissolves in 6 M ammonia.

D. Without heating completely dissolve 0.2 g in 15 ml of a 70 per cent w/w solution of sulphuric acid, pour the solution with stirring into 100 ml of iced water. In a test-tube kept in ice, mix thoroughly 1 ml of the solution with 8 ml of sulphuric acid, added dropwise. Heat in a water-bath for exactly 3 minutes and cool immediately in ice. When the mixture is cool, carefully add 0.6 ml of a solution containing 3 g of ninhydrin in 100 ml of a 4.55 per cent w/v solution of sodium metabisulphite, mix well and allow to stand at 25°; a pink colour is produced immediately which becomes violet within 100 minutes.

E. Place 1 ml of solution A on a glass plate. After evaporation of the water a thin film is produced.

**Tests**

**pH** (2.4.24). 5.5 to 8.0, determined in solution A.
Appearance of solution. Solution A is not more opalescent than opalescence standard OS3 (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

Apparent viscosity. 75 to 140 per cent of the stated value, determined by the following method. Weigh accurately a quantity equivalent to 2.0 g of the dried substance and add, with constant stirring, to 50 ml of water previously heated to 90°. Allow to cool, dilute to 100 ml with water and continue stirring until solution is complete. Adjust the weight of the solution to 100 g and centrifuge the solution to expel any trapped air. Determine the viscosity, Method C, at 20° using a shear rate of 10 s⁻¹ (2.4.28).

Heavy metals (2.3.12). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Chlorides (2.3.12). Dilute 5.0 ml of solution A to 15 ml with water. The resulting solution complies with the limit test for chlorides (0.5 per cent).

Sulphated ash (2.3.18). Not more than 3.0 per cent.

Loss on drying (2.4.19). Not more than 10.0 per cent, determined on 0.5 g by drying in an oven at 105°.

Storage. Store protected from moisture.

Labelling. The label states the apparent viscosity in millipascal seconds of a 2 per cent w/v solution.

Hyoscine Butylbromide

Scopolamine Butylbromide

\[
\text{C}_{21}\text{H}_{30}\text{BrNO}_4 \quad \text{Mol. Wt. 440.4}
\]

Hyoscine Butylbromide is (1S,3S,5R,6R,7S,8R)-6,7-epoxy-8-butyl-3-[(S)-tropoyloxy]tropanium bromide.

Hyoscine Butylbromide contains not less than 98.0 per cent and not more than 101.0 per cent of \( \text{C}_{21}\text{H}_{30}\text{BrNO}_4 \), calculated on the dried basis.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with hyoscine butylbromide RS or with the reference spectrum of hyoscine butylbromide.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.15 per cent w/v solution in 0.01 M hydrochloric acid shows absorbance at about 252 nm, 257 nm and 264 nm and a less well-defined maximum at about 247 nm; absorbance at about 252 nm, about 0.50, at about 257 nm, about 0.67 and at about 264 nm, about 0.50.

C. To 1 mg add 0.2 ml of fuming nitric acid and evaporate to dryness on a water-bath. Dissolve the residue in 2 ml of acetone and add 0.1 ml of a 3 per cent w/v solution of potassium hydroxide in methanol; a violet colour is produced.

D. Gives the reactions of bromides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in carbon dioxide-free water is clear (2.4.1), and colourless (2.4.1).

pH (2.4.24). 5.5 to 6.5, determined in a 10.0 per cent w/v solution.

Specific optical rotation (2.4.22). –18.0° to –20.0°, determined in a 5.0 per cent w/v solution.

Apo-compounds. Ratio of the absorbance (2.4.7) of a 0.1 per cent w/v solution in 0.01 M hydrochloric acid at the maximum at about 247 nm to that at the maximum at about 264 nm is not more than 0.94.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with microcrystalline cellulose.

Mobile phase. The upper layer obtained by shaking together 50 volumes of 1-butanol, 25 volumes of water and 5 volumes of anhydrous formic acid.

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of methanol (50 per cent).

Reference solution (a). A solution containing 0.004 per cent w/v of the substance under examination in 10 ml of methanol (50 per cent).

Reference solution (b). A solution containing 0.002 per cent w/v of hyoscine hydrobromide RS in methanol (50 per cent).

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with dilute potassium iodobismuthate solution. In the chromatogram obtained with the test solution any spot corresponding to hyoscine
Hyoscine Butylbromide Injection

Scopolamine Butylbromide Injection

Hyoscine Butylbromide Injection is a sterile solution of Hyoscine Butylbromide in Water for Injections.

Hyoscine Butylbromide Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of hyoscine butylbromide, C₂₁H₃₀BrNO₄.

Identification

Evaporate to dryness a volume of the injection containing 0.1 g of Hyoscine Butylbromide, shake the residue with 20 ml of chloroform, filter, evaporate the filtrate to dryness and triturate the residue with 5 ml of acetoniitrile. Evaporate to dryness and dry the residue at 50°C at a pressure not exceeding 0.7 kPa for 1 hour. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with hyoscine butylbromide RS or with the reference spectrum of hyoscine butylbromide.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.15 per cent w/v solution in 0.01 M hydrochloric acid shows absorption maxima at about 252 nm, 257 nm and 264 nm and a less well-defined maximum at about 247 nm; absorbance at about 252 nm, about 0.50, at about 257 nm, about 0.67 and at about 264 nm, about 0.50.

C. To 1 mg add 0.2 ml of fuming nitric acid and evaporate to dryness on a water-bath. Dissolve the residue in 2 ml of acetone and add 0.1 ml of a 3 per cent w/v solution of potassium hydroxide in methanol; a violet colour is produced.

Tests

pH (2.4.24). 3.7 to 5.5.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with microcrystalline cellulose.

Mobile phase. The upper layer obtained by shaking together 50 volumes of 1-butanol, 25 volumes of water and 5 volumes of anhydrous formic acid.

Test solution. The injection diluted if necessary with methanol (50 per cent) to contain 2 per cent w/v of Hyoscine Butylbromide.

Reference solution (a). Dilute 1 volume of the test solution to 20 volumes with methanol (50 per cent).

Reference solution (b). A solution containing 0.002 per cent w/v of hyoscine hydrobromide RS in methanol (50 per cent).

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with dilute potassium iodobismuthate solution. In the chromatogram obtained with the test solution any spot corresponding to hyoscine hydrobromide is not more intense than the spot in the chromatogram obtained with reference solution (b) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a).

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Dilute a volume of the injection containing about 60 mg of Hyoscine Butylbromide to 50.0 ml with water and dilute 5.0 ml of this solution to 200.0 ml with water. To 10.0 ml of the resulting solution add 10 ml of water, 15 ml of dichloromethane, 15 ml of a 0.01 per cent w/v solution of hexanitrodiphenylamine in dichloromethane and 5 ml of 5 M sodium hydroxide and shake for 2 minutes. Allow the layers to separate and reserve the organic layer. Extract the aqueous layer with successive quantities, each of 5 ml, of dichloromethane and dry the filtrate until no further colour is extracted from the aqueous layer. Add the dichloromethane extracts to the reserved organic layer, filter through absorbent cotton, add sufficient dichloromethane to produce 50.0 ml and measure the absorbance of the resulting solution at the maximum at about 420 nm (2.4.7), using as the blank a solution prepared by repeating the procedure without the injection. Calculate the content of C₂₁H₃₀BrNO₄ from the absorbance obtained by repeating the procedure using 10.0 ml of a 0.003 per cent w/v solution of hyoscine butylbromide RS and beginning at the words “add 10 ml of water...”.

Storage. Store at a temperature not exceeding 30°C protected from light and moisture.
Hyoscine Butylbromide Tablets

Hyoscine Butylbromide Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of hyoscine butylbromide, C21H30BrNO4. The tablets may be coated.

Identification

Shake a quantity of the powdered tablets containing 50 mg of hyoscine butylbromide with 20 ml of chloroform, filter, evaporate the filtrate to dryness and triturate the residue with 5 ml of acetonitrile. Evaporate to dryness and dry the residue at 50°C at a pressure not exceeding 0.7 kPa for 1 hour. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with hyoscine butylbromide RS or with the reference spectrum of hyoscine butylbromide.

B. When examined in the range 230 nm to 360 nm, a 0.15 per cent w/v solution in 0.01 M hydrochloric acid shows absorption maxima at about 252 nm, 257 nm and 264 nm and a less well-defined maximum at about 247 nm; absorbance at about 252 nm, about 0.50, at about 257 nm, about 0.67 and at about 264 nm, about 0.50 (2.4.7).

C. To 1 mg add 0.2 ml of fuming nitric acid and evaporate to dryness on a water-bath. Dissolve the residue in 2 ml of acetone and add 0.1 ml of a 3 per cent w/v solution of potassium hydroxide in methanol; a violet colour is produced.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with microcrystalline cellulose.

Mobile phase. The upper layer obtained by shaking together 50 volumes of 1-butanol, 25 volumes of water and 5 volumes of anhydrous formic acid.

Test solution. Shake a quantity of the powdered tablets containing 40 mg of Hyoscine Butylbromide with 2 ml of methanol (50 per cent) and filter.

Reference solution (a). Dilute 1 volume of the test solution to 500 volumes with methanol (50 per cent).

Reference solution (b). A solution containing 0.002 per cent w/v of hyoscine hydrobromide RS in methanol (50 per cent).

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with dilute potassium iodobismuthate solution. In the chromatogram obtained with the test solution any spot corresponding to hyoscine hydrobromide is not more intense than the spot in the chromatogram obtained with reference solution (b) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a).

Uniformity of content. Comply with the test stated under Tablets.

Shake one tablet with 100 ml of water for 30 minutes, add sufficient water to produce 250.0 ml and filter. To 10.0 ml of the resulting solution add 10 ml of water, 15 ml of dichloromethane, 15 ml of a 0.01 per cent w/v solution of hexanitrodiphenylamine in dichloromethane and 5 ml of 5 M sodium hydroxide and shake for 2 minutes. Allow the layers to separate and reserve the organic layer. Extract the aqueous layer with successive quantities, each of 5 ml, of dichloromethane until no further colour is extracted from the aqueous layer. Add the dichloromethane extracts to the reserved organic layer, filter through absorbent cotton, add sufficient dichloromethane to produce 50.0 ml and measure the absorbance of the resulting solution at the maximum at about 420 nm (2.4.7), using as the blank a solution prepared by repeating the procedure without the injection. Calculate the content of C21H30BrNO4 from the absorbance obtained by repeating the procedure using 10.0 ml of a 0.003 per cent w/v solution of hyoscine butylbromide RS and beginning at the words “add 10 ml of water...”.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 3 mg of Hyoscine Butylbromide and shake with 50 ml of water for 30 minutes, add sufficient water to produce 100.0 ml and filter. To 10.0 ml of the resulting solution add 10 ml of water, 15 ml of dichloromethane, 15 ml of a 0.01 per cent w/v solution of hexanitrodiphenylamine in dichloromethane and 5 ml of 5 M sodium hydroxide and shake for 2 minutes. Allow the layers to separate and reserve the organic layer. Extract the aqueous layer with successive quantities, each of 5 ml, of dichloromethane until no further colour is extracted from the aqueous layer. Add the dichloromethane extracts to the reserved organic layer, filter through absorbent cotton, add sufficient dichloromethane to produce 50.0 ml and measure the absorbance of the resulting solution at the maximum at about 420 nm (2.4.7), using as the blank a solution prepared by repeating the procedure without the injection. Calculate the content of C21H30BrNO4 from the absorbance obtained by repeating the procedure using 10.0 ml of a 0.003 per cent w/v solution of hyoscine butylbromide RS and beginning at the words “add 10 ml of water....”.

Storage. Store at a temperature not exceeding 30°C protected from light and moisture.
Hyoscine Hydrobromide
Scopolamine Hydrobromide

\[ \text{C}_{17}\text{H}_{21}\text{NO}_4\text{HBr,3H}_2\text{O} \quad \text{Mol. Wt. 438.3} \]

Hyoscine Hydrobromide is (1S,3S,5R,6R,7S)-6,7-epoxytropan-3-yl-tropane hydrobromide trihydrate.

Hyoscine Hydrobromide contains not less than 99.0 per cent and not more than 101.0 per cent of \( \text{C}_{17}\text{H}_{21}\text{NO}_4\text{HBr} \), calculated on the anhydrous basis.

**Description.** Colourless crystals or a white, crystalline powder; odourless; efflorescent.

**Identification**

*Test A* may be omitted if *tests B, C and D* are carried out. *Tests B and C* may be omitted if *tests A and D* are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hyoscine hydrobromide RS*.

B. To about 1 mg add 0.2 ml of *fuming nitric acid* and evaporate to dryness on a water-bath. Dissolve the residue in 2 ml of *acetone* and add 0.1 ml of a 3 per cent w/v solution of *potassium hydroxide in methanol*; a violet colour is produced.

C. Gives the reaction of alkaloids (2.3.1).

D. Gives the reactions of bromides (2.3.1).

**Tests**

**pH** (2.4.24). 4.0 to 5.5, determined in a 5.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). –24.0° to –27.0°, determined in a 5.0 per cent w/v solution.

**Apohyoscine.** Dissolve 0.1 g in sufficient 0.01 \( \text{M} \) hydrochloric acid to produce 100 ml and measure the absorbance (2.4.7) at about 245 nm; absorbance, about 0.36, calculated on the anhydrous basis (0.5 per cent).

**Related substances and decomposition products.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

**Mobile phase.** A mixture of 50 volumes of *chloroform*, 30 volumes of *acetone*, 10 volumes of *methanol* and 2 volumes of *strong ammonia solution*.

**Test solution.** Dissolve 0.2 g of the substance under examination in 10 ml of *methanol*.

**Reference solution (a).** Dilute 1 ml of the test solution to 100 ml with *methanol*.

**Reference solution (b).** Dilute 25 ml of reference solution (a) to 50 ml with *methanol*.

Apply to the plate 10 \( \mu \)l of each solution. After development, dry the plate at 105° for 15 minutes, allow to cool and spray with *dilute potassium iodobismuthate solution* until spots appear. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b). Ignore any yellow spot remaining on the line of application.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). 10.0 to 13.0 per cent, determined on 0.2 g.

**Assay.** Weigh accurately about 0.4 g, dissolve in 10 ml of *anhydrous glacial acetic acid*, warming if necessary, cool the solution and add 20 ml of *dioxan*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03843 g of \( \text{C}_{17}\text{H}_{21}\text{NO}_4\text{HBr} \).

**Storage.** Store protected from light and moisture in well-filled containers of small capacity in a refrigerator at a temperature not exceeding 15°.

Hyoscine Hydrobromide Injection
Scopolamine Hydrobromide Injection

Hyoscine Hydrobromide Injection is a sterile solution of Hyoscine Hydrobromide in Water for Injection.

Hyoscine Hydrobromide Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of hyoscine hydrobromide, \( \text{C}_{17}\text{H}_{21}\text{NO}_4\text{HBr,3H}_2\text{O} \).

**Identification**

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

**Mobile phase.** A mixture of 50 volumes of *chloroform*, 40 volumes of *acetone* and 10 volumes of *diethylamine*. 
Test solution. Evaporate a volume of the injection containing 5 mg of Hyoscine Hydrobromide to dryness on a water-bath, triturate the residue with 1 ml of ethanol (95 per cent), allow to stand and use the supernatant liquid.

Reference solution. A solution containing 0.5 per cent w/v of hyoscine hydrobromide RS in ethanol (95 per cent).

Apply to the plate 5 µl of each solution. After development, dry the plate in air, heat it at 105° for 20 minutes, allow to cool and spray with dilute potassium iodobismuthate solution. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the chromatogram obtained with test solution (b) shows a peak with the same retention time as the peak derived from hyoscine hydrobromide in the chromatogram obtained with the reference solution.

C. Evaporate a suitable volume to dryness. To 1 ml of a 1 per cent w/v solution of the residue add 1 ml of 5 M ammonia, shake with chloroform and evaporate the chloroform solution to dryness on a water-bath. To the residue add 1.5 ml of a 2 per cent w/v solution of mercuric chloride in ethanol (60 per cent); a white precipitate is produced which dissolves on warming (distinction from atropine and hyoscyamine).

D. Gives reaction A of bromides (2.3.1).

Tests

pH (2.4.24). 4.0 to 6.0.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by gas chromatography (2.4.13).

Test solution (a). Add 1 of a 0.3 per cent w/v solution of atropine sulphate RS (internal standard) in methanol (solution A) and 1 ml of 5 M ammonia to a volume of the injection containing about 5 mg of Hyoscine Hydrobromide, dilute if necessary to 15 ml with water. Extract with two quantities, each of 10 ml, of chloroform, shake the combined extracts with 2 g of anhydrous sodium sulphate, filter and evaporate the filtrate to dryness. Dissolve the residue in 2.0 ml of dichloromethane. To 1.0 ml of this solution add 0.2 ml of a mixture of 4 volumes of N,O-bis(trimethylsilyl)acetamide and 1 volume of trimethylchlorosilane, mix and allow to stand for 30 minutes.

Test solution (b). Prepare in the same manner as test solution (a) but omitting the addition of solution A.

Reference solution. Add 1 ml of solution A and 1 ml of 5 M ammonia to 15.0 ml of a 0.033 per cent w/v solution of hyoscine hydrobromide RS and complete the procedure described under test solution (a) beginning at the words “Extract with two quantities,...".

Chromatographic system
- a glass column 1.5 m x 4 mm, packed with acid-washed, diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w phenyl methyl silicone fluid (50 per cent phenyl),
- temperature: column, 230°, inlet port and detector, 280°,
- flow rate. 30 ml per minute of the carrier gas.

Calculate the content of C₁₇H₂₁NO₄.HBr.₃H₂O in the injection.

Storage. Store at a temperature not exceeding 15°, protected from light.

Hyoscine Hydrobromide Tablets

Scopolamine Hydrobromide Tablets

Hyoscine Hydrobromide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of hyoscine hydrobromide, C₁₇H₂₁NO₄.HBr.₃H₂O.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 50 volumes of chloroform, 40 volumes of acetone and 10 volumes of diethylamine.

Test solution. Shake a quantity of the powdered tablets containing 10 mg of Hyoscine Hydrobromide with 2 ml of ethanol (95 per cent) and centrifuge.

Reference solution. A solution containing 0.5 per cent w/v of hyoscine hydrobromide RS in ethanol (95 per cent).

Apply to the plate 5 µl of each solution. After development, dry the plate in air, heat it at 105° for 20 minutes, allow to cool and spray with dilute potassium iodobismuthate solution. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the chromatogram obtained with test solution (b) shows a peak with the same retention time as the peak derived from hyoscine hydrobromide in the chromatogram obtained with the reference solution.

C. Extract a quantity of the powdered tablets containing 1 mg of Hyoscine Hydrobromide with 5 ml of ethanol (95 per cent), filter and evaporate the filtrate to dryness on a water-bath. Cool, add 0.2 ml of fuming nitric acid and again evaporate to dryness on a water-bath; a yellow residue is produced. To the cooled residue add 2 ml of acetone and 0.2 ml of a 3 per cent w/v solution of potassium hydroxide in methanol; a deep violet
colour is produced. (Atropine and hyoscyamine also yield this reaction; the reaction is masked by other alkaloids).

D. The powdered tablets give reaction A of bromides (2.3.1).

Tests

Uniformity of content. Comply with the test stated under Tablets.

For tablets containing 600 µg of Hyoscine Hydrobromide -

Test solution (a). Powder one tablet and triturate with 5 ml of 0.1 M hydrochloric acid. Add 1.0 ml of a 0.0375 per cent w/v solution of atropine sulphate RS (internal standard) in methanol (solution A), extract with two quantities, each of 5 ml, of chloroform and discard the chloroform extracts. Add 1 ml of 5 M ammonia. Extract with two quantities, each of 5 ml, of chloroform, shake the combined extracts with 1 g of anhydrous sodium sulphate, filter and evaporate the filtrate to dryness. Dissolve the residue in 0.5 ml of a mixture of 20 volumes of dichloromethane, 4 volumes of N,O-bis(trimethylsilyl) acetamide and 1 volume of trimethylchlorosilane, mix and allow to stand for 30 minutes.

Test solution (b). Prepare in the same manner as test solution (a) but omitting the addition of solution A.

Reference solution. Add 1 ml of a 0.0375 per cent w/v solution of atropine sulphate RS (solution A) and 1 ml of 5 M ammonia to 5.0 ml of a 0.012 per cent w/v solution of hyoscine hydrobromide RS and complete the procedure described under test solution (a) beginning at the words “extract with two quantities, ...”.

Carry out the procedure described in the Assay. Calculate the content of C17H21NO4.HBr.3H2O in the tablet.

For tablets containing less than 600 µg of Hyoscine Hydrobromide -

Use the same procedure but with correspondingly smaller concentrations of hyoscine hydrobromide RS and atropine sulphate RS.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by gas chromatography (2.4.13).

Test solution (a). Shake a quantity of the powdered tablets containing about 5 mg of Hyoscine Hydrobromide with 10 ml of 0.1 M hydrochloric acid. Add 1 ml of a 0.3 per cent w/v solution of atropine sulphate RS (internal standard) in methanol (solution A), extract with two quantities, each of 10 ml, of chloroform and discard the chloroform extracts. Add 1 ml of 5 M ammonia. Extract with two quantities, each of 10 ml, of chloroform, shake the combined extracts with 2 g of anhydrous sodium sulphate, filter and evaporate the filtrate to dryness. Dissolve the residue in 2.0 ml of dichloromethane. To 1.0 ml of this solution add 0.2 ml of a mixture of 4 volumes of N,O-bis(trimethylsilyl) acetamide and 1 volume of trimethylchlorosilane, mix and allow to stand for 30 minutes.

Test solution (b). Prepare in the same manner as test solution (a) but omitting the addition of solution A.

Reference solution. Add 1 ml of solution A and 1 ml of 5 M ammonia to 15.0 ml of a 0.033 per cent w/v solution of hyoscine hydrobromide RS and complete the procedure described under test solution (a) beginning at the words “Extract with two quantities,”

Chromatographic system

– a glass column 1.5 m x 4 mm, packed with acid-washed, diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w phenyl methyl silicone fluid (50 per cent phenyl),
– temperature: column 230°, inlet port and detector 280°,
– flow rate. 30 ml per minute of the carrier gas.

Calculate the content of C17H21NO4.HBr.3H2O in the tablets.

Storage. Store at a temperature not exceeding 15°, protected from light.
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Ibuprofen

\[
\text{C}_{13}\text{H}_{18}\text{O}_2 \quad \text{Mol. Wt. 206.3}
\]

Ibuprofen is \((RS)-2-(4\text{-isobutylphenyl})\text{propionic acid.}\)

Ibuprofen contains not less than 98.5 per cent and not more than 101.0 per cent of \(\text{C}_{13}\text{H}_{18}\text{O}_2\), calculated on the dried basis.

**Description**. A white or almost white, crystalline powder or colourless crystals; odour, slight.

**Identification**

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ibuprofen \(\text{RS}\) or with the reference spectrum of ibuprofen.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.05 per cent w/v solution in 0.1 \(\text{M sodium hydroxide}\) shows absorption maxima at about 264 nm and 272 nm, and a shoulder at about 258 nm. The ratio of the absorbance at about 264 nm to that at the shoulder at about 258 nm is 1.20 to 1.30. The ratio of the absorbance at the maximum at about 272 nm to that at the shoulder at about 258 nm is 1.00 to 1.10.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

**Mobile phase**. A mixture of 75 volumes of \(n\)-hexane, 25 volumes of ethyl acetate and 5 volumes of glacial acetic acid.

**Test solution**. Dissolve 0.5 g of the substance under examination in 100 ml of dichloromethane.

**Reference solution**. A 0.5 per cent w/v solution of *ibuprofen RS* in dichloromethane.

Apply to the plate 5 \(\mu\)l of each solution. After development, dry the plate at 120° for 30 minutes, lightly spray the plate with a 1 per cent w/v solution of potassium permanganate in 1 \(\text{M sulphuric acid}\), heat at 120° for 20 minutes and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

**Tests**

**Appearance of solution**. A 10.0 per cent w/v solution in ethanol (95 per cent) is clear (2.4.1), and colourless (2.4.1).

**Optical rotation** (2.4.22). +0.05° to -0.05°, determined in a 2.5 per cent w/v solution in *methanol*.

**Related substances**. Determine by liquid chromatography (2.4.14).

**Test solution**. Dissolve 20 mg of the substance under examination in 2 ml of *acetonitrile* and add sufficient of the mobile phase to produce 10 ml.

**Reference solution (a)**. Dilute 1 ml of the test solution to 100 ml with the mobile phase.

**Reference solution (b)**. Dissolve 20 mg of *ibuprofen RS* in 2 ml of *acetonitrile*, add 1 ml of 0.006 per cent w/v solution of 2-(4-butylphenyl)propionic acid *RS* in *acetonitrile* and add sufficient mobile phase to produce 10 ml.

**Chromatographic system**

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 \(\mu\)m),
- mobile phase: a mixture of 600 volumes of \(\text{water}\), 340 volumes of \(\text{acetonitrile}\) and 0.5 volume of \(\text{phosphoric acid}\) diluted to 1000 volumes with \(\text{water}\) after equilibration,
- flow rate. 2 ml per minute,
- spectrophotometer set at 214 nm,
- a 20 \(\mu\)l loop injector.

Adjust the sensitivity so that the height of the principal peak in the chromatogram obtained with reference solution (a) is about 80 per cent of full-scale deflection on the recorder. Record the chromatogram for 1.5 times the retention time of the principal peak. Equilibrate the column with the mobile phase for about 45 minutes before starting the chromatography.

The retention time of ibuprofen is about 20 minutes. In the chromatogram obtained with reference solution (b) measure the height \(a\) of the peak due to 2-(4-butylphenyl)propionic acid and the height \(b\) of the lowest point of the curve separating this peak from that due to ibuprofen. The test is not valid unless \(a\) is greater than 1.5\(b\). If necessary, adjust the concentration of acetonitrile in the mobile phase to obtain the required resolution. Verify the repeatability by making five separate injections of 20 \(\mu\)l of reference solution (a). The test is not valid unless the relative standard deviation of the area of the principal peak is less than 2.0 per cent.

In the chromatogram obtained with the test solution, the area of any peak corresponding to 2-(4-butylphenyl)propionic acid is not greater than that of the peak due to 2-(4-butylphenyl)propionic acid in the chromatogram obtained with reference solution (b), the area of any other secondary peak is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) and the sum of the areas of any such peaks is not greater than 0.7 times the area of the principal peak in the chromatogram obtained with reference solution (a). Ignore any peak the area of which is less than
0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over phosphorus pentoxide at a pressure not exceeding 0.1 kPa.

**Assay.** Weigh accurately about 0.4 g, dissolve in 100 ml of ethanol (95 per cent) and titrate with 0.1 M sodium hydroxide using 0.2 ml of phenolphthalein solution as indicator. Carry out a blank titration.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.02063 g of C_{13}H_{18}O_{2}.

**Ibuprofen Tablets**

Ibuprofen Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of ibuprofen, C_{13}H_{18}O_{2}. The tablets are coated.

**Identification**

A. Extract a quantity of the powdered tablets containing 0.5 g of Ibuprofen with 20 ml of acetone, filter and evaporate the filtrate to dryness in a current of air without heating. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ibuprofen RS or with the reference spectrum of ibuprofen.

B. The residue obtained in test A, after recrystallisation from light petroleum (40° to 60°), melts at about 75° (2.4.21).

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel H.

**Mobile phase.** A mixture of 75 volumes of n-hexane, 25 volumes of ethyl acetate and 5 volumes of glacial acetic acid.

**Test solution.** Extract a quantity of the powdered tablets containing 0.2 g of Ibuprofen with three quantities, each of 10 ml, of chloroform, filter, evaporate the combined filtrate to about 1 ml and add sufficient chloroform to produce 2 ml.

**Reference solution.** Dilute 1 volume of the test solution to 100 volumes with chloroform.

Apply to the plate 5 µl of each solution. After development, dry the plate in air, lightly spray the plate with a 1 per cent w/v solution of potassium permanganate in 1 M sulphuric acid, heat at 120° for 20 minutes and examine in ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution. Ignore any spot with an R_{f} value relative to ibuprofen of about 1.2.

**Dissolution** (2.5.2).

**Apparatus.** No 1

**Medium.** 900 ml of phosphate buffer pH 7.2

**Speed and time.** 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Dilute a suitable volume of the filtrate with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 221 nm (2.4.7). Calculate the content of C_{13}H_{18}O_{2}.

D. Not less than 50 per cent of the stated amount of C_{13}H_{18}O_{2}.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.5 g of Ibuprofen, extract with 60 ml of chloroform for 15 minutes and filter through a sintered-glass crucible of porosity 3. Wash the residue with three quantities, each of 10 ml, of chloroform and gently evaporate the filtrate just to dryness in a current of air. Dissolve the residue in 100 ml of ethanol (95 per cent), previously neutralized to phenolphthalein solution, and titrate with 0.1 M sodium hydroxide using phenolphthalein solution as indicator.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.02063 g of C_{13}H_{18}O_{2}.

Idoxuridine

![Idoxuridine Structure](image)

C_{9}H_{11}IN_{2}O_{5}  
Mol. Wt. 354.1

Idoxuridine is 2'-deoxy-5-iodouridine.

Idoxuridine contains not less than 98.0 per cent and not more than 101.0 per cent of C_{9}H_{11}IN_{2}O_{5}, calculated on the dried basis.
Description. A white or almost white, crystalline powder; odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with idoxuridine RS or with the reference spectrum of idoxuridine. Examine the substances as dispersions containing 1 mg in 0.3 g of potassium bromide IR.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.004 per cent w/v solution in 0.01 M sodium hydroxide shows an absorption maximum only at about 279 nm; absorbance at about 279 nm, about 0.65.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).

D. Heat about 5 mg in a test-tube over a naked flame; a violet vapour is evolved.

Tests

Appearance of solution. A 1.0 per cent w/v solution in 1 M sodium hydroxide is clear (2.4.1), and colourless (2.4.1).

pH (2.4.24). 5.5 to 6.5, determined in a 0.1 per cent w/v solution.

Specific optical rotation (2.4.22). +28.0° to +32.0°, determined in a 1.0 per cent w/v solution in 1 M sodium hydroxide.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 50 volumes of 2-propanol, 40 volumes of chloroform and 10 volumes of strong ammonia solution.

Test solution (a). Dissolve 0.4 g of the substance under examination in 10 ml of a mixture of 5 volumes of methanol and 1 volume of strong ammonia solution.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with the same solvent mixture.

Reference solution (a). Dilute 5 ml of test solution (b) to 100 ml with the same solvent mixture.

Reference solution (b). A solution containing 0.02 per cent w/v each of 5-iodouracil RS and 2′-deoxyuridine RS in the same solvent mixture.

Reference solution (c). A solution containing 0.4 per cent w/v of idoxuridine RS in the same solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of cold air and examine in ultraviolet light at 254 nm. The spots due to 5-iodouracil and 2′-deoxyuridine in the chromatogram obtained with reference solution (b) are more intense than any corresponding spots in the chromatogram obtained with test solution (a). Any other secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

Iodide. Not more than 0.1 per cent, determined by the following method. Dissolve 0.25 g in 25 ml of 0.1 M sodium hydroxide, 5 ml of dilute hydrochloric acid and sufficient water to produce 50 ml, allow to stand for 10 minutes and filter. To 25 ml of the filtrate add 5 ml of hydrogen peroxide solution (10 vol) and 10 ml of chloroform and shake. Any pink colour produced in the organic layer is not more intense than that obtained by repeating the procedure using 1 ml of a 0.033 per cent w/v solution of potassium iodide in place of the substance under examination.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 60° over phosphorus pentoxide at a pressure of 1.5 to 2.5 kPa.

Assay. Weigh accurately about 0.3 g, dissolve in 50 ml of dimethylformamide and titrate with 0.1 M tetrabutylammonium hydroxide, determining the end-point potentiometrically (2.4.25). Carry out a blank titration. 1 ml of 0.1 M tetrabutylammonium hydroxide is equivalent to 0.03541 g of C₉H₁₁IN₂O₅.

Storage. Store protected from light.

Idoxuridine Eye Drops

Idoxuridine Eye Drops are a sterile solution of Idoxuridine in Purified Water.

Idoxuridine Eye Drops contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of idoxuridine, C₉H₁₁IN₂O₅.

Identification

A. Dilute a suitable volume with 0.01 M sodium hydroxide to produce a solution containing 0.003 per cent w/v of Idoxuridine. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum only at about 279 nm.

B. In the Assay, the chromatogram obtained with the reference solution (a) shows a peak that corresponds to the peak due to idoxuridine in the chromatogram obtained with the test solution.
**Tests**

**Other tests.** Comply with the tests stated under Eye Drops.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Add 2 ml of a 10 per cent v/v solution of ethanol (95 per cent) to 15.0 ml of a solution prepared by diluting an accurately measured volume of the eye drops with water if necessary to give a final concentration of 0.1 per cent w/v of Idoxuridine (solution A) and dilute to 20.0 ml with water.

**Reference solution (a).** Shake 0.1 g of Idoxuridine RS with 50 ml of water until dissolved and then dilute to 100.0 ml with water. To 15.0 ml of this solution add 2.0 ml of a solution prepared by diluting 10 ml of a 1.2 per cent w/v of sulphathiazole (internal standard) in ethanol (95 per cent) to 100 ml with water (solution B), and dilute to 20.0 ml with water.

**Reference solution (b).** Add 2.0 ml of solution B to 15.0 ml of solution A and dilute to 20.0 ml with water.

**Chromatographic system**
- a stainless steel column 30 cm x 4 mm, packed with octadecylsilyl silica gel (10 µm),
- mobile phase: a mixture of 87 volumes of water and 13 volumes of methanol,
- flow rate. 1.7 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Calculate the content of C9H11IN2O5 in the eye drops.

**Storage.** Store at a temperature not exceeding 30°. The eye drops should not be allowed to freeze.

**Labelling.** The label states that the eye drops should not be used for continuous periods of treatment exceeding 21 days.

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**Imipenem**

**Description.** A white to almost white or pale yellow powder.

**Identification**

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with imipenem monohydrate RS or with the reference spectrum of imipenem monohydrate.

**Tests**

**Appearance of solution.** A 1.0 per cent w/v solution in phosphate buffer pH 7.0 is not more opalescent than opalescence standard OS2 (2.4.1) and not more intensely coloured than reference solution BYS6 (2.4.1).

**pH (2.4.24).** 4.5 to 7.0 determined in 0.5 per cent w/v solution in water.

**Specific optical rotation (2.4.22).** + 84.0° to + 89.0°, determined in a 0.5 per cent w/v solution in phosphate buffer pH 7.

**Related substances.** Determine by liquid chromatography (2.4.14).

**NOTE**  Prepare the solutions immediately before use.

**Solvent mixture.** A solution prepared by dissolving 1.36 g of potassium dihydrogen phosphate in 1000 ml of water, adjust to pH 6.8 with 10 per cent w/v of potassium hydroxide and filter.

**Test solution.** Dissolve about 50 mg of the substance under examination in 25 ml of the solvent mixture.

**Reference solution (a).** Heat 20 ml of the test solution at 60° for 5 minutes previously adjusted to pH 10.0 with 10 per cent w/v of sodium hydroxide.

**Reference solution (b).** A 0.002 per cent w/v solution of imipenem monohydrate RS in the solvent mixture.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as ACE, AQ),
- mobile phase: A. methanol,
  B. a buffer solution prepared by dissolving 6.25 g of dipotassium hydrogen phosphate and 2.5 g of potassium dihydrogen phosphate in 900 ml of water. Adjust to pH 7.0 with orthophosphoric acid or 10 per cent w/v of potassium hydroxide, dilute to 1000 ml with water and filter,
  - flow rate.1.2 ml per minute,
  - a linear gradient programme using the conditions given below,
  - spectrophotometer set at 210 nm and 300 nm,
  - a 20 µl loop injector.

---

**Imipenem**

\[
\text{C}_{12}\text{H}_{17}\text{N}_{3}\text{O}_{4}\text{S},\text{H}_{2}\text{O}\quad \text{Mol. Wt. 317.4}
\]


Imipenem contains not less than 98.0 per cent and not more than 101.0 per cent of C12H17N3O4S, calculated on the anhydrous basis.
Inject reference solution (a) at 300 nm. The relative retention time between the peak due to thienamycin and imipenem is about 0.76. The test is not valid unless the resolution between the peak due to thienamycin and imipenem is at least 4.0. The column efficiency is not less than 3000 theoretical plates, and the tailing factor is not more than 1.5.

Inject reference solution (b) at 210 nm. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution (b). Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak, other than that of the principal peak and any peak corresponding to thienamycin, is not more than 0.7 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.7 per cent); the sum of the areas of all the peaks, other than that of the principal peak and any peak corresponding to thienamycin, is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.2 per cent, determined on 1.0 g.

Water (2.3.43). 5.0 per cent to 8.0 per cent, determined on 0.2 g.

Assay. Determine by liquid chromatography (2.4.14). Keep the solutions in an ice-bath and use within 8 hours of preparation.

Test solution. Dissolve about 40 mg of the substance under examination in 100 ml of the mobile phase.

Reference solution. A 0.04 per cent w/v solution of imipenem monohydrate RS in the mobile phase.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm) (such as Bondapak C18),
- mobile phase: a solution prepared by dissolving 54 mg of monobasic potassium phosphate in 360 ml of water, adjusted to pH 6.8 with 0.5 M sodium hydroxide or 0.5 M orthophosphoric acid, dilute to 400 ml with water and filter,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 300 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 600 theoretical plates, and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the content of C₁₂H₁₇N₃O₄S.

Imipenem intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial Endotoxins (2.2.3). Not more than 0.17 Endotoxin unit per mg of imipenem.

Imipenem intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store in an airtight container in a refrigerator (2° to 8°).

Labelling. The label states, where applicable, that the substance is sterile and is free from bacterial endotoxins.

Imipenem and Cilastatin Injection

Imipenem and Cilastatin Injection is a sterile mixture of Imipenem, Cilastatin Sodium, and Sodium Bicarbonate.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Imipenem and Cilastatin Injection contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amounts of imipenem, C₁₂H₁₇N₃O₄S and cilastatin, C₁₆H₂₆N₂O₅S.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.
**Identification**

In the Assay, the principal peaks in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

**Tests**

**pH** (2.4.24). 6.5 to 8.5, when constituted as directed on the label.

**Bacterial endotoxins** (2.2.3). Not more than 0.17 Endotoxin Unit per mg of imipenem and not more than 0.17 Endotoxin Unit per mg of cilastatin.

**Sterility** (2.2.11). Complies with the test for sterility.

**Loss on drying** (2.4.19). Not more than 3.5 per cent, determined on 0.1 g, by drying in an oven at 60º for 3 hours at a pressure not exceeding 0.7 kPa.

**Assay.** Determine by liquid chromatography (2.4.14).

*NOTE – Prepare the following solutions immediately before use.*

**Test solution.** Determine the weight of the contents of 10 containers. Weigh accurately a quantity of the mixed contents of the 10 containers containing about 50 mg of Imipenem dissolve in *buffer solution pH 6.8* and dilute to 100.0 ml with the same solution.

**Reference solution (a).** A 0.05 per cent w/v solution of imipenem monohydrate RS in *buffer solution pH 6.8*.

**Reference solution (b).** A 0.05 per cent w/v solution of cilastatin ammonium RS in *buffer solution pH 6.8*.

**Chromatographic system**

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 50º,
- mobile phase: dissolve 2.0 g of sodium 1-hexanesulphonate in 800 ml of buffer solution pH 6.8, adjust the pH to 6.8 with 0.5 M sodium hydroxide or 0.5 M phosphoric acid, dilute to 1000 ml with *buffer solution pH 6.8* and filter,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 10 µl loop injector.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 1.5, the column efficiency in not less than 600 theoretical plates, and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and reference solutions (a) and (b).

Calculate the contents of C₁₂H₁₇N₃O₅S and C₁₆H₂₆N₂O₅S in the injection.

**Storage.** Store protected from moisture, in a single dose or multiple dose container.

**Labelling.** The label states that the constituted solution should be solubilized in a suitable parenteral fluid prior to intravenous infusion.

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**Imipramine Hydrochloride**

C₁₀H₁₂N₂HCl    Mol. Wt. 316.9

Imipramine Hydrochloride is 10,11-dihydro-5H-dibenz[b,f]azepine-5-(dimethylaminopropyl) hydrochloride.

Imipramine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of C₁₀H₁₂N₂HCl, calculated on the dried basis.

**Description.** A white or slightly yellow, crystalline powder; almost odourless.

**Identification**

*Test A may be omitted if tests B, C, D and E are carried out.*

**A.** Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with imipramine hydrochloride RS or with the reference spectrum of imipramine hydrochloride.

**B.** When examined in the range 230 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution in 0.01 M hydrochloric acid shows an absorption maximum only at about 250 nm and a shoulder at about 270 nm; absorbance at about 250 nm, about 0.52.

**C.** Dissolve 5 mg in 2 ml of nitric acid; an intense blue colour is produced.

**D.** Dissolve 50 mg in 3 ml of water and add 1 drop of a 2.5 per cent w/v solution of quinhydrone in methanol; no red colour is produced within 15 minutes.

**E.** 20 mg gives reaction A of chlorides (2.3.1).

**Tests**

**Appearance of solution.** Triturate with a glass rod 3.0 g with 20 ml of carbon dioxide-free water and dilute to 30 ml with the same solvent (solution A). Solution A is clear (2.4.1).
Immediately after preparation dilute the solution with an equal volume of water. The resulting solution is not more intensely coloured than reference solution BYS6 (2.4.1).

**pH** (2.4.24). 3.6 to 5.0, determined in solution A immediately after preparation.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 55 volumes of ethyl acetate, 35 volumes of glacial acetic acid, 5 volumes of hydrochloric acid and 5 volumes of water.

**Prepare the following solutions immediately before use.**

**Test solution.** Dissolve 0.25 g of the substance under examination in 10 ml of methanol.

**Reference solution (a).** A 0.005 per cent w/v solution of the substance under examination in methanol.

**Reference solution (b).** A 0.005 per cent w/v solution of iminodibenzyl RS in methanol.

Apply to the plate 10 µl of each solution. After development, remove the plate, allow the solvent to evaporate for 5 minutes, spray with a 0.5 per cent w/v solution of potassium dichromate in sulphuric acid (20 per cent) and examine immediately. In the chromatogram obtained with the test solution any spot corresponding to iminodibenzyl is not more intense than the spot in the chromatogram obtained with reference solution (b) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.5 g, dissolve in 50 ml of chloroform, add 10 ml of mercuric acetate solution and titrate with 0.1 M perchloric acid, using 0.5 ml of metanil yellow solution as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.03169 g of C₁₉H₂₄N₂.HCl.

**Storage.** Store protected from light.

### Identification

Triturate a quantity of the powdered tablets containing about 0.25 g of Imipramine Hydrochloride with 10 ml of chloroform, filter, evaporate the filtrate to low bulk, add ether until a turbidity is produced, and allow to stand. The precipitate, after recrystallisation from acetone, melts at about 172° (2.4.21), and complies with the following tests.

A. Dissolve 5 mg in 2 ml of nitric acid; an intense blue colour is produced.

B. Dissolve 50 mg in 3 ml of water and add 1 drop of a 2.5 per cent w/v solution of quinhydrone in methanol; no red colour is produced within 15 minutes.

C. 20 mg gives reaction A of chlorides (2.3.1).

### Tests

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 55 volumes of ethyl acetate, 35 volumes of glacial acetic acid, 5 volumes of hydrochloric acid and 5 volumes of water.

**Prepare the following solutions immediately before use.**

**Test solution.** Shake a quantity of the powdered tablets containing 0.2 g of Imipramine Hydrochloride with three quantities, each of 10 ml, of chloroform, filter the combined chloroform extracts, evaporate to dryness and dissolve the residue in 10 ml of methanol.

**Reference solution (a).** Dilute 3 volumes of the test solution to 1000 volumes with methanol.

**Reference solution (b).** A 0.006 per cent w/v solution of iminodibenzyl RS in methanol.

Apply to the plate 10 µl of each solution. After development, remove the plate, allow the solvent to evaporate for 5 minutes, spray with a 0.5 per cent w/v solution of potassium dichromate in sulphuric acid (20 per cent) and examine immediately. In the chromatogram obtained with the test solution any spot corresponding to iminodibenzyl is not more intense than the spot in the chromatogram obtained with reference solution (b) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Uniformity of content.** (For tablets containing 10 mg or less). Comply with the test stated under Tablets.

Powder one tablet, shake with 25 ml of 0.1 M hydrochloric acid for 30 minutes, add sufficient 0.1 M hydrochloric acid to produce 100.0 ml and filter. Dilute 10.0 ml of the filtrate to 50.0 ml with 0.1 M hydrochloric acid and measure the absorbance of the resulting solution at the maximum at about 250 nm (2.4.7).
Calculate the content of C_{19}H_{24}N_{2}HCl in the tablet taking 264 as the specific absorbance at 250 nm.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 25 mg of Imipramine Hydrochloride, shake with 75 ml of 0.1 M hydrochloric acid for 30 minutes, dilute to 100.0 ml with the same solvent and filter through a sintered-glass filter. Dilute 10.0 ml to 100.0 ml with 0.1 M hydrochloric acid and measure the absorbance of the resulting solution at the maximum at about 250 nm (2.4.7). Calculate the content of C_{19}H_{24}N_{2}, HCl taking 264 as the specific absorbance at 250 nm.

### Indinavir Sulphate

![Indinavir Sulphate molecule]

C_{36}H_{47}N_{5}O_{4}, H_{2}SO_{4}  \quad \text{Mol. Wt. 711.9}

Indinavir Sulphate is \([1\{1,5,7,2R,5(S)\}-2,3,5\text{-trideoxy-}\text{N-(2,3-dihydro-2-hydroxy-1H-inden-1-yl)}-5\{2\text{-[1,1-dimethylethyl]amino}\text{carbonyl}\}-4\text{-[3-pyridinylmethyl]1-piperazinyl\}}\text{-2\text{-}(phenylmethyl)-D-erythro-pentonamide sulphate.}

Indinavir Sulphate contains not less than 98.5 per cent and not more than 101.5 per cent of C_{36}H_{47}N_{5}O_{4}H_{2}SO_{4}, calculated on the anhydrous and ethanol-free basis.

**Description.** A white or almost white powder; hygroscopic.

**Identification.** A white or almost white powder; hygroscopic.

**Tests**

**pH** (2.4.24). 2.8 to 3.2, determined in a 1.0 per cent w/v solution in carbon dioxide-free water.

**Specific optical rotation** (2.4.22). +122° to +129°, determined at about 365 nm in a 1.0 per cent w/v solution in water, calculated on the anhydrous and ethanol-free basis.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 0.1 g of the substance under examination in 100 ml of the mobile phase.

**Reference solution (a).** A 0.0001 per cent w/v solution of indinavir RS in the mobile phase.

**Reference solution (b).** Transfer 15 mg of indinavir RS to a 50-ml volumetric flask and add 0.1 ml of 5 M hydrochloric acid. Keep this solution for about 1 hour at room temperature and make up to volume with the mobile phase.

**Chromatographic system**

- a stainless steel column 20 cm x 4.6 mm, packed with base deactivated octylsilyl silica gel (5 µm),
- column temperature. 40°,
- mobile phase: a filtered and degassed mixture of 40 volumes of acetonitrile and 60 volumes of a solution containing 0.37 per cent w/v of sodium citrate and 0.16 per cent w/v of citric acid, the pH of which has been adjusted to 5.0 with 1 M sodium hydroxide or 1 M phosphoric acid,
- flow rate. 1 ml per minute,
- spectrophotometer set at 260 nm,
- a 50 µl loop injector.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to indinavir and any impurity at a relative retention time of about 1.4 is not less than 2.

Inject reference solution (a). The test is not valid unless the capacity factor for indinavir peak is not less than 2.0, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5 per cent.

Inject the test solution. Calculate the content of each impurity in the chromatogram obtained with the test solution by comparing with the area of the principal peak obtained with reference solution (a). The content of any individual impurity is not more than 0.1 per cent and the sum of all impurities is not more than 0.5 per cent.

**Monoethyl sulphate content.** Determine by liquid chromatography (2.4.14).

**Prepare the following solutions freshly.**

**Test solution.** Dissolve about 0.125 g of the substance under examination in 25 ml of water and filter through a fine porosity membrane.

**Reference solution (a).** Weigh and transfer about 65 mg of potassium monoethyl sulphate to a 50-ml volumetric flask, dissolve and dilute to volume with water. Dilute 5 ml of this solution to 50 ml with water.
Reference solution (b). Dilute 5 ml of the test solution to 50 ml with water. Further dilute 25 ml of this solution to 100 ml with water and filter through a fine porosity membrane.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with rigid, spherical styrene-divinylbenzene copolymer, 5 to 10 µm (such as Metrosep A supp 3.6.1005.320),
- mobile phase: a mixture of 10 volumes of acetone and 90 volumes of a buffer prepared by dissolving 0.25 g each of sodium carbonate and sodium bicarbonate in 1000 ml of water,
- flow rate. 0.5 ml per minute,
- a conductivity detector,
- a 20 µl loop injector.

Inject reference solution (b). The test is not valid unless the relative standard deviation of replicate injections is not more than 2.0 per cent and the tailing factor for monoethyl sulphate anion peak is not more than 2.0.

Inject the test solution. Calculate the content of monoethyl sulphate present from the declared content of monoethyl sulphate present in potassium monoethyl sulphate.

1 mg of potassium monoethyl sulphate corresponds to 0.77 mg of monoethyl sulphate. The content of monoethyl sulphate is not more than 0.05 per cent w/w.

Ethanol. 5.0 to 8.0 per cent, calculated on the anhydrous basis, determined by the following method.

Determine by gas chromatography (2.4.13).

Test solution. Add 5 ml of a 1.5 per cent w/v solution of n-propanol (internal standard) to 1.0 g of the substance under examination in a 25-ml volumetric flask and dilute to volume with water.

Reference solution. A 0.1 per cent w/v solution of ethanol.
Transfer 5 ml of this solution and 5 ml of the internal standard to a 25-ml volumetric flask and make up to volume with water.

Chromatographic system
- a stainless steel column 1.8 m x 2 mm, packed with ethylvinyl benzene divinyl benzene copolymer, mesh size 80/100,
- temperature: inlet port. 180°,
- flame ionisation detector,
- flow rate. 20 ml per minute of the carrier gas (nitrogen).

Separately inject 10 µl of the test solution and the reference solution. Calculate the ethanol content by comparing the ratio of the area of the peak corresponding to ethanol and the area of the internal standard peak in the chromatogram obtained with the test solution with that of the reference solution.

Sulphate. 13.2 to 14.4 per cent w/w, calculated on the anhydrous and ethanol-free basis, determined by the following method.

Weigh accurately about 0.5 g, dissolve in 50 ml of dimethylformamide and titrate with 0.1 M sodium hydroxide, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.0480 g of sulphate.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 1.5 per cent, determined on 0.2 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately about 60 mg of the substance under examination in 100.0 ml of the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated octylsilyl silica gel (5 µm),
- column temperature. 40°,
- mobile phase: a filtered and degassed mixture of 45 volumes of acetonitrile and 55 volumes of a buffer prepared by dissolving 3 g of phosphoric acid and 1.7 ml of dibutylamine in 900 ml of water, adjusting the pH to 6.5 with 1 M sodium hydroxide and making up the volume to 1000.0 ml with water,
- flow rate. 1 ml per minute,
- spectrophotometer set at 260 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the column efficiency determined from the indinavir peak is not less than 4000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of C₃₆H₄₇N₅O₄·H₂SO₄.
1 mg of indinavir corresponds to 1.16 mg of indinavir sulphate.

Storage. Store protected from light.

Indinavir Capsules

Indinavir Sulphate Capsules

Indinavir Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of indinavir, C₃₆H₄₇N₅O₄·H₂SO₄.

Identification

A. Shake a quantity of the contents of the capsules containing 0.1 g of Indinavir Sulphate with 80 ml of water, dilute to 100 ml
with water and filter. Dilute 5 ml of the filtrate to 100 ml with water. When examined in the range 200 nm to 300 nm (2.4.7), the resulting solution shows an absorption maximum at about 260 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to indinavir in the chromatogram obtained with the reference solution.

**Tests**

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Mix well the contents of 20 capsules and shake a quantity of the mixed contents containing about 50 mg of indinavir with about 60 ml of a solution prepared by mixing 40 volumes of acetonitrile and 60 volumes of 0.05 M dipotassium hydrogen phosphate, the pH of which is adjusted to 7.5 with dilute phosphoric acid (solution A) in a 100-ml volumetric flask, mix with the aid of ultrasound for 10 minutes, dilute to volume with solution A and filter.

**Reference solution (a).** Weigh accurately a quantity of indinavir sulphate RS containing about 50 mg of indinavir in a 100-ml volumetric flask, dissolve and dilute to volume with solution A. Dilute 1 ml of this solution to 100 ml with solution A.

**Reference solution (b).** Dissolve a quantity of indinavir sulphate RS containing about 50 mg of indinavir and 5 mg of indinavir 4-epimer RS and dilute to 100 ml with solution A.

**Chromatographic system**
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: filtered and degassed mixtures of acetonitrile and 0.05 M dipotassium hydrogen phosphate, adjusting the pH of the solution to 7.5 with dilute phosphoric acid,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 260 nm,
- a 20 µl loop injector.

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<th>Time (in min)</th>
<th>Phosphate buffer (pH 7.5) (per cent v/v)</th>
<th>Acetonitrile (per cent v/v)</th>
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Inject reference solution (b). The test is not valid unless the column efficiency determined from the indinavir peak is not less than 10,000 theoretical plates, the tailing factor is not more than 1.5 and the resolution between indinavir and indinavir 4-epimer peaks is not less than 1.5.

Inject reference solution (a) and the test solution. Examine the chromatogram obtained with reference solution (a) for any extraneous peaks and ignore the corresponding peaks observed in the chromatogram obtained with the test solution.

Any secondary peak observed in the chromatogram obtained with the test solution should not be more than 1.0 per cent and the sum of the areas of all the secondary peaks should not be more than 2.5 per cent when calculated by percentage area normalisation.

**Dissolution** (2.5.2).

**Apparatus.** No 1

Medium. 900 ml of a buffer prepared by dissolving 21 g of citric acid in 880 ml of water, adjusting the pH to 3.8 with a 50 per cent w/v solution of sodium hydroxide and making up to 1000 ml with water

Speed and time. 50 rpm and 30 minutes. Use sinkers.

Withdraw a suitable volume of the medium and filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate if necessary, with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 260 nm (2.4.7). Calculate the content of indinavir, C₃₆H₄₇N₅O₄, in the medium from the absorbance obtained from a solution of known concentration of indinavir sulphate RS in the same solvent.

D. Not less than 75 per cent of the stated amount of C₃₆H₄₇N₅O₄.

**Other tests.** Comply with the tests stated under Capsules.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Mix well the contents of 20 capsules and shake a quantity of the mixed contents containing about 200 mg of indinavir with about 60 ml of the mobile phase, mix with the aid of ultrasound for 10 minutes, dilute to 100.0 ml with the mobile phase, mix and filter. Dilute 10.0 ml of the filtrate to 100.0 ml with the mobile phase.

**Reference solution.** Weigh accurately a quantity of indinavir sulphate RS containing about 50 mg of indinavir in a 50-ml volumetric flask, dissolve and dilute to volume with the mobile phase. Dilute 10.0 ml of this solution to 50.0 ml with the mobile phase.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated octylsilyl silica gel (5 µm),
mobile phase: a filtered and degassed mixture of 45 volumes of acetonitrile and 60 volumes of 0.05 M dipotassium hydrogen phosphate, with the pH adjusted to 7.5 with dilute phosphoric acid,

- flow rate. 1 ml per minute,
- spectrophotometer set at 260 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the column efficiency determined from the indinavir peak is not less than 6000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the content of C₃₆H₄₇N₅O₄ in the capsules.

Storage. Store protected from moisture at a temperature not exceeding 30°

Indomethacin
Indometacin

\[
\text{C}_{19}\text{H}_{16}\text{ClNO}_4 \quad \text{Mol. Wt. 357.8}
\]

Indomethacin is 1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl acetic acid.

Indomethacin contains not less than 98.0 per cent and not more than 101.0 per cent of C₁₉H₁₆ClNO₄, calculated on the dried basis.

Description. A white to pale yellow, crystalline powder; odourless or almost odourless.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with indomethacin RS or with the reference spectrum of indomethacin. Examine the substances in the solid state without recrystallisation.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0025 per cent w/v solution in a mixture of 90 volumes of methanol and 10 volumes of 1 M hydrochloric acid shows an absorption maximum only at about 320 nm; absorbance at about 320 nm, about 0.45.

C. Dissolve 0.1 g in 10 ml of ethanol (95 per cent), heating gently if necessary. To 0.1 ml add 2 ml of a freshly prepared mixture of 1 volume of a 25 per cent w/v solution of hydroxylamine hydrochloride and 3 volumes of 2 M sodium hydroxide. Add 2 ml of 2 M hydrochloric acid and 1 ml of ferric chloride solution and mix; a violet-pink colour develops.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with a suspension of silica gel HF254 in a 4.68 per cent w/v solution of sodium dihydrogen phosphate.

Mobile phase. A mixture of 70 volumes of ether and 30 volumes of light petroleum (50° to 70°).

Prepare the following solutions immediately before use.

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of methanol.

Reference solution. A 0.01 per cent w/v solution of the substance under examination in methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.45 g, dissolve in 75 ml of acetone and titrate under nitrogen with carbonate-free 0.1 M sodium hydroxide using 0.2 ml of phenolphthalein solution as indicator. Carry out a blank titration.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.03578 g of C₁₉H₁₆ClNO₄.

Storage. Store protected from light.

Indomethacin Capsules

Indomethacin Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of indomethacin, C₁₉H₁₆ClNO₄.
Identification

A. Shake a quantity of the contents of the capsules containing 0.1 g of Indomethacin with 5 ml of chloroform, filter and evaporate the filtrate to dryness. Dry the residue at 60° at a pressure not exceeding 0.7 kPa for 1 hour. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with indomethacin RS or with the reference spectrum of indomethacin.

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum only at about 320 nm.

C. Mix a quantity of the contents of the capsules containing 25 mg of Indomethacin with 2 ml of water and add 2 ml of 2 M sodium hydroxide; a bright yellow colour is produced which fades rapidly.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with a suspension of silica gel HF254 in a 4.68 per cent w/v solution of sodium dihydrogen phosphate.

Mobile phase. A mixture of 70 volumes of ether and 30 volumes of light petroleum (50° to 70°).

Prepare the following solutions immediately before use.

Test solution. Shake a quantity of the contents of the capsules containing 0.1 g of Indomethacin with 5 ml of chloroform, filter and use the filtrate.

Reference solution. Dilute 1 volume of the test solution to 200 volumes with chloroform.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Dissolution (2.5.2).

Apparatus. No 1

Medium. 750 ml of a freshly prepared mixture of 1 volume of phosphate buffer pH 7.2 and 4 volumes of water

Speed and time. 100 rpm and 20 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Dilute the filtrate suitably with the medium and measure the absorbance of the resulting solution at the maximum at about 320 nm (2.4.7). Calculate the content of C_{19}H_{16}ClNO_{4} in the medium from the absorbance obtained from a solution of known concentration of indomethacin RS in the same medium.

D. Not less than 80 per cent of the stated amount of C_{19}H_{16}ClNO_{4}.

Other tests. Comply with the tests stated under Capsules.

Assay. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 50 mg of Indomethacin, add 10 ml of water and allow to stand for 10 minutes, with occasional swirling. Add 75 ml of methanol, shake well, add sufficient methanol to produce 100.0 ml and filter if necessary. To 5.0 ml of the filtrate add sufficient of a mixture of equal volumes of methanol and phosphate buffer pH 7.2 to produce 100.0 ml. Measure the absorbance of the resulting solution at the maximum at about 320 nm (2.4.7). Calculate the content of C_{19}H_{16}ClNO_{4} taking 193 as the specific absorbance at 320 nm.

Storage. Store protected from moisture.

Indomethacin Suppositories

Indomethacin Suppositories contain Indomethacin in a suitable suppository basis.

Indomethacin Suppositories contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of indomethacin, C_{19}H_{16}ClNO_{4}.

Identification

A. Dissolve a quantity of the powdered suppositories containing 0.1 g of Indomethacin as completely as possible in 50 ml of hot water; filter, wash the residue with hot water and allow to dry in air. Dissolve the residue in 5 ml of chloroform and evaporate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with indomethacin RS or with the reference spectrum of indomethacin.

B. Shake a quantity containing 25 mg of Indomethacin with 5 ml of water until the base dissolves; a white suspension is produced. Add 2 ml of 2 M sodium hydroxide; a bright yellow colour is produced which fades rapidly.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Prepare the following solutions freshly.

Test solution (a). Powder or cut into small pieces a suitable number of suppositories, dissolve a quantity containing 0.1 g of Indomethacin in sufficient methanol to produce 50 ml.
Reference solution (a). Dilute 3 volumes of test solution (a) to 100 volumes with the mobile phase.

Chromatographic system
- a stainless steel column 30 cm x 4 mm, packed with octadeclisilc silica gel (10 µm) (such as Bondapack C18),
- mobile phase: a mixture of 60 volumes of methanol and 40 volumes of 0.2 per cent v/v solution of phosphoric acid,
- flow rate. 2 ml per minute,
- spectrophotometer set at 320 nm,
- a 20 µl loop injector.

The sum of the areas of any secondary peaks that elute before the principal peak in the chromatogram obtained with test solution (a) is not greater than the area of the peak in the chromatogram obtained with reference solution (a).

Repeat the procedure but using the following freshly prepared solutions and a detection wavelength of about 240 nm.

Test solution (b). Dilute 10 volumes of test solution (a) to 20 volumes with the mobile phase.

Reference solution (b). A solution containing 0.001 per cent w/v of 4-chlorobenzoic acid in the mobile phase.

In the chromatogram obtained with test solution (b) the sum of the areas of any secondary peaks that elute before the principal peak, other than those determined in test solution (a), is not greater than the area of the peak in the chromatogram obtained with reference solution (b).

The column efficiency, determined using the principal peak in the chromatogram obtained with reference solution (a), should be not less than 7500 theoretical plates per metre.

Disintegration (2.5.1). Use a weighed suppository and phosphate buffer pH 6.8 in place of water and operate the apparatus for 90 minutes. At the end of this period remove the suppository, dry with filter paper and weigh. Repeat the operation with two further weighed suppositories. Not less than 75 per cent of each suppository is dissolved.

Other tests. Comply with the tests stated under Suppositories.

Assay. Weigh 10 suppositories and powder or cut into small pieces. Weigh accurately a quantity of the powder or small pieces containing about 0.1 g of Indomethacin, add 50 ml of methanol, shake until the dispersion is complete and, if necessary, filter. To 2.0 ml add sufficient of a mixture of equal volumes of methanol and phosphate buffer pH 7.2 to produce 100.0 ml. Measure the absorbance of the resulting solution at the maximum at about 320 nm (2.4.7). Calculate the content of C_{19}H_{16}ClNO_4 taking 193 as the specific absorbance at 320 nm.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Insulin

Crystalline Insulin

C_{256}H_{381}N_{65}O_{76}S_{6} (porcine)  
Mol. Wt. 5777.6

C_{254}H_{377}N_{65}O_{75}S_{6} (bovine)  
Mol. Wt. 5733.5

Insulin is the specific natural antidiabetic principle obtained from the pancreas of either the pig or the ox and purified.

Insulin contains not less than 26.5 Units per mg of porcine insulin, C_{256}H_{381}N_{65}O_{76}S_{6}, or of bovine insulin, C_{254}H_{377}N_{65}O_{75}S_{6}, as appropriate, calculated on the dried basis.

Description. A white or almost white powder.

Identification

A. In the Assay, the principal peak due to insulin in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the appropriate reference solution.

B. Complies with the test for peptide mapping (2.3.47).

Tests

Light absorption (2.4.7). Absorbance of a 0.05 per cent w/v solution in 0.01 M hydrochloric acid at the maximum at about 276 nm, 0.48 to 0.56.
**Other tests.** Complies with the tests for Impurities with molecular masses greater than that of insulin, Related proteins and Total zinc stated under Insulin Preparations.

**Sulphated ash** (2.3.18). Not more than 2.5 per cent, calculated on the dried basis, determined on 0.2 g.

**Loss on drying** (2.4.19). Not more than 10.0 per cent, determined on 0.2 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa.

**Assay.** Determine as described under Assay of Insulins (2.3.46).

**Test solution.** Dissolve a suitable quantity of the substance under examination in 0.01 M hydrochloric acid to obtain a concentration of 4.0 mg per ml.

**Insulin intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.**

**Bacterial endotoxins** (2.2.3). Not more than 20 Endotoxin Units per mg.

**Storage.** Store protected from light, at a temperature not exceeding −20° until released by the manufacturer. When thawed, it should be stored in a refrigerator (2° to 8°) and used for the manufacture of preparations within a short period of time.

**Labelling.** The label states (1) the animal source or sources of the insulin; (2) where applicable, that the material is free from bacterial endotoxins; (3) the storage conditions.

**NOTE** — 0.0345 mg of porcine or 0.0342 mg of bovine insulin is equivalent to 1 Unit of insulin.

### Human Insulin

![Human Insulin structure](image)

**C$_{257}$H$_{383}$N$_{65}$O$_{77}$S$_{6}$**  
Mol. Wt. 5808.0

Human insulin contains not less than 27.5 Units per mg of human insulin, C$_{257}$H$_{383}$N$_{65}$O$_{77}$S$_{6}$, calculated on the dried basis.

**Description.** A white or almost white powder.

**Identification**

A. In the Assay, the principal peak due to insulin in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b) or (c), as appropriate.

B. Complies with the test for peptide mapping (2.3.47).

**Tests**

**Light absorption** (2.4.7). Absorbance of a 0.05 per cent w/v solution in 0.01 M hydrochloric acid at the maximum at about 276 nm, 0.48 to 0.56.

**Total zinc.** Not more than 1.0 per cent, calculated on the dried basis, determined by the following method. To 5 ml of a 0.5 per cent w/v solution in 0.01 M hydrochloric acid add 10 ml of alkaline borate buffer pH 9.0, 3 ml of zincon solution and sufficient water to produce 50 ml. Allow to stand for 1 hour and measure the absorbance of the resulting solution at about 620 nm, using as the blank a solution prepared by treating 5 ml of water instead of the substance being examined in a similar manner. Calculate the content of zinc from the absorbance obtained by repeating the procedure using a suitable aliquot of a mixture of 4 volumes of zinc sulphate solution and 6 volumes of water.

**Other tests.** Complies with the tests for Impurities with molecular masses greater than that of insulin, Related proteins and Total zinc stated under Insulin Preparations.
Storage. Store protected from light, at a temperature not exceeding –18° until released by the manufacturer. When thawed, it should be stored in a refrigerator (2° to 8°) and used for the manufacture of preparations within a short period of time.

Labelling. The label states (1) whether the material is produced by enzymatic modification of porcine insulin or by rDNA technology; (2) where applicable, that the material is free from bacterial endotoxins; (3) the storage conditions.

NOTE - 0.0347 mg of human insulin is equivalent to 1 Unit of insulin.

Insulin Injection

Neutral Insulin; Neutral Insulin Injection; Soluble Insulin; Plain Insulin

Insulin injection is a neutral, sterile solution of bovine, porcine or human insulin.

Insulin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated number of Units of Insulin.

Description. A colourless liquid, free from turbidity and foreign matter; during storage, traces of a very fine sediment may be deposited.

Identification

In the chromatograms obtained in the assay, the position of the peak due to insulin in the chromatogram obtained with the test solution corresponds to that of the principal peak obtained with the appropriate reference solution.

Tests

pH (2.4.24). 6.8 to 7.8.

Total zinc. Not more than 40 μg per 100 Units of insulin, determined by either of the methods stated under Insulin Preparations.

Test solution. Dilute a volume of the gently shaken preparation under examination containing 200 Units to 25.0 ml with water. Dilute if necessary to a suitable concentration with water.

Other tests. Complies with the tests stated under Insulin Preparations.

Assay. Determine as described under Assay of Insulins (2.3.46).

Test solution. Add 4 μl of 6 M hydrochloric acid per millilitre of the injection to obtain a clear solution. For a preparation containing more than 100 IU ml, an additional dilution with 0.01 M hydrochloric acid is necessary to avoid overloading the column. Dilute a suitable volume of the injection with 0.01 M hydrochloric acid to obtain a concentration of 40 Units per ml.

Storage. Store in multiple dose containers in a refrigerator (2° to 8°). It should not be allowed to freeze.

Labelling. The label states (1) whether the preparation is acidified or neutral; (2) the strength in terms of the number of Units per ml; (3) the animal source or sources of the insulin; (4) that the preparation should not be allowed to freeze; (5) the storage conditions.

Biphasic Insulin Injection

Biphasic Insulin Injection is a sterile suspension of crystals containing bovine insulin in a solution of porcine insulin.

Biphasic Insulin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated number of Units of Insulin.

Description. A white suspension. When examined under a microscope, the majority of the particles appear as rhombohedral crystals, with a maximum dimension of the crystals greater than 10 μm but rarely exceeding 40 μm.

Identification

In the chromatograms obtained in the Assay the position of the peaks due to the two insulins in the chromatogram obtained with the test solution correspond to those of the principal peaks in the chromatogram obtained with the appropriate reference solution.

Tests

pH (2.4.24). 6.6 to 7.2.

Total zinc. 26.0 μg to 37.5 μg per 100 Units of insulin, determined by either of the methods stated under Insulin Preparations.

Insulin in the supernatant. 22.0 per cent to 28.0 per cent of insulin in solution, determined as stated under Insulin Preparations.

Other tests. Complies with the tests stated under Insulin Preparations.

Assay. Determine as described under Assay of Insulins (2.3.46).

Test solution. To 10 ml of the preparation under examination add 40 μl of 5 M hydrochloric acid, mix well, allow to stand for 1 hour to ensure solution of the sediment and dilute with 0.03 M hydrochloric acid to obtain a solution containing 40 Units per ml.
Biphasic Isophane Insulin Injection

Biphasic isophane Insulin Injection is a sterile buffered suspension of either porcine or human insulin, complexed with protamine sulphate or any other suitable protamine, in a solution of insulin of the same species.

It is prepared by the procedure described under Insulin Preparations. It is produced by mixing, in defined ratios, soluble insulin injection and isophane insulin injection.

Biphasic isophane Insulin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated number of Units of Insulin.

Description. A white suspension which on standing deposits a white sediment and leaves a colourless or almost colourless supernatant liquid; the sediment is readily resuspended by gently shaking. When examined under a microscope, the particles are seen to be rod-shaped crystals, the majority with a maximum dimension greater than 1 µm but rarely exceeding 60 µm, free from large aggregates.

Identification

In the chromatograms obtained in the Assay the position of the peak due to insulin in the chromatogram obtained with the test solution corresponds to that of the principal peak in the chromatogram obtained with the appropriate reference solution.

Tests

pH (2.4.24). 6.9 to 7.5.

Total zinc. Not more than 40 µg per 100 Units of insulin, determined by either of the methods stated under Insulin Preparations.

Other tests. Complies with the tests stated under Insulin Preparations.

Assay. Determine as described under Assay of Insulins (2.3.46). Test solution. To 10 ml of the preparation under examination add 40 µl of 5 M hydrochloric acid, mix well, allow to stand for 1 hour to ensure solution of the sediment and dilute with 0.03 M hydrochloric acid to obtain a solution containing 40 Units per ml.

Storage. Store in multiple dose containers in a refrigerator (2° to 8°). It should not be allowed to freeze.

Labelling. The label states (1) the strength in terms of the number of Units per ml; (2) the animal source or sources of the insulin; (3) that the preparation should not be allowed to freeze; (4) that the container should be gently shaken before a dose is withdrawn; (5) the storage conditions.

Isophane Insulin Injection

Isophane Insulin; Isophane Insulin (NPH)

Isophane insulin injection is a sterile suspension of bovine, porcine or human insulin, complexed with protamine sulphate or another suitable protamine.

Protamine Zinc Insulin Injection

Protamine Zinc Insulin

Protamine Zinc Insulin Injection is a sterile buffered suspension of porcine, bovine or human insulin in the form of
a complex obtained by the addition of Protamine Sulphate or another suitable protamine and Zinc Chloride or another suitable zinc salt.

Protamine Zinc Insulin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated number of Units of Insulin.

Production

It may be prepared by assaying a sterile solution of crystalline insulin having a potency not less than 23 Units per mg, calculated on the dried basis, adjusting its potency so that when diluted with the other constituents in sterile form, it contains the requisite number of Units per ml, and adding aseptically to it a suitable protamine in the proportion of 1.0 to 1.7 mg of protamine sulphate for each 100 Units of Insulin. It contains Sodium Phosphate as buffering agent, sufficient of a suitable substance to render the preparation isotonic with blood and a sufficient amount of a suitable bactericide. It is distributed aseptically into sterile containers which are then sealed so as to exclude micro-organisms.

Description. A white suspension which on standing deposits a white sediment and leaves an almost colourless supernatant liquid. The sediment is readily resuspended on gentle shaking. When examined under a microscope, approximately half the number of particles are seen to have no uniform shape, with a maximum diameter rarely more than 2 µm. The remaining particles are seen to be rod-shaped crystals, the majority with a maximum diameter more than 10 µm but rarely more than 100 µm.

Identification

In the Assay, the principal peak due to insulin in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the appropriate reference solution.

Tests

pH (2.4.24). 6.9 to 7.5.

Total zinc. Not more than 40 µg per 100 Units of insulin, determined by either of the methods stated under Insulin Preparations.

Other tests. Complies with the requirements stated under Insulin Preparations.

Assay. Determine as described under Assay of Insulins (2.3.46). Test solution. To 10 ml of the preparation under examination add 40 µl of 5 M hydrochloric acid, mix well, allow to stand for 1 hour to ensure solution of the sediment and dilute with 0.03 M hydrochloric acid to obtain a solution containing 40 Units per ml.

Storage. Store in multiple dose containers in a refrigerator (2° to 8°). It should not be allowed to freeze.

Labelling. The label states (1) the strength in terms of the number of Units per ml; (2) the animal source or sources of the insulin; (3) that the preparation should not be allowed to freeze; (4) that the container should be gently shaken before a dose is withdrawn; (5) the storage conditions.

Insulin Zinc Suspension

I.Z.S; Insulin Zinc Suspension (Mixed); I.Z.S (Mixed); Insulin Lente

Insulin Zinc Suspension is a sterile, buffered suspension of Insulin in the form of a complex obtained by the addition of zinc chloride to insulin in a manner such that the insulin is in a form insoluble in water. It may be prepared by mixing aseptically about 3 volumes of Insulin Zinc Suspension (Amorphous) and about 7 volumes of Insulin Zinc Suspension (Crystalline) and distributing the mixture aseptically into sterile containers which are then sealed so as to exclude micro-organisms.

Insulin Zinc Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated number of Units of Insulin.

Description. A white suspension which on standing deposits a white sediment and leaves an almost colourless supernatant liquid. The sediment is readily resuspended on gentle shaking. When examined under a microscope, the majority of the particles in the suspension are seen as rhombohedral crystals, with a maximum dimension greater than 10 mm but rarely exceeding 40 mm; a considerable number of particles have no uniform shape and do not exceed 2 mm in maximum dimension.

Identification

In the Assay, the principal peak due to insulin in the chromatogram obtained with test solution has a retention time similar to that of the principal peak in the chromatogram obtained with reference solution (a), reference (b) or reference solution (c), as appropriate.

Tests

pH (2.4.24). 6.9 to 7.5.

Total zinc. Not more than 0.0095 per cent w/v (for preparations containing 40 Units per ml) and not more than 0.014 per cent w/v (for preparations containing 80 Units per ml), determined by the following method. Take a volume of the well-shaken suspension containing 200 Units of insulin and add 1 ml of 0.1M hydrochloric acid, 10 ml of alkaline borate buffer pH 9.0, 1 ml of 0.1M sodium hydroxide, 2 ml of a 0.0009 per cent
Determine by liquid chromatography Insulin in solution and distil the liberated ammonia into 20 ml of the flask to an ammonia distillation apparatus, mix the contents an excess of than 0.2 ml of sulphuric acid of sulphate nitrogen-free mercuric oxide possible to a long-necked, round-bottomed flask, add 0.3 g of centrifuge. Transfer the supernatant liquid as completely as buffered acetone solution, liquid. Suspend the residue in 3.3 ml of a volume containing 400 Units and reject the supernatant liquid. Add 1 g of zinc powder, shake and allow to stand for 10 minutes. Add an excess of sodium hydroxide solution, immediately connect the flask to an ammonia distillation apparatus, mix the contents and distil the liberated ammonia into 20 ml of 0.005 M sulphuric acid prepared with carbon dioxide-free water. Rinse the condenser tube into the flask containing the distillate, add sufficient carbon dioxide-free water to produce a total volume of about 50 ml and titrate the excess of sulphuric acid with 0.01 M sodium hydroxide to pH 6.0, using a glass electrode. Centrifuge a further volume containing 400 Units and reject the supernatant liquid. Dissolve the residue in 10 ml of a 5 per cent w/v solution of nitrogen-free sulphuric acid, transfer to a long-necked, round-bottomed flask, and repeat the procedure described above beginning at the words “add 0.3 g of nitrogen-free mercuric oxide,......”. Calculate the percentage of insulin extractable with the buffered acetone solution from the formula 100A/B, where A is the volume of 0.005 M sulphuric acid used in the first determination and B is the volume used in the second determination.

The result of the test is not valid unless in carrying out the first determination omitting the insulin preparation, not more than 0.2 ml of 0.005 M sulphuric acid is required.

Insulin in solution. Determine by liquid chromatography (2.4.14).

Test solution. (for preparations containing 100 Units per ml) centrifuge a well-shaken suspension of the preparation under examination, transfer 2.0 ml of the supernatant liquid to a 5-ml volumetric flask, dilute to volume with 0.03 M hydrochloric acid and mix well.

Reference solution (a). Prepare as test solution but using 2.5 ml of the supernatant liquid in place of 2.0 ml.

Reference solution (b). Use 5 ml of the supernatant liquid.

Reference solution (c). Weigh accurately 4.5 mg of bovine insulin RS into a 100-ml volumetric flask containing 50 ml of 0.025 M hydrochloric acid, dissolve by shaking for 5 minutes, dilute to volume with 0.025 M hydrochloric acid and mix to obtain a solution containing approximately 1 Unit per ml.

Chromatographic system as described in the Assay.

The area of the peak due to insulin in the chromatogram obtained with test solution, reference solution (a) or reference (b), as the case may be, is not more than that of the principal peak in the chromatogram obtained with reference solution (c).

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. To 10 ml of the preparation under examination add 40 ml of 5 M hydrochloric acid, mix well, allow to stand for 1 hour to ensure solution of the sediment and dilute with 0.03 M hydrochloric acid to obtain a solution containing 20 Units per ml.

Reference solution (a). A 0.08 per cent w/v of bovine insulin RS in 0.025 M hydrochloric acid.

Reference solution (b). A 0.08 per cent w/v of porcine insulin RS in 0.025 M hydrochloric acid.

Reference solution (c). A solution containing 0.04 per cent w/v of bovine insulin RS and 0.04 per cent w/v of porcine insulin RS in 0.025 M hydrochloric acid for a preparation containing both bovine and porcine insulin.

Reference solution (d). A 0.04 per cent w/v each of human insulin RS and porcine insulin RS in 0.025 M hydrochloric acid.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane chemically bonded to porous silica (5 µm) (Ultrasphere ODS is suitable),
- column. temperature 45°,
- mobile phase. a mixture of 72.5 volumes of 0.1 M sodium dihydrogen phosphate adjusted to pH 2.0 with phosphoric acid and 27.5 volumes of acetonitrile,
- flow rate 1 ml per minute.
- spectrophotometer set at 214 nm,
- a 50 l l loop injector.
The test is not valid unless the resolution factor between the peaks corresponding to human insulin and porcine insulin is at least 1.2 in the chromatogram obtained with reference solution (d). If necessary, adjust the concentration of acetonitrile in the mobile phase by slight decrease or increase until the required resolution is obtained. In the chromatogram obtained with reference solution (d) the two principal peaks, in order of emergence, are due to human insulin and porcine insulin and any smaller peaks appearing immediately following each of the principal peaks are due to the corresponding monodesamido derivatives.

Inject either of reference solutions (a), (b) and (c), as appropriate, alternatively six times. The test is not valid unless the relative standard deviation of the area of the principal peak is not more than 2 per cent.

Inject the test solution. If necessary, make further adjustments in the composition of the mobile phase so that the antimicrobial preservatives present in test solution are well separated from insulin and show shorter retention times. A small reduction in the concentration of acetonitrile increases the retention time of insulin peak relatively more than those of the preservatives. If necessary, after having carried out the chromatography of a solution, wash the column with a mixture of equal volumes of acetonitrile and water for a sufficient time in order to elute any interfering substances before injecting the next solution.

Calculate the content of insulin from the area of the peaks due to the bovine, porcine or human insulin and that of any peak due to the monodesamido derivative of the insulin from the declared content of insulin in bovine insulin RS, porcine insulin RS or human insulin RS, as appropriate. For preparations containing both bovine and porcine insulin use the sum of the areas of both the bovine and porcine insulin peaks and of any peak due to the desamido derivative of either insulin.

Storage. Store in multiple dose containers at a temperature between 2° and 8°. It should not be allowed to freeze.

Labelling. The label states (1) the strength in terms of the number of Units per ml; (2) the animal source or sources of the insulin; (3) that the preparation should not be allowed to freeze; (4) that the container should be gently shaken before a dose is withdrawn; (5) the storage conditions.

Insulin Zinc Suspension (Amorphous)

Amorph. I.Z.S.; Prompt Insulin Zinc Suspension

Insulin Zinc Suspension (Amorphous) is a sterile, buffered suspension of insulin in the form of a complex obtained by the addition of zinc chloride to insulin in a manner such that the solid phase of the suspension is amorphous. It may be prepared by adding aseptically to crystalline insulin having a potency not less than 23 Units per mg, calculated on the dried basis, a suitable quantity of zinc chloride, an appropriate amount of a suitable substance to render the preparation isotonic with blood and a sufficient quantity of a suitable bactericide. It is distributed aseptically into sterile containers which are then sealed so as to exclude micro-organisms.

Insulin Zinc Suspension (Amorphous) contains not less than 90.0 per cent and not more than 110.0 per cent of the stated number of Units of Insulin.

Description. A white suspension which on standing deposits a white sediment and leaves an almost colourless supernatant liquid. The sediment is readily resuspended on gentle shaking. When examined under a microscope, the particles in the suspension are seen to have no uniform shape and rarely exceed 2 mm in maximum dimension.

Identification

In the Assay, the principal peak due to insulin in the chromatogram obtained with test solution has a retention time similar to that of the principal peak in the chromatogram obtained with reference solution (a), reference (b) or reference solution (c), as appropriate.

Tests

pH (2.4.24). 6.9 to 7.5.

Total zinc. Not more than 0.0095 per cent w/v (for preparations containing 40 Units per ml) and not more than 0.014 per cent w/v (for preparations containing 80 Units per ml), determined by the following method. Take a volume of the well-shaken suspension containing 200 Units of insulin and add 1 ml of 0.1 M hydrochloric acid, 10 ml of alkaline borate buffer pH 9.0, 1 ml of 0.1 M sodium hydroxide, 2 ml of a 0.0009 per cent w/v solution of trypsin in 0.01 M hydrochloric acid. Mix, allow to stand for 10 minutes and add 3 ml of zincon solution and sufficient water to produce 50 ml. Allow to stand for 1 hour and measure the absorbance of the resulting solution at about 620 nm (2.4.7), using as the blank a solution prepared by treating 5 ml of water instead of the substance under examination in a similar manner. Calculate the content of zinc from the absorbance obtained by repeating the procedure using a suitable aliquot of a mixture of 4 volumes of zincon sulphate solution and 6 volumes of water.

Zinc in solution. Not more than 70 per cent of the total zinc (for preparations containing 40 Units per ml) and not more than 55 per cent of the total zinc (for preparations containing 80 Units per ml), determined by the method described in the test for Total zinc, using 1 ml of the clear supernatant liquid obtained by centrifuging and beginning at the words “add 1 ml of 0.1 M hydrochloric acid......”.

Insulin in solution. Determine by liquid chromatography (2.4.14).
Test solution. (for preparations containing 100 Units per ml) centrifuge a well-shaken suspension of the preparation under examination, transfer 2.0 ml of the supernatant liquid to a 5-ml volumetric flask, dilute to volume with 0.03 M hydrochloric acid and mix well.

Reference solution (a). Prepare as test solution but using 2.5 ml of the supernatant liquid in place of 2.0 ml.

Reference solution (b). Use 5 ml of the supernatant liquid.

Reference solution (c). Weigh accurately 4.5 mg of bovine insulin RS into a 100-ml volumetric flask containing 50 ml of 0.025 M hydrochloric acid, dissolve by shaking for 5 minutes, dilute to volume with 0.025 M hydrochloric acid and mix to obtain a solution containing approximately 1 Unit per ml.

Chromatographic system as described in the Assay.

The area of the peak due to insulin in the chromatogram obtained with test solution, reference solution (a) or reference (b), as the case may be, is not more than that of the principal peak in the chromatogram obtained with reference solution (c).

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. To 10 ml of the preparation under examination add 40 ml of 5 M hydrochloric acid, mix well, allow to stand for 1 hour to ensure solution of the sediment and dilute with 0.03 M hydrochloric acid to obtain a solution containing 20 Units per ml.

Reference solution (a). A 0.08 per cent w/v of bovine insulin RS in 0.025 M hydrochloric acid.

Reference solution (b). A 0.08 per cent w/v of porcine insulin RS in 0.025 M hydrochloric acid.

Reference solution (c). A solution containing 0.04 per cent w/v of bovine insulin RS and 0.04 per cent w/v of porcine insulin RS in 0.025 M hydrochloric acid for a preparation containing both bovine and porcine insulin.

Reference solution (d). A 0.04 per cent w/v each of human insulin RS and porcine insulin RS in 0.025 M hydrochloric acid.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm packed with octadecysilane chemically bonded to porous silica (5 \( \mu \)m),

- column temperature 45\(^{\circ}\),

- mobile phase. a mixture of 72.5 volumes of 0.1 M sodium dihydrogen phosphate adjusted to pH 2.0 with phosphoric acid and 27.5 volumes of acetonitrile,

- flow rate 1 ml per minute,

- spectrophotometer set at 214 nm,

- a 50 μl loop injector.

The test is not valid unless the resolution factor between the peaks corresponding to human insulin and porcine insulin is at least 1.2 in the chromatogram obtained with reference solution (d). If necessary, adjust the concentration of acetonitrile in the mobile phase by slight decrease or increase until the required resolution is obtained. In the chromatogram obtained with reference solution (d) the two principal peaks, in order of emergence, are due to human insulin and porcine insulin and any smaller peaks appearing immediately following each of the principal peaks are due to the corresponding monodesamido derivatives.

Inject either of reference solutions (a), (b) and (c), as appropriate, alternatively six times. The test is not valid unless the relative standard deviation of the area of the principal peak is not more than 2 per cent.

Inject the test solution. If necessary, make further adjustments in the composition of the mobile phase so that the antimicrobial preservatives present in test solution are well separated from insulin and show shorter retention times. A small reduction in the concentration of acetonitrile increases the retention time of insulin peak relatively more than those of the preservatives.

If necessary, after having carried out the chromatography of a solution, wash the column with a mixture of equal volumes of acetonitrile and water for a sufficient time in order to elute any interfering substances before injecting the next solution.

Calculate the content of insulin from the area of the peaks due to the bovine, porcine or human insulin and that of any peak due to the monodesamido derivative of the insulin from the declared content of insulin in bovine insulin RS, porcine insulin RS or human insulin RS, as appropriate. For preparations containing both bovine and porcine insulin use the sum of the areas of both the bovine and porcine insulin peaks and of any peak due to the desamido derivative of either insulin.

Storage. Store in multiple dose containers at a temperature between 2\(^{\circ}\) and 8\(^{\circ}\). It should not be allowed to freeze.

Labelling. The label states (1) the strength in terms of the number of Units per ml; (2) the animal source or sources of the insulin; (3) that the preparation should not be allowed to freeze; (4) that the container should be gently shaken before a dose is withdrawn; (5) the storage conditions.

Insulin Zinc Suspension (Crystalline)

Cryst. I.Z.S.; Extended Insulin Zinc Suspension

Insulin Zinc Suspension (Crystalline) is a sterile, buffered suspension of Insulin in the form of a complex obtained by the addition of zinc chloride to insulin in a manner such that
the insulin is in the form of crystals insoluble in water. It may be prepared by adding aseptically to crystalline insulin having a potency not less than 23 Units per mg, calculated with reference to the dried substance, a suitable quantity of zinc chloride, an appropriate amount of a suitable substance to render the preparation isotonic with blood and a sufficient quantity of a suitable bactericide. The solution is partially neutralised to allow crystallisation to occur and the pH of the crystalline suspension is adjusted to about 7.2. The suspension is distributed aseptically into sterile containers which are then sealed so as to exclude micro-organisms.

Insulin Zinc Suspension (Crystalline) contains not less than 90.0 per cent and not more than 110.0 per cent of the stated number of Units of Insulin.

Description. A white suspension which on standing deposits a white sediment and leaves an almost colourless supernatant liquid. The sediment is readily resuspended on gentle shaking. When examined under a microscope, the particles in the suspension are seen to be rhombohedral crystals, the majority having a maximum dimension greater than 10 mm but rarely exceedings 40 mm.

Identification

In the Assay, the principal peak due to insulin in the chromatogram obtained with test solution has a retention time similar to that of the principal peak in the chromatogram obtained with reference solution (a), reference (b) or reference solution (c), as appropriate.

Tests

pH (2.4.24). 6.9 to 7.5.

Total zinc. Not more than 0.0095 per cent w/v (for preparations containing 40 Units per ml) and not more than 0.014 per cent w/v (for preparations containing 80 Units per ml), determined by the following method. Take a volume of the well-shaken suspension containing 200 Units of insulin and add 1 ml of 0.1 M hydrochloric acid, 10 ml of alkaline borate buffer pH 9.0, 1 ml of 0.1 M sodium hydroxide, 2 ml of a 0.0009 per cent w/v solution of trypsin in 0.01 M hydrochloric acid. Mix, allow to stand for 10 minutes and add 3 ml of zincon solution and sufficient water to produce 50 ml. Allow to stand for 1 hour and measure the absorbance of the resulting solution at about 620 nm (2.4.7), using as the blank a solution prepared by treating 5 ml of water instead of the substance under examination in a similar manner. Calculate the content of zinc from the absorbance obtained by repeating the procedure using a suitable aliquot of a mixture of 4 volumes of zinc sulphate solution and 6 volumes of water.

Zinc in solution. Not more than 70 per cent of the total zinc (for preparations containing 40 Units per ml) and not more than 55 per cent of the total zinc (for preparations containing 80 Units per ml), determined by the method described in the test for Total zinc, using 1 ml of the clear supernatant liquid obtained by centrifuging and beginning at the words “add 1 ml of 0.1 M hydrochloric acid,…. “.

Insulin extractable with buffered acetone solution. Not more than 15 per cent, determined by the following method. Centrifuge a volume containing 400 Units and reject the supernatant liquid. Suspend the residue in 3.3 ml of water, add 6.6 ml of buffered acetone solution, stir for 3 minutes and again centrifuge. Transfer the supernatant liquid as completely as possible to a long-necked, round-bottomed flask, add 0.3 g of nitrogen-free mercuric oxide, 3 g of anhydrous sodium sulphate, and 6 ml of nitrogen-free sulphuric acid, heat over a low flame until the liquid is colourless and boil for a further 30 minutes. Allow to cool, dilute carefully with water, add 1 g of zinc powder, shake and allow to stand for 10 minutes. Add an excess of sodium hydroxide solution, immediately connect the flask to an ammonia distillation apparatus, mix the contents and distil the liberated ammonia into 20 ml of 0.005 M sulphuric acid prepared with carbon dioxide-free water. Rinse the condenser tube into the flask containing the distillate, add sufficient carbon dioxide-free water to produce a total volume of about 50 ml and titrate the excess of sulphuric acid with 0.01 M sodium hydroxide to pH 6.0, using a glass electrode. Centrifuge a further volume containing 400 Units and reject the supernatant liquid. Dissolve the residue in 10 ml of a 5 per cent w/v solution of nitrogen-free sulphuric acid, transfer to a long-necked, round-bottomed flask, and repeat the procedure described above beginning at the words “add 0.3 g of nitrogen-free mercuric oxide,…..”. Calculate the percentage of insulin extractable with the buffered acetone solution from the formula 100A/B, where A is the volume of 0.005 M sulphuric acid used in the first determination and B is the volume used in the second determination.

The result of the test is not valid unless in carrying out the first determination omitting the insulin preparation, not more than 0.2 ml of 0.005 M sulphuric acid is required.

Insulin in solution. Determine by liquid chromatography (2.4.14).

Test solution. (for preparations containing 100 Units per ml) centrifuge a well-shaken suspension of the preparation under examination, transfer 2.0 ml of the supernatant liquid to a 5-ml volumetric flask, dilute to volume with 0.03 M hydrochloric acid and mix well.

Reference solution (a). Prepare as test solution but using 2.5 ml of the supernatant liquid in place of 2.0 ml.

Reference solution (b). Use 5 ml of the supernatant liquid.

Reference solution (c). Weigh accurately 4.5 mg of bovine insulin RS into a 100-ml volumetric flask containing 50 ml of
**Invert Sugar Injection**

Invert Sugar Injection is a sterile solution of a mixture of equal amounts of Dextrose and Fructose in Water for Injections, or an equivalent sterile solution produced by the hydrolysis of Sucrose in Water for Injections. It contains no antimicrobial agent.

Invert Sugar Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the labelled amount of \( \text{C}_6\text{H}_{12}\text{O}_6 \).

**Description.** A clear, colourless or faintly straw-coloured solution.

**Identification**

To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.
Tests

pH (2.4.24). 3.0 to 6.5.

5-Hydroxymethylfurfural and Related substances. Dilute a volume containing 1.0 g of invert sugar to 500.0 ml with water and measure the absorbance (2.4.7) of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.25.

Heavy metals (2.3.13). A solution prepared by evaporating a volume containing 4.0 g of invert sugar to 10 ml and adding 2 ml of dilute acetic acid and sufficient water to produce 25 ml complies with the limit test for heavy metals, Method A (5 ppm).

Chlorides (2.3.12). 2.0 ml of the injection complies with the limit test for chlorides (120 ppm).

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Completeness of inversion.

NOTE — Invert Sugar Injection that is produced by mixing Dextrose and Fructose is exempt from this test.

Determine by liquid chromatography (2.4.14).

Test solution. Transfer an accurately measured volume of the preparation under examination containing about 2.5 g of invert sugar to a 100-ml volumetric flask, dilute to volume with water and mix.

Reference solution. Prepare a solution in water containing known concentrations of about 0.25 mg of sucrose and about 12.5 mg of dextrose per ml.

Chromatographic system
- a stainless steel column 30 cm x 7.8 mm, packed with a strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the calcium form (9 µm),
- mobile phase: filtered and degassed water,
- flow rate. 1 ml per minute,
- column temperature. constant at about 40°,
- refractive index detector,
- a 20 µl loop injector.

Inject the reference solution. The sucrose elutes first and the peak is baseline separated from the dextrose peak. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution and measure the responses for the sucrose peak. Calculate the content of sucrose in the volume taken of the preparation under examination. Not more than 1.5 per cent of the quantity of invert sugar in the volume taken of the preparation under examination, based on the value stated on the label, is found.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Transfer 50.0 ml of cupri-tartaric solution into a 400-ml beaker, add 48 ml of water, mix and add 2.0 ml of the preparation under examination that has been diluted quantitatively with water, if necessary, to a 5.0 per cent concentration. Cover the beaker with a watch glass, heat the solution, regulating the heat so that boiling begins in 4 minutes and continue boiling for 2 minutes. Filter the hot solution at once through a tared porcelain filtering crucible, wash the precipitate with water maintained at 60°, then with 10 ml of ethanol (95 per cent). Dry at 105° to constant weight. Carry out a blank determination and make any necessary correction. The corrected weight of the precipitate so obtained is not less than 204.0 mg and not more than 224.4 mg.

Storage. Store in single dose containers at a temperature not exceeding 30°.

Labelling. The label states (1) whether it is produced by hydrolysis of Sucrose or by mixing Dextrose and Fructose; (2) the strength as the percentage w/v of invert sugar; (3) total osmolar concentration in mOsmol per litre; (4) that the injection should not be used if it contains visible particles.

Invert Sugar and Sodium Chloride Injection

Sodium Chloride and Invert Sugar Intravenous Infusion

Invert Sugar and Sodium Chloride Injection is a sterile solution of a mixture of equal amounts of Dextrose and Fructose in Water for Injections to which the required amount of Sodium Chloride is added. Invert sugar may be prepared by acid hydrolysis of Sucrose.

Invert Sugar and Sodium Chloride Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of sodium chloride, NaCl, and invert sugar, C6H12O6. It contains no antimicrobial agent.

Description. A clear, colourless or faintly straw-coloured solution.

Identification

A. To 1 ml add 0.05 ml of potassium cupri-tartrate solution; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. Gives reaction A of chlorides and reaction B of sodium salts (2.3.1).

Tests

pH (2.4.24). 3.0 to 6.5.
5-Hydroxymethylfurfural and Related substances. Dilute a volume containing 1.0 g of invert sugar to 500.0 ml with water and measure the absorbance (2.4.7) of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.25.

Completeness of inversion

NOTE — Invert Sugar and Sodium Chloride Injection that is produced by mixing Dextrose and Fructose is exempt from this test.

Determine by liquid chromatography (2.4.14).

Test solution. Transfer an accurately measured volume of the preparation under examination containing about 2.5 g of invert sugar to a 100-ml volumetric flask, dilute to volume with water and mix.

Reference solution. Prepare a solution in water containing known concentrations of about 0.25 mg of sucrose and about 12.5 mg of dextrose per ml.

Chromatographic system

– a stainless steel column 30 cm x 7.8 mm, packed with a strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the calcium form (9 µm),
– mobile phase: filtered and degassed water,
– flow rate. 1.0 ml per minute,
– column temperature. constant at about 40°,
– refractive index detector,
– a 20 µl loop injector.

Inject the reference solution. The sucrose elutes first and the peak is baseline separated from the dextrose peak. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution and measure the responses for the sucrose peak. Calculate the content of sucrose in the volume taken of the preparation under examination. Not more than 1.5 per cent of the quantity of invert sugar in the volume taken of the preparation under examination, based on the value stated on the label, is found.

Heavy metals (2.3.13). A solution prepared by evaporating a volume containing 4.0 g of invert sugar to 10 ml and adding 2 ml of dilute acetic acid and sufficient water to produce 25 ml complies with the limit test for heavy metals, Method A (5 ppm).

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. For sodium chloride — Titrate an accurately measured volume containing about 0.1 g of Sodium Chloride with 0.1 M silver nitrate using potassium chromate solution as indicator. 1 ml of 0.1 M silver nitrate is equivalent to 0.005844 g of NaCl.

For invert sugar — Transfer 50.0 ml of cupri-tartaric solution into a 400-ml beaker, add 48 ml of water, mix and add 2.0 ml of the preparation under examination that has been diluted quantitatively with water, if necessary, to a 5.0 per cent concentration. Cover the beaker with a watch glass, heat the solution, regulating the heat so that boiling begins in 4 minutes and continue boiling for 2 minutes. Filter the hot solution at once through a tared porcelain filtering crucible, wash the precipitate with water maintained at 60°, then with 10 ml of ethanol (95 per cent). Dry at 105° to constant weight. Carry out a blank determination and make any necessary correction. The corrected weight of the precipitate so obtained is not less than 204.0 mg and not more than 224.4 mg.

Storage. Store in single dose containers at a temperature not exceeding 30°.

Labelling. The label states (1) whether it is produced by hydrolysis of Sucrose or by mixing Dextrose and Fructose; (2) the strength as the percentage w/v of sodium chloride and invert sugar; (3) total osmolar concentration in mOsmol per litre; (4) approximate concentrations, in millimoles per litre, of the sodium and chloride ions; (5) that the injection should not be used if it contains visible particles.

Iodine

I₂

Mol. Wt. 253.8

Iodine contains not less than 99.5 per cent and not more than 100.5 per cent of I.

Description. Greyish violet brittle plates or small crystals with a metallic sheen; odour, irritant. It volatalises slowly at room temperature.

Identification

A. When heated gently it gives violet vapours which condense forming a bluish-black crystalline sublimate.

B. A saturated solution yields a blue colour in the presence of starch solution which disappears when the solution is heated and reappears when it is cooled.

Tests

Bromides and chlorides. Not more than 250 ppm, determined by the following method. Triturate 3.0 g with 20 ml of water, filter, wash the filter, dilute the filtrate to 30 ml with water and add 1 g of zinc powder. When the solution is decolorised, filter and wash the filter with sufficient water to produce 40 ml of filtrate. To 10 ml of this solution add 3 ml of 10 M ammonia and 6 ml of silver nitrate solution, filter, wash the filter with water and dilute to 20 ml with water. To 10 ml of the filtrate add
1.5 ml of nitric acid. After 1 minute any opalescence produced is not more intense than that produced in a solution prepared at the same time by mixing 10.75 ml of water, 0.25 ml of 0.01 M hydrochloric acid, 0.2 ml of 2 M nitric acid and 0.3 ml of silver nitrate solution.

Non-volatile matter. Not more than 0.1 per cent, determined by heating 1.0 g in a porcelain dish on a water-bath until the iodine has volatilised and drying the residue at 105°C.

Assay. Weigh accurately about 0.2 g, transfer to a flask containing 1 g of potassium iodide and 2 ml of water, add 1 ml of 2 M acetic acid, dissolve completely and add 50 ml of water. Titrate with 0.1 M sodium thiosulphate using starch solution as indicator.

1 ml of 0.1 M sodium thiosulphate is equivalent to 0.01269 g of I.

Storage. Store in ground-glass-stoppered containers or in earthenware containers with waxed bungs.

Ipratropium Bromide

![Ipratropium Bromide structure](image)

C_{20}H_{30}BrNO_3 \cdot H_2O  
Mol. Wt. 430.4

Ipratropium Bromide is (1R, 3r, 5S, 8r)-3-[[2RS]-3-hydroxy-2-phenylpropanoyloxy]-8-methyl-8-(1-methylethyl)-8-azoniabicyclo[3.2.1]octane bromide monohydrate

Ipratropium Bromide contains not less than 99.0 per cent and not more than 100.5 per cent of C_{20}H_{30}BrNO_3 \cdot H_2O, calculated on the anhydrous basis.

Description. A white or almost white crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ipratropium bromide RS or with the reference spectrum of ipratropium bromide.

Tests

pH (2.4.24). 5.0 to 7.5, determined in a 1.0 per cent w/v solution in carbon dioxide-free water.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in 10 ml of the mobile phase.

Reference solution. A 0.001 per cent w/v solution of ipratropium bromide RS in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (4 µm),
- mobile phase: a mixture of 87 volumes of buffer solution pH 5.5 prepared by dissolving 1.24 g of sodium dihydrogen phosphate and 0.17 g of tetratropyl ammonium chloride in 87 ml of water and adjusting pH to 5.5 with 18 per cent w/v solution of disodium hydrogen phosphate, and 13 volumes of methanol,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.5.

Inject the test solution. Any individual impurity is not more than 0.5 per cent and the sum of all the impurities is not more than 1.0 per cent.

Water (2.3.43). Not more than 5.0 per cent, determined on 0.5 g.

Sulphated Ash (2.3.18). Not more than 0.1 per cent.

Assay. Weigh accurately about 0.35 g of the substance under examination, dissolve in 50 ml of water and add 3 ml of dilute nitric acid. Titrate with 0.1 M silver nitrate, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M silver nitrate is equivalent to 0.04124 g of C_{20}H_{30}BrNO_3.

Storage. Store protected from light and moisture.

Irinotecan Hydrochloride Trihydrate

![Irinotecan Hydrochloride Trihydrate structure](image)

C_{33}H_{38}N_4O_6 \cdot HCl \cdot 3H_2O  
Mol. Wt. 677.18

Irinotecan Hydrochloride Trihydrate is (4S)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo-1H-pyrano
[3',4':6,7']indolizino[1,2-b]quinolin-9-yl [1,4'-bipiperidine]-1'-carboxylate hydrochloride.

Irinotecan Hydrochloride Trihydrate contains not less than 98.0 per cent and not more than 102.0 per cent of C$_{33}$H$_{38}$N$_{4}$O$_{6}$·HCl, calculated on the anhydrous basis.

**Description.** A pale yellow to yellow crystalline powder.

**CAUTION —** Irinotecan Hydrochloride Trihydrate is potentially cytotoxic. Great care should be taken in handling the powder and preparing solutions.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with irinotecan hydrochloride trihydrate RS.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

**Tests**

**Appearance of solution.** A 1.0 per cent w/v solution is clear (2.4.1) and not more intensely coloured than the reference solution GYS3 (2.4.1).

**pH** (2.4.24). 3.0 to 5.0, determined in 1.0 per cent w/v solution in water.

**Specific optical rotation** (2.4.22). + 60.0º to + 73.0º, determined in 1.0 per cent w/v solution in water.

**Light absorption.** Dissolve 0.1 g of the substance under examination in 10 ml of methanol. The absorbance of the resulting solution, at about 430 nm (2.4.7) is not more than 0.17.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Solvent mixture.** Dissolve 6.41 g of disodium hydrogen phosphate dihydrate and 0.73 g 1-heptane sulphonic acid sodium in 720 ml of water. Adjust the pH to 3.0 with orthophosphoric acid and dilute to 1000 ml with acetonitrile.

**Test solution.** Dissolve 40 mg of the substance under examination in 100 ml of solvent mixture.

**Reference solution (a).** A 0.04 per cent w/v solution of irinotecan hydrochloride trihydrate RS in solvent mixture.

**Reference solution (b).** Dilute 1 ml of reference solution (a) to 100 ml with solvent mixture.

**NOTE — Use freshly prepared solutions.**

**Chromatographic system**

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to silica (5 µm),
- mobile phase: A. dissolve 8.90 g of disodium hydrogen phosphate dihydrate and 1.01 g of 1-heptane sulphonic acid sodium in 900 ml of water. Adjust the pH to 3.0 with orthophosphoric acid and dilute to 1000 ml with water and filter,
- B. acetonitrile,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 254 nm,
- a 10 µl loop injector.

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<th>Time (in min.)</th>
<th>mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
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**Reference solution (c)-(i)** weigh accurately about 10 mg of 7-ethyl-10-hydroxycamptothecin into a 25-ml volumetric flask. Dissolve to dilute to volume with N,N-dimethylformamide (solution-A). Weigh 20 mg of irinotecan hydrochloride RS in 10 ml of solution A and dilute to volume 50 ml with solvent mixture.

The system is not valid unless the solution between 7-ethyl-10-hydroxycamptothecin.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.2 times the area of the peak in the chromatogram obtained with the reference solution (b) (0.2 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

**Test solution.** Weigh accurately about 0.5 g of the substance under examination in 5 ml of N,N'-dimethylformamide. Mix 1 ml of the solution with 5 ml of water in to 10 ml of headspace vail.

**Reference solution (a).** To 50 ml of water, add 12.6 µl of ethanol, 126 µl of acetone, dilute to 100 ml with water.

**Reference solution (b).** Dilute 80 µl of chloroform to 10 ml with N,N'-dimethylformamide. Dilute 1 ml of the solution to 100 ml with water. Further, dilute 1 ml of this solution to 10 ml with water.

**Reference solution (c).** Dilute 10 ml each of reference solutions (a) and (b) to 100 ml with water. To 5 ml of this solution, add 1 ml of N,N'-dimethylformamide in to 10 ml of headspace vail.
Chromatographic system
- a capillary column 30 m x 0.53 mm, 3 µm packed with mega bore coated with a mixture of 6 per cent cyanopropylphenyl and 94 per cent dimethylpolysiloxane (3 µm),
- temperature: column, 50º for 10 minutes increase @ 30º per minute to 220º hold for 15 minutes, inlet port 180º and detector, 260º,
- nitrogen as carrier gas with a flow rate, 4.8 ml per min.

Head space conditions
- Vial equilibrium temperature 85º, loop temperature 110º, Transfer line 160º, vial equilibrium 0.5 minute, sample loop fill 0.2 minute, loop equilibrium 0.05 minute, sample injection 1 minute, vial pressure 10 psi.
- a flame ionisation detector,
- nitrogen as carrier gas.

Inject 1 ml of the reference solution (c). The test is not valid unless the resolution between the peaks due to ethanol and acetone, the peaks due to acetone and chloroform is not more than 1.5 and the tailing factor is not more than 1.5 for each component.

Inject 1 ml of the test solution and reference solution (c). In the chromatogram obtained with test solution, the area of peaks due to ethanol, acetone and chloroform is not more than the area of peaks obtained in the chromatogram due to reference solution (c).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

Total Chloride. 5.0 per cent to 5.5 per cent, Weigh accurately about 500 mg of the Irinotecan Hydrochloride Trihydrate, dissolve in 10 ml of methanol, add 20 ml of water and 20 ml of glacial acetic acid and titrate with 0.1 M silver nitrate solution, using eosin yellow solution as indicator. Carry out a blank titration.

1 ml of 0.1 M silver nitrate is equivalent to 0.003545 g of chloride.

Loss on ignition (2.4.20). Not more than 0.1 per cent.

Water (2.3.43). 8.0 per cent to 9.0 per cent, determined on 0.1 g.

Microbial Contamination (2.2.9). The total aerobic microbial count does not exceed 100 cfu per g. It meets the requirements of the tests for the absence of Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella species, and Escherichia coli.

Bacterial endotoxins (2.2.3). Not more than 0.275 Endotoxin Unit per mg of irinotecan hydrochloride trihydrate.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 6.41 g of disodium hydrogen phosphate dihydrate and 0.73 g 1-heptane sulphonic acid sodium in 720 ml of water. Adjust the pH to 3.0 with orthophosphoric acid, dilute to 1000 ml with acetonitrile, filter.

Test solution. Dissolve 40 mg of the substance under examination in 100 ml of solvent mixture.

Reference solution. A 0.04 per cent w/v solution of irinotecan hydrochloride trihydrate RS in solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. dissolve 8.90 g of disodium hydrogen phosphate dihydrate and 1.01 g of 1-heptane sulphanic acid sodium in 900 ml of water. Adjust the pH to 3.0 with orthophosphoric acid and dilute to 1000 ml with water,
- B. acetonitrile,
- flow rate, 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 254 nm,
- a 10 µl loop injector.

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</table>

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution. Calculate the content of C33H38N4O6.HCl.

Storage. Store protected from light, at a temperature not exceeding 25º.
hydrochloride, C_{33}H_{38}N_{4}O_{6}.HCl, calculated on the anhydrous basis.

**Description.** A light yellow coloured solution, free from visible particles.

**Identification**

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

A. It gives the reaction of chlorides (2.3.1).

**Tests**

**Appearance of solution** (2.4.1). Prepare a solution of equivalent to 1.0 per cent of the substance in water. The solution is clear or not more intensely coloured than the reference solution GYS3.

**pH** (2.4.24). 3.0 to 3.8.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dilute a measured volume containing 40 mg of irinotecan hydrochloride to 100 ml with mobile phase.

**Reference solution (a).** A 0.04 per cent w/v solution of irinotecan hydrochloride trihydrate RS with mobile phase.

**Reference solution (b).** Dilute 1 ml of reference solution (a) to 100 ml with mobile phase.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 72 volumes of buffer solution prepared by dissolving 1.1 g of 1-heptane sulphonic acid sodium monohydrate and 7.1 g of disodium hydrogen orthophosphate anhydrous in 1000 ml water. Adjusted the pH to 3.0 with orthophosphoric acid and 28 volumes of acetonitrile, filter.
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- a 10 µl loop injector.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 1.4. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution. Calculate the content of C_{33}H_{38}N_{4}O_{6}.HCl.

**Storage.** Store protected from light, at a temperature not exceeding 25º.

**Iron and Ammonium Citrate**

**Ferric Ammonium Citrate**

Iron and Ammonium Citrate is a complex ammonium ferric citrate.

Iron and Ammonium Citrate contains not less than 20.5 per cent and not more than 22.5 per cent of iron, Fe.

**Description.** Thin, transparent, dark red scales or granules or a brownish red powder; odourless; deliquescent in moist air and is affected by light.

**Identification**

A. Ignite 0.1 g gently and dissolve the residue in 5 ml of hydrochloric acid; the solution gives the reactions of ferric salts (2.3.1).

B. Warm 50 mg with 5 M sodium hydroxide; ammonia is evolved and the solution gives the reactions of citrates (2.3.1).

**Tests**

**Arsenic** (2.3.10). Mix 2.5 g with 1.5 g of anhydrous sodium carbonate, add 10 ml of bromine solution and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite and dissolve the cooled residue in 20 ml of brominated hydrochloric acid and 10 ml of water. Transfer to a small
flask, add sufficient stannous chloride \( \text{AsT} \) to remove the yellow colour, connect to a condenser and distil 22 ml. To the distillate add 40 ml of water and 0.15 ml of stannous chloride \( \text{AsT} \). The resulting solution complies with the limit test for arsenic (4 ppm).

**Lead.** Dissolve 2.0 g in 20 ml of hydrochloric acid and 8 ml of water, add 0.5 ml of nitric acid, heat just to boiling, cool, transfer to a separating funnel and extract with three quantities, each of 20 ml, of ether. Reject the ether extracts. Transfer the acid solution to a narrow-necked flask, rinse the separating funnel with 5 ml of the ether extracts. Transfer the acid solution to a narrow-necked flask, rinse the separating funnel with an additional 20 ml of ether. Reject the ether extracts. Transfer the acid solution to a narrow-necked flask, rinse the separating funnel with 5 ml of water and add the rinsings to the flask. Heat to remove the dissolved ether, cool, make alkaline with ammonia solution, add 1 ml of potassium cyanide solution, dilute to 50 ml with water and add 0.1 ml of sodium sulphide solution. Any colour produced is not more intense than that produced by mixing 10 ml of hydrochloric acid, 0.5 ml of nitric acid and 6 ml of lead standard solution (10 ppm Pb), making alkaline with ammonia solution and adding 1 ml of potassium cyanide solution and 0.1 ml of sodium sulphide solution (30 ppm).

**Zinc.** Dissolve 2.0 g in a mixture of 20 ml of hydrochloric acid and 8 ml of water, add 0.5 ml of nitric acid, heat just to boiling, cool and extract with three quantities, each of 20 ml, of ether. If the acid solution is still more than faintly yellow, extract with an additional 20 ml of ether. Reject the ether extracts. Transfer the acid solution to a narrow-necked flask, rinse the separating funnel with 5 ml of water and add the rinsings to the flask. Heat to remove the dissolved ether, cool, make alkaline with ammonia solution, add 1 ml of potassium cyanide solution, dilute to 50 ml with water and add 0.1 ml of sodium sulphide solution. Any colour produced is not more intense than that produced by mixing 10 ml of hydrochloric acid, 0.5 ml of nitric acid and 6 ml of lead standard solution (10 ppm Pb), making alkaline with ammonia solution and adding 1 ml of potassium cyanide solution and 0.1 ml of sodium sulphide solution (30 ppm).

**Iron Dextran Injection**

Iron Dextran Injection is a sterile colloidal solution containing a complex of ferric hydroxide with dextrans of average molecular weight between 5000 and 7500.

Iron Dextran Injection contains not less than 4.75 per cent and not more than 5.25 per cent w/v of iron, Fe, and not less than 17.0 per cent and not more than 23.0 per cent w/v of dextrans.

**Description.** A dark brown solution.

**Identification**

A. To 0.2 ml, previously diluted to 5 ml with water; add 5 M ammonia; no precipitate is produced.

B. Mix 1 ml with 100 ml of water. To 5 ml of this solution add 0.1 ml of hydrochloric acid, boil for 30 seconds, cool rapidly, add 2 ml of strong ammonia solution and 5 ml of hydrogen sulphide solution, boil to remove hydrogen sulphide, cool and filter. Boil 5 ml of the filtrate with 5 ml of potassium cupri-tartrate solution; the solution remains greenish in colour and no precipitate is produced. Boil a further 5 ml of the filtrate with 0.5 ml of hydrochloric acid for 5 minutes, cool, add 2.5 ml of 5 M sodium hydroxide and 5 ml of potassium cupri-tartrate solution and boil again; a reddish precipitate is produced.

C. To 1 ml add 20 ml of water and 5 ml of hydrochloric acid and boil for 5 minutes. Cool, add an excess of strong ammonia solution and filter. Wash the precipitate with water, dissolve in the minimum volume of 2 M hydrochloric acid and add sufficient water to produce 20 ml. The resulting solution gives reaction B of ferric salts (2.3.1).

**Tests**

**pH (2.4.24).** 5.2 to 6.5.

**Arsenic (2.3.10).** To 10.0 ml in a round-bottomed, long-necked flask add 20 ml of water and 20 ml of nitric acid and heat until the vigorous evolution of brown fumes ceases. Cool, add 20
ml of sulphuric acid and heat again until fumes are evolved, adding nitric acid dropwise from time to time until oxidation is complete. Cool, add 60 ml of water, bring to boil and continue boiling until the volume of liquid is reduced to about 40 ml. Cool and dilute to 100 ml with water. Reserve a portion of the solution (solution A) for the test for Heavy metals. Boil gently 40 ml of this solution until the volume is reduced to about 15 ml, cool and add 15 ml of stannous chloride solution AsT. Connect to a condenser and distil 15 ml into 25 ml of water. To the distillate add 0.2 ml of bromine solution, remove the excess of bromine with a few drops of stannous chloride solution AsT and add 20 ml of water. The resulting solution complies with the limit test for arsenic. Use 0.8 ml of arsenic standard solution (10 ppm As) to prepare the standard (2 ppm).

Heavy metals (2.3.13). To 16.0 ml of solution A reserved in the test for Arsenic add 50 ml of hydrochloric acid and extract with four quantities, each of 20 ml, of isobutyl acetate, discarding the extracts. Evaporate the acid solution to dryness and dissolve the residue in 20 ml of water. The resulting solution complies with the limit test for heavy metals, Method D (25 ppm). Use lead standard solution (2 ppm) to prepare the standard.

Copper. To 5.0 ml add 5 ml of nitric acid and heat until the vigorous evolution of brown fumes ceases. Cool, add 2 ml of sulphuric acid and heat again until fumes are evolved, adding nitric acid dropwise at intervals until oxidation is complete. Cool, add 25 ml of hydrochloric acid, warm to dissolve, cool and extract with four quantities, each of 25 ml, of isobutyl acetate, discarding the extracts. Evaporate the acid solution to dryness, adding nitric acid dropwise if charring occurs. Dissolve the residue in 10 ml of 1 M hydrochloric acid, reserving a portion (solution B) for the test for Zinc. To 1 ml add 25 ml of water and 1 g of citric acid, make alkaline to litmus paper with 5 M ammonia, dilute to 50 ml with water; add 1 ml of sodium diethylthiocarbamate solution and allow to stand for 5 minutes. Any colour produced is not more intense than that produced by treating in the same manner a mixture of 3 ml of copper standard solution (10 ppm Cu) and 1 ml of 1 M hydrochloric acid beginning at the words “add 25 ml of water.....” (60 ppm).

Zinc. To 5.0 ml of solution B reserved in the test for Copper add 15 ml of 1 M sodium hydroxide, boil, filter, wash the residue with water and dilute the combined filtrate and washings to 25 ml with water. To 5 ml add 5 ml of 1 M hydrochloric acid and 2 g of ammonium chloride, dilute to 50 ml with water; add 1 ml of freshly prepared dilute potassium ferrocyanide solution and allow to stand for 20 minutes. Any opalescence produced is not more than that produced when 1 ml of freshly prepared dilute potassium ferrocyanide solution is added to a solution prepared from 3 ml of zinc standard solution (25 ppm Zn), 3 ml of 1 M sodium hydroxide, 6 ml of 1 M hydrochloric acid and 2 g of ammonium chloride diluted to 50 ml with water and allowed to stand for 20 minutes (150 ppm).

Chlorides. To 5.0 ml add 75 ml of water and 0.05 ml of nitric acid and titrate immediately with 0.1 M silver nitrate, determining the end-point potentiometrically (2.4.25). 6.8 to 9.6 ml of 0.1 M silver nitrate is required.

Iron absorption. Prepare a site over the semitendinosus muscle of one leg of each of two rabbits, each weighing between 1.5 and 2.5 kg, by clipping the fur and disinfecting the exposed skin. Inject each site with a dose of 0.4 ml per kg of body weight in the following manner. Place the needle in the distal end of the semitendinosus muscle at an angle such as to ensure that the full length of the needle is used, then pass it through the sartorius and vastus medialis muscles. House the rabbits separately. Sacrifice the rabbits after 7 days and remove the legs into which the injection were made. Carefully dissect the muscles and examine the site of injection. The muscle is only lightly stained and no heavy black deposit of unabsorbed iron compounds or leakage along fascial planes is observed.

Skin the leg, dissect the flesh from the bone and cut into small pieces. Transfer the pieces to a 1000-ml beaker, add 75 ml of 2 M sodium hydroxide and sufficient water to submerge them, cover the beaker with a watch glass and boil until most of the solid matter has disintegrated. Cool cautiously, add 50 ml of sulphuric acid, heat the mixture almost to boiling and add carefully 10 ml of fuming nitric acid about 1 ml at a time, until no charring occurs when the excess of nitric acid has been boiled off. Cool, add 175 ml of water, boil until solution is complete, cool and dilute to 250.0 ml with water. To 5.0 ml of the solution add 3 ml of sulphuric acid, heat to fuming and complete the oxidation by adding small quantities of nitric acid until the solution is colourless. Cool, add 20 ml of water, boil for 3 minutes and add 10 ml of ammonium citrate solution, 10 ml of ammonium thioglycollate solution followed by dilute ammonia solution dropwise until the iron colour is fully developed. Add 1 ml excess of dilute ammonia solution and sufficient water to produce 100.0 ml. Measure the absorbance of the resulting solution at about 530 nm (2.4.7). For the reference solution, add 10 ml of ammonium citrate solution, 10 ml of ammonium thioglycollate solution and the same quantities of dilute ammonia solution as used above to 20 ml of water, and dilute to 100.0 ml. Measure the absorbance of this solution at about 530 nm (2.4.7). From the difference between the absorbances, calculate the amount of Fe present in the legs from a reference curve prepared by treating suitable aliquots of a solution of ferric ammonium sulphate containing 0.01 per cent w/v of Fe by the above procedure beginning at the words “add 10 ml of ammonium citrate solution.....”.

Repeat the determination of Fe on the corresponding legs into which injection was not made beginning at the words “Carefully dissect the muscles.....”. From the difference between...
the two amounts of Fe, calculate the proportion of injected iron, as Fe, remaining in the leg tissues. Not more than 20 per cent of the injected iron remains.

Abnormal toxicity. Inject 0.10 ml into a tail vein of each of 10 mice; not more than 3 mice die within 5 days of injection. If more than 3 mice die within 5 days, repeat the test on another group of 20 mice. Not more than 10 of the 30 mice used in the combined tests die within 5 days of injection.

Bacterial endotoxins (2.2.3). Not more than 0.50 Endotoxin Unit per mg of iron.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. For iron — Weigh accurately about 2.0 g, add 10 ml of water and 5 ml of sulphuric acid and stir for several minutes. Allow to stand for 5 minutes, cool and dilute to 50 ml with water. Prepare a suitable zinc amalgam by covering 300 g of zinc shots with a 2 per cent w/v solution of mercuric chloride and stir for 10 minutes. Decant the solution, wash the residue three times with water and transfer it to a column (30 cm x 18 mm) fitted with a sintered-glass disc (porosity No. 0). Activate the zinc amalgam by passing through the column 500 ml of sulphuric acid (5 per cent v/v). Pass the prepared solution slowly through the column and wash successively with 50 ml of water, 10 ml of sulphuric acid (5 per cent v/v) and 50 ml of water. Titrate the combined eluates with 0.1 M ceric ammonium sulphate using ferroin solution as indicator.

1 ml of 0.1 M ceric ammonium sulphate is equivalent to 0.005585 g of Fe.

Determine the weight per ml of the injection (2.4.29), and calculate the percentage w/v of Fe.

For dextrans — Weigh accurately about 1.0 g, dilute to 500.0 ml with water, dilute 10.0 ml of this solution to 100.0 ml with water, transfer 3.0 ml of the resulting solution to a test-tube and cool to 0°C. Add, to form a lower layer, 6.0 ml of a solution prepared and maintained at 0°C containing 0.2 per cent w/v of anthrone in a mixture of 19 volumes of sulphuric acid and 1 volume of water, mix and immediately heat on a water-bath for 5 minutes. Cool and measure the absorbance of the resulting solution at about 625 nm (2.4.7). Repeat the operation using 3.0 ml of water in place of the dilution of the injection. From the difference between the absorbances, calculate the content of dextrose present using a calibration curve prepared by treating suitable amounts of dextrose in the same manner.

1 g of dextrose is equivalent to 0.94 g of dextrans.

Determine the weight per ml of the injection (2.4.29), and calculate the percentage w/v of dextrans.

Labelling. The label states the strength in terms of the equivalent amount of iron, Fe, in a suitable dose-volume.

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Isoniazid

Isonicotinylhydrazine; INH

\[
\begin{align*}
\text{H}_2\text{N}_2\text{O} & \quad \text{Mol. Wt. 137.1} \\
\text{C}_6\text{H}_7\text{N}_3\text{O} & 
\end{align*}
\]

Isoniazid is isonicotinic acid hydrazide.

Isoniazid contains not less than 98.0 per cent and not more than 101.0 per cent of \( \text{C}_6\text{H}_7\text{N}_3\text{O} \), calculated on the dried basis.

Description. Colourless crystals or a white, crystalline powder; odourless.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with isoniazid RS or with the reference spectrum of isoniazid.

B. Dissolve 0.1 g in 2 ml of water, add a warm solution of 0.1 g of vanillin in 10 ml of water, allow to stand and scratch the inside of the container with a glass rod; a yellow precipitate is produced. The precipitate after recrystallisation from 5 ml of ethanol (70 per cent) and drying at 105°C melts at 226°C to 231°C (2.4.21).

C. Melts at 170°C to 174°C (2.4.21).

Tests

Appearance of solution. A 5.0 per cent w/v solution in carbon dioxide-free water is clear (2.4.1), and not more intensely coloured than reference solution BYS7 (2.4.1).

pH (2.4.24). 6.0 to 8.0, determined in a 5.0 per cent w/v solution.

Hydrazine. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 50 volumes of ethyl acetate, 20 volumes of acetone, 20 volumes of methanol and 10 volumes of water.

Test solution. Dissolve 1 g of the substance under examination in sufficient of a mixture of equal volumes of acetone and water to produce 10 ml.

Reference solution. Dissolve 50 mg of hydrazine sulphate in 50 ml of water and dilute to 100 ml with acetone; to 10 ml of this solution add 0.2 ml of the test solution and dilute to 100 ml with a mixture of equal volumes of acetone and water.
After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution. Spray with dimethylaminobenzaldehyde reagent and examine in daylight. The additional spot (due to hydrazine) in the chromatogram obtained with the reference solution is more intense than any corresponding spot in the chromatogram obtained with the test solution.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 50 mg of the substance under examination in 100 ml of water.

**Chromatographic system**

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 96 volumes of a solution prepared by dissolving 1.4 g disodium hydrogen phosphate and 1 ml of triethylamine to 1000 ml with water, adjusting the pH to 6.0 with orthophosphoric acid and 4 volumes of acetonitrile,
- flow rate. 1 ml per minute,
- spectrophotometer set at 265 nm,
- a 20 µl loop injector.

Inject the test solution. Any individual impurity is not more than 0.2 per cent and the sum of all impurities found is not more than 1.0 per cent.

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve about 25.0 mg of the substance under examination in 50.0 ml of water. Dilute 5.0 ml of this solution to 25.0 ml with water.

**Reference solution.** Dissolve 25.0 mg of the isoniazid RS in 50.0 ml of water. Dilute 5.0 ml of this solution to 25.0 ml with water.

Use the chromatographic system described under the test for Related substances.

Inject the reference solution. The tailing factor is not more than 2.0. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the content of C,H,N,O.

**Storage.** Store protected from light.

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**Isoniazid Tablets**

Isonicotinylhydrazid Tablets; INH Tablets

Isoniazid Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of isoniazid, C,H,N,O.

**Identification**

A. Shake a quantity of the powdered tablets containing 0.1 g of Isoniazid with 10 ml of ethanol (95 per cent) for 15 minutes, centrifuge and decant the supernatant liquid. Extract the residue with two further quantities, each of 10 ml, of ethanol (95 per cent) and evaporate the combined extracts to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with isoniazid RS or with the reference spectrum of isoniazid.

B. Shake a quantity of the powdered tablets containing 1 mg of Isoniazid with 50 ml of ethanol (95 per cent) and filter. To 5 ml of the filtrate add 0.1 g of borax and 5 ml of a 5 per cent w/v solution of 1-chloro-2,4-dinitrobenzene in ethanol (95 per cent), evaporate to dryness on a water-bath and continue heating for a further 10 minutes. To the residue add 10 ml of methanol and mix; a reddish purple colour is produced.

**Tests**

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh and powder 20 tablets. Weigh a quantity of the powdered tablets containing 50 mg of Isoniazid, dissolve in 100 ml of the mobile phase. Use the chromatographic system described under Assay.

Inject the test solution. Any individual impurity is not more than 1.0 per cent and the sum of all impurities found is not more than 2.0 per cent.

**Dissolution** (2.5.2).

Apparatus. No 1 Medium. 900 ml of water Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 mµ, rejecting the first 1 ml of the filtrate. Dilute suitably with water and measure the absorbance of the resulting solution at the maximum at about 263 nm (2.4.7). Calculate the content of C,H,N,O taking 307 as the specific absorbance at 263 nm.

D. Not less than 80 per cent of the stated amount of C,H,N,O.

**Other tests.** Comply with the tests stated under Tablets.
**Assay.** Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 25.0 mg of Isoniazid and dissolve in 50.0 ml of the mobile phase. Dilute 5.0 ml of this solution to 25.0 ml with the mobile phase.

*Reference solution.* A 0.01 per cent w/v solution of the isoniazid RS in the mobile phase.

**Chromatographic system**
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm) (such as Intersil ODS-3),
- mobile phase: mix 96 volumes of a solution prepared by dissolving 1.4 g disodium hydrogen orthophosphate anhydrous and 1.0 ml of triethylamine to 1000 ml with water and adjusting the pH to 6.0 with orthophosphoric acid, and 4 volumes of acetonitrile and filter,
- flow rate. 1 ml per minute,
- spectrophotometer set at 265 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency is not less than 2000 theoretical plates. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the content of C6H7N3O in the tablets.

**Storage.** Store protected from light.

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**Isoprenaline Hydrochloride**

**Isoproterenol Hydrochloride**

\[
\text{H}_2\text{N} - \text{CH}_3
\]

\[
\text{HO} - \text{CH}_3
\]

\[
\text{HO}
\]

C₁₁H₁₇NO₃,HCl  Mol. Wt. 247.7

Isoprenaline Hydrochloride is (RS)-1-(3,4-dihydroxy-phenyl)-2-isopropylaminoethanol hydrochloride.

Isoprenaline Hydrochloride contains not less than 98.0 per cent and not more than 101.5 per cent of C₁₁H₁₇NO₃,HCl, calculated on the dried basis.

**Description.** A white or almost white, crystalline powder; almost odourless. Gradually darkens on exposure to air and light; even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures. Aqueous solutions become pink to brownish on standing exposed to air and almost immediately after being made alkaline.

**Identification**

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with isoprenaline hydrochloride RS or with the reference spectrum of isoprenaline hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution shows an absorption maximum only at about 280 nm; absorbance at about 280 nm, about 0.5.

C. To 2 ml of a freshly prepared 1 per cent w/v solution add 0.1 ml of ferric chloride test solution; an emerald-green colour is produced which, on the gradual addition of sodium bicarbonate solution, changes first to blue and then to red.

D. Gives the reactions of chlorides (2.3.1).

**Tests**

**Isoprenaline.** Absorbance of a 0.2 per cent w/v solution in 0.005 M sulphuric acid at about 310 nm, not more than 0.15 (2.4.7).

**Sulphates (2.3.17).** Dissolve 0.5 g in 100 ml of water; 15 ml of the resulting solution complies with the limit test for sulphates (0.2 per cent).

**Sulphated ash (2.3.18).** Not more than 0.1 per cent.

**Loss on drying (2.4.19).** Not more than 1.0 per cent, determined on 1.0 g by drying over phosphorus pentoxide at a pressure not exceeding 0.7 kPa for 4 hours.

**Assay.** Weigh accurately about 0.5 g, dissolve in 50 ml of anhydrous glacial acetic acid with the aid of the minimum of heat and titrate with 0.1 M perchloric acid, using crystal violet solution as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02477 g of C₁₁H₁₇NO₃,HCl.

**Storage.** Store protected from light.

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**Isoprenaline Injection**

Isoprenaline Hydrochloride Injection; Isoproterenol Injection; Isoproterenol Hydrochloride Injection

Isoprenaline Injection is a sterile solution of Isoprenaline Hydrochloride in Water for Injections. It may contain suitable stabilising agents.
Isoprenaline Hydrochloride Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of isoprenaline hydrochloride, C\(_{11}\)H\(_{17}\)NO\(_3\),HCl.

**Description.** A colourless or very pale yellow solution.

**Identification**

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase.* A mixture of 50 volumes of *ethyl acetate*, 30 volumes of *2-propanol*, 16 volumes of *water* and 4 volumes of *strong ammonia solution*.

*Test solution.* Use the injection diluted if necessary with sufficient *methanol (80 per cent)* to produce a solution containing 0.02 per cent w/v of isoprenaline hydrochloride.

*Reference solution.* A solution containing 0.02 per cent w/v of isoprenaline hydrochloride *RS* in *methanol (80 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable, place it for a few minutes in an atmosphere saturated with *diethylamine* and spray with *diazotised nitroaniline solution*. The chromatogram obtained with the test solution exhibits an elongated zone corresponding to that obtained with the reference solution.

B. To 2 ml add 0.1 ml of *ferric chloride test solution*; an emerald-green colour develops which, on gradual addition of *sodium bicarbonate solution*, changes first to blue and then to red.

**Tests**

**pH** (2.4.24). 2.5 to 3.0.

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** To an accurately measured volume containing about 5 mg of Isoprenaline Hydrochloride add sufficient *water* to produce 50.0 ml. To 20.0 ml add 0.5 ml of *ferrous sulphate-citrate solution* and 2 ml of *glycine buffer solution*, mix and allow to stand for 20 minutes. Add sufficient *water* to produce 25.0 ml, mix and measure the absorbance of the resulting solution at the maximum at about 540 nm (2.4.7). Calculate the content of C\(_{11}\)H\(_{17}\)NO\(_3\),HCl from the absorbance obtained by repeating the determination using 2.0 ml of a 0.1 per cent w/v solution of *isoprenaline hydrochloride RS* in place of the substance under examination.

**Storage.** Store protected from light at a temperature not exceeding 30°.

---

**Isoprenaline Sulphate**

**Isoproterenol Sulphate**

\[
\text{HO} \quad \text{OH} \quad \text{OH}
\]

\[
\begin{align*}
\text{H} & \quad \text{H} & \quad \text{N} & \quad \text{CH}_3 \\
\text{HO} & \quad \text{HO} & \quad \text{CH}_3
\end{align*}
\]

\[
\begin{array}{c}
\text{(C\(_{11}\)H\(_{17}\)NO\(_3\))\text{2,H}_{2}\text{SO}_4,2H}_2\text{O} \\
\text{Mol. Wt. 247.7}
\end{array}
\]

Isoprenaline Sulphate is (RS)-1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol sulphate dihydrate.

Isoprenaline Sulphate contains not less than 98.0 per cent and not more than 102.0 per cent of (C\(_{11}\)H\(_{17}\)NO\(_3\))\text{2,H}_2\text{SO}_4, calculated on the anhydrous basis.

**Description.** A white or almost white, crystalline powder.

**Identification**

*Test A may be omitted if tests B, C and D are carried out.* *Tests B and C may be omitted if tests A and D are carried out.*

A. Dissolve 0.5 g in 1.5 ml of *water*, add 3.5 ml of *2-propanol* scratch the walls of the container with a glass rod to induce crystallisation, collect the crystals and dry over phosphorus pentoxide at 60° at a pressure of 1.5 to 2.5 kPa. The crystals comply with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *isoprenaline sulphate RS* treated in the same manner.

B. To 1 ml of a 1 per cent w/v solution add 0.1 ml of *ferric chloride test solution*; an emerald-green colour is produced which, on gradual addition of *sodium bicarbonate solution*, changes first to blue and then to red.

C. To 5 ml of a freshly prepared 1 per cent w/v solution add 0.15 ml of *silver nitrate solution*; a greyish precipitate is produced on standing for 10 minutes and the solution becomes pink.

D. A 10 per cent w/v solution gives the reaction of sulphates (2.3.1).

**Tests**

**Appearance of solution.** A freshly prepared 10.0 per cent w/v solution in carbon dioxide-free water is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

**pH** (2.4.24). 4.0 to 5.5, determined in a freshly prepared 1.0 per cent w/v solution in carbon dioxide-free water.
Isoprenalone. Absorbance of a 0.2 per cent w/v solution in 0.005 M sulphuric acid at about 310 nm, not more than 0.2 (2.4.7).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 5.0 to 7.5, determined on 0.2 g.

Assay. Weigh accurately about 0.8 g, dissolve in 40 ml of anhydrous glacial acetic acid, warming gently if necessary and titrate with 0.1 M perchloric acid, using crystal violet solution as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.05206 g of \( (C_{11}H_{17}NO_3)_2H_2SO_4 \).

Storage. Store protected from light.

Isoprenaline Tablets

Isoprenaline Sulphate Tablets; Isoproterenol Tablets; Isoproterenol Sulphate Tablets

Isoprenaline Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of isoprenaline sulphate, \( (C_{11}H_{17}NO_3)_2H_2SO_4 \).

Identification

A. Extract a quantity of the powdered tablets containing about 50 mg of Isoprenaline Sulphate with 5 ml of water and filter. Reserve the filtrate for test C. To 1 ml of the filtrate add 0.1 ml of ferric chloride test solution; an emerald-green colour is produced which, on gradual addition of sodium bicarbonate solution, changes first to blue and then to red.

B. Extract a quantity of the powdered tablets containing about 50 mg of Isoprenaline Sulphate with 5 ml of water and filter. To the filtrate add 0.15 ml of silver nitrate solution; a greyish precipitate is produced on standing for 10 minutes and the solution becomes pink.

C. To 2 ml of the filtrate reserved from test A add 0.5 ml of dilute hydrochloric acid and 0.5 ml of barium chloride solution; a white precipitate is formed.

Tests

Uniformity of content. Comply with the test stated under Tablets.

Crush one tablet and shake with 50 ml of water for 15 minutes. Add sufficient water to produce 100.0 ml, mix and filter. To 20.0 ml of the filtrate add 0.5 ml of ferrous sulphate-citrate solution and 2 ml of glycine buffer solution and allow to stand for 20 minutes. Dilute to 25.0 ml with water and mix. Measure the absorbance of the resulting solution at the maximum at about 540 nm (2.4.7). Calculate the content of \( (C_{11}H_{17}NO_3)_2H_2SO_4 \) from the absorbance obtained by repeating the determination using 2.0 ml of a 0.1 per cent w/v solution of isoprenaline sulphate RS in place of the substance under examination.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Isoprenaline Sulphate and shake with 50 ml of water for 15 minutes. Add sufficient water to produce 100.0 ml, mix and filter. Dilute 20.0 ml of the filtrate to 200.0 ml with water. To 20.0 ml of the resulting solution add 0.5 ml of ferrous sulphate-citrate solution and 2 ml of glycine buffer solution and allow to stand for 20 minutes. Dilute to 25.0 ml with water and mix. Measure the absorbance of the resulting solution at the maximum at about 540 nm (2.4.7). Calculate the content of \( (C_{11}H_{17}NO_3)_2H_2SO_4 \) from the absorbance obtained by repeating the determination using 2.0 ml of a 0.1 per cent w/v solution of isoprenaline sulphate RS in place of the substance under examination.

Storage. Store protected from light.

Isopropyl Alcohol

2-Propanol; Propan-2-ol

\[ \text{C}_3\text{H}_7\text{O} \]

Mol. Wt. 60.1

Isopropyl Alcohol is propan-2-ol.

Description. A clear, colourless liquid; odour, characteristic and spirituous; flammable.

Identification

A. Mix 1 ml of a 10 per cent v/v solution with 2 ml of mercuric sulphate solution and heat just to boiling; a white or yellowish white precipitate is produced.

B. Gently heat 1 ml with 4 ml of dilute potassium dichromate solution and 1 ml of sulphuric acid; acetone, recognisable by its odour, is evolved.

Tests

Acidity or alkalinity. Gently boil 25 ml for 5 minutes with 25 ml of carbon dioxide-free water and cool, taking precautions to exclude carbon dioxide. Not more than 0.06 ml of 0.1 M sodium hydroxide is required to make the resulting solution alkaline to phenolphthalein solution.
**Distillation range** (2.4.8). Not less than 95.0 per cent v/v distils between 81° and 83°.

**Refractive index** (2.4.27). 1.377 to 1.378, determined at 20°.

**Weight per ml** (2.4.29). 0.782 g to 0.786 g, determined at 20°.

**Aldehydes and ketones.** Mix in a cylinder 25 ml with 25 ml of water and 50 ml of hydroxylamine solution, allow to stand for 5 minutes and titrate with 0.1 M sodium hydroxide until the colour is the same as that of a mixture of 50 ml of hydroxylamine solution and 50 ml of water contained in a similar cylinder, each being viewed down the vertical axis of the cylinder. Not more than 2.0 ml of 0.1 M sodium hydroxide is required.

**Benzene and related substances.** Determine by gas chromatography (2.4.13).

**Test solution.** The substance under examination.

**Reference solution (a).** A 0.1 per cent v/v solution of 2-butanol reagent in the substance under examination.

**Reference solution (b).** A solution containing 0.1 per cent v/v of each of 2-butanol reagent and 1-propanol in the substance under examination.

**Reference solution (c).** A 0.0002 per cent v/v solution of benzene in the substance under examination.

Chromatographic system
- a glass column 1.8 m x 2 mm, packed with acid-washed diatomaceous support (80 to 100 mesh) coated with 15 per cent w/w of polyethylene glycol 400,
- temperature: column, 50°, inlet port, 150°,
- flow rate, 30 ml per minute of the carrier gas,
- flame ionisation detector at 200°.

Inject separately 2 μl of each of the test solution and reference solution (a). The chromatogram obtained with the test solution shows no peak with retention time similar to the peak due to 2-butanol (retention time relative to isopropyl alcohol, about 1.5) obtained with solution (2). Inject 2 μl of reference solution (b) and adjust the sensitivity of the system so that the heights of the peaks due to 2-butanol and 1-propanol in the chromatogram obtained with reference solution (b) are not less than 50 per cent of the full scale of the recorder. The test is not valid unless the resolution between the peaks due to 2-butanol and 1-propanol in the chromatogram obtained with reference solution (b) is at least 1.2.

Inject alternately 2 μl each of the test solution and reference solution (c). The area of any peak due to benzene in the chromatogram obtained with the test solution is not greater than the difference between the area of the peak due to benzene in the chromatogram obtained with reference solution (c) and that of the peak due to benzene in the chromatogram obtained with the test solution.

In the chromatogram obtained with reference solution (a) the sum of areas of any peaks other than the principal peak and the peaks due to 2-butanol is not greater than 3 times the area of the peak due to 2-butanol (0.3 per cent).

**Non-volatile matter.** Not more than 0.002 per cent w/v, determined by evaporation of 100 ml on a water-bath and drying the residue at 105°.

**Water-insoluble matter.** Mix 1 volume with 19 volumes of water; no opalescence is produced.

**Water** (2.3.43). Not more than 0.5 per cent, determined on 5 g.

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**Diluted Isosorbide Dinitrate**

Diluted Sorbide Dinitrate; Diluted Sorbide Nitrate

![Chemical Structure](image)

C₆H₈N₂O₈ Mol. Wt. 236.1

Diluted Isosorbide Dinitrate is a dry mixture of 1,4:3,6-dianhydro-α-glucitol 2,5-dinitrate with Lactose, Mannitol or other suitable inert diluent. It may contain a suitable stabilising agent.

Diluted Isosorbide Dinitrate contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of isosorbide dinitrate, C₆H₈N₂O₈ and usually contains 20 per cent to 50 per cent of isosorbide dinitrate.

**Description.** A fine, white, crystalline powder; odourless or almost odourless.

**CAUTION — Undiluted isosorbide dinitrate is a powerful explosive and can be exploded with percussion or excessive heat. Proper precautions must be taken in handling it and only exceedingly small amounts should be isolated.**

**Identification**

A. Shake a quantity containing 50 mg of isosorbide dinitrate with 15 ml of acetone for 2 minutes. Filter, evaporate the filtrate to dryness at a temperature not exceeding 35° and dry the residue over phosphorus pentoxide at a pressure of 0.7 kPa for 16 hours. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with diluted isosorbide dinitrate RS treated in the same manner or with the reference spectrum of isosorbide dinitrate.
B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. Toluene.

Test solution. Extract a quantity containing 2 mg of isosorbide dinitrate with 1 ml of ether and centrifuge.

Reference solution. Prepare in the same manner as the test solution but using diluted isosorbide dinitrate RS in place of the substance under examination.

Apply to the plate 20 µl of each solution. After development, dry the plate in a current of air, spray with a 1 per cent w/v solution of diphenylamine in methanol and expose for 15 minutes to ultraviolet light at 254 and 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Extract a quantity containing 10 mg of isosorbide dinitrate with 10 ml of ether and filter. Evaporate the filtrate to dryness at a temperature not exceeding 35° and dissolve the residue in 0.15 ml of sulphuric acid (50 per cent) containing a trace of diphenylamine; an intense blue colour is produced.

Tests

Inorganic nitrates. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel H.

Mobile phase. A mixture of 60 volumes of toluene, 30 volumes of acetone and 15 volumes of glacial acetic acid.

Test solution. A solution of the substance under examination in ethanol (95 per cent) containing the equivalent of 2.0 per cent w/v of isosorbide dinitrate.

Reference solution. Prepare freshly a 0.01 per cent w/v solution of potassium nitrate in ethanol (90 per cent).

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of air until the acetic acid is completely removed. Spray copiously with freshly prepared potassium iodide and starch solution. Expose the plate to ultraviolet light at 254 nm for 15 minutes. Examine in daylight. Any spot corresponding to potassium nitrate in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Related substances. Determine by liquid chromatography (2.4.14) by the method described under Assay, changing the detector setting to 210 to 215 nm and using a 10 µl injector.

Under the stated conditions, the retention times are: isosorbide dinitrate, about 5 minutes; isosorbide 2-nitrate, about 8 minutes; isosorbide 5-nitrate, about 11 minutes.

Inject 10 µl of reference solution (c). Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (c) is not less than 20 per cent of the full scale of the recorder.

Inject 10 µl of reference solution (e). The test is not valid unless in the chromatogram obtained with reference solution (e), the resolution between the peaks corresponding to isosorbide dinitrate and isosorbide 2-nitrate is at least 6.0.

Inject 10 µl of test solution (a), 10 µl of reference solution (c) and 10 µl of reference solution (d). In the chromatogram obtained with test solution (a) the area of any peak corresponding to isosorbide 2-nitrate is not greater than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent); the area of any peak corresponding to isosorbide 5-nitrate is not greater than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying over phosphorus pentoxide at a pressure not exceeding 0.7 kPa for 16 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution (a). Mix an accurately weighed quantity of the substance under examination containing about 25 mg of isosorbide dinitrate with 20 ml of the mobile phase with the aid of ultrasound for 15 minutes and dilute to 25.0 ml with the mobile phase. Filter the solution through a suitable membrane filter.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with the mobile phase.

Reference solution (a). Mix a quantity of isosorbide dinitrate RS containing 25.0 mg of isosorbide dinitrate with 20 ml of the mobile phase with the aid of ultrasound for 15 minutes and dilute to 25.0 ml with the mobile phase. Filter the solution through a suitable membrane filter.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase.

Reference solution (c). Dissolve 10.0 mg of isosorbide 2-nitrate RS in the mobile phase and dilute to 10.0 ml with the mobile phase. Dilute 0.1 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (d). Dissolve 20.0 mg of isosorbide mononitrate RS in the mobile phase and dilute to 10.0 ml with the mobile phase. Dilute 0.1 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (e). Dissolve 5 mg of isosorbide 2-nitrate RS in the mobile phase and dilute to 10 ml with the mobile phase. To 1 ml of this solution add 0.5 ml of reference solution (a) and dilute to 10 ml with the mobile phase.
Chromatographic system
- A stainless steel column 25 cm x 4.6 mm packed with aminopropylmethylsilyl silica gel (10 µm),
- Mobile phase: a mixture of 15 volumes of ethanol and 85 volumes of trimethylpentane,
- Flow rate: 1 ml per minute,
- Spectrophotometer set at 230 nm,
- A 20 µl loop injector.

Inject reference solution (b). Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained is not less than 50 per cent of the full scale of the recorder. If the areas of the peaks from two successive injections do not agree to within 1.0 per cent, then inject a further four times and calculate, for the six injections, the relative standard deviation. The assay is not valid unless the relative standard deviation for the six injections is at most 2.0 per cent.

Inject alternately test solution (b) and reference solution (b). Calculate the content of isosorbide dinitrate as a percentage of the declared content.

Storage. Store protected from light, at a temperature not exceeding 15°.

Labelling. The label states the percentage content of isosorbide dinitrate.

**Isosorbide Dinitrate Tablets**

Sorbide Dinitrate Tablets; Sorbide Nitrate Tablets

Isosorbide Dinitrate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of isosorbide dinitrate, C₆H₈N₂O₈.

Identification

**CAUTION** - Undiluted isosorbide dinitrate is a powerful explosive and can be exploded with percussion or excessive heat. Proper precautions must be taken in handling it and only exceedingly small amounts should be isolated.

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** Toluene.

**Test solution.** Extract a quantity of the powdered tablets containing 2 mg of isosorbide dinitrate with 1 ml of ether and centrifuge.

**Reference solution.** Dissolve 2 mg of isosorbide dinitrate RS in 1 ml of ether.

Apply to the plate 20 µl of each solution. After development, dry the plate in a current of air, spray with a 1 per cent w/v solution of diphenylamine in methanol and expose for 15 minutes to ultraviolet light at 254 nm and 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution B. Extract a quantity of the powdered tablets containing 10 mg of isosorbide dinitrate with 10 ml of ether and filter. Evaporate the filtrate to dryness at a temperature not exceeding 35° and dissolve the residue in 0.15 ml of sulphuric acid (50 per cent) containing a trace of diphenylamine; an intense blue colour is produced.

**Tests**

**Inorganic nitrates.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel H.

**Mobile phase.** A mixture of 60 volumes of toluene, 30 volumes of acetone and 15 volumes of glacial acetic acid.

**Test solution.** Shake a quantity of the powdered tablets containing 0.1 g of isosorbide nitrate with 5 ml of ethanol (95 per cent) and filter.

**Reference solution.** Prepare freshly a 0.01 per cent w/v solution of potassium nitrate in ethanol (90 per cent).

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of air until the acetic acid is completely removed. Spray copiously with freshly prepared potassium iodide and starch solution. Expose the plate to ultraviolet light at 254 nm for 15 minutes. Examine in daylight. Any spot corresponding to potassium nitrate in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Add 20 ml of the mobile phase to a quantity of the powdered tablets containing 25 mg of isosorbide dinitrate, mix with the aid of ultrasound for 15 minutes and dilute to 25 ml with the same solvent. Filter through a glass-fibre filter (such as Whatman GF/C) and use the filtrate.

**Reference solution (a).** A solution containing 0.0005 per cent w/v of isosorbide 2-nitrate RS in the mobile phase.

**Reference solution (b).** A solution containing 0.0005 per cent w/v of isosorbide mononitrate RS in the mobile phase.

**Reference solution (c).** A solution containing 0.005 per cent w/v each of isosorbide dinitrate RS and isosorbide 2-nitrate RS in the mobile phase.

Chromatographic system
- A stainless steel column 25 cm x 4.6 mm packed with aminopropylmethylsilyl silica gel (10 µm),
Calculate the content of C₆H₈N₂O₈.

Chromatographic system
- mobile phase: a mixture of 15 volumes of ethanol and 85 volumes of 2,2,4-trimethylpentane,
- flow rate, 1 ml per minute,
- spectrophotometer set at 215 nm,
- a 20 µl loop injector.

Inject reference solution (c). The test is not valid unless, in the chromatogram obtained, the resolution between the peaks corresponding to isosorbide dinitrate and isosorbide 2-nitrate is at least 6.0.

In the chromatogram obtained with the test solution, the area of any peak corresponding to isosorbide 2-nitrate is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the area of any peak corresponding to isosorbide 5-nitrate is not greater than the area of the principal peak obtained with reference solution (b) (0.5 per cent).

**Dissolution** (2.5.2). (for tablets intended to be swallowed whole).

Apparatus. No 1

Medium. 900 ml of 0.1 M hydrochloric acid

Speed and time. 50 rpm and 30 minutes.

Withdraw 10 ml of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate.

Determine by liquid chromatography (2.4.14).

**Test solution.** The filtrate from the dissolution medium.

**Reference solution.** A solution of isosorbide dinitrate RS in the dissolution medium containing the same concentration of isosorbide dinitrate as that expected in the dissolution medium in the vessel.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm packed with octadecylsilyl silica gel (5 µm) (such as Hypersil ODS),
- mobile phase: a mixture of equal volumes of methanol and water,
- flow rate, 1 ml per minute,
- spectrophotometer set at 222 nm.
- a 100 µl loop injector.

Calculate the content of C₆H₈N₂O₈.

If not less than 80 per cent of the stated amount of C₆H₈N₂O₈.

**Uniformity of content.** Comply with the test stated under Tablets.

Crush one tablet, add 5 ml of glacial acetic acid, shake for 1 hour and centrifuge. To a suitable volume of the supernatant liquid containing 1.0 mg of isosorbide dinitrate add sufficient glacial acetic acid, if necessary, to produce 1.0 ml, add 2 ml of phenoldisulphonic acid solution, allow to stand for 15 minutes, add 25 ml of water, make alkaline with strong ammonia solution, cool and add sufficient water to produce 50.0 ml. Measure the absorbance of the resulting solution at about 405 nm (2.4.7), using as the blank 1.0 ml of glacial acetic acid treated in a similar manner beginning at the words “add 2 ml of phenoldisulphonic acid solution...”. Dissolve 0.2 g of potassium nitrate, previously dried at 105°, in 5 ml of water and add sufficient glacial acetic acid to produce 25.0 ml. To 5.0 ml add sufficient glacial acetic acid to produce 50.0 ml. Using 1.0 ml of this solution repeat the procedure beginning at the words “add 2 ml of phenoldisulphonic acid solution...”. Calculate the content of C₆H₈N₂O₈ from the values of the absorbances so obtained.

1 ml of the potassium nitrate solution is equivalent to 0.000934 g of C₆H₈N₂O₈.

Calculate the content of C₆H₈N₂O₈ in the tablet.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh and powder 20 tablets. Add 20 ml of the mobile phase to an accurately weighed quantity of the powdered tablets containing about 25 mg of isosorbide dinitrate, mix with the aid of ultrasound for 15 minutes and dilute to 25.0 ml with the same solvent. Filter through a glass-fibre filter (such as Whatman GF/C) and dilute 1 volume to 10 volumes with the mobile phase.

**Reference solution (a).** Add 20 ml of the mobile phase to a quantity of isosorbide dinitrate RS containing 25 mg of isosorbide dinitrate, mix with the aid of ultrasound for 15 minutes and dilute to 25 ml with the mobile phase. Filter through a glass-fibre filter (such as Whatman GF/C) and dilute 1 volume to 10 volumes with the mobile phase.

**Reference solution (b).** A solution containing 0.005 per cent w/v each of isosorbide dinitrate RS and isosorbide 2-nitrate RS in the mobile phase.

Follow the procedure described under Related substances but using a detection wavelength of 230 nm.

The test is not valid unless, in the chromatogram obtained with reference solution (b), the resolution between the peaks corresponding to isosorbide dinitrate and isosorbide 2-nitrate is at least 6.0.

Calculate the content of C₆H₈N₂O₈ in the tablets from the chromatograms obtained.

**Storage.** Store at a temperature not exceeding 30°.

**Labelling.** The label states whether the tablets are to be swallowed whole, chewed before swallowing or allowed to dissolve in the mouth.
Isoxsuprine Hydrochloride

C₁₈H₂₃NO₃,HCl  Mol. Wt. 337.9

Isoxsuprine Hydrochloride is (1RS,2SR)-1-(4-hydroxyphenyl)-2-[(1RS)-1-methyl-2-phenoxyethylamino]propan-1-ol hydrochloride.

Isoxsuprine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of C₁₈H₂₃NO₃,HCl, calculated on the dried basis.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with isoxsuprine hydrochloride RS or with the reference spectrum of isoxsuprine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.01 per cent w/v solution in 0.1 M hydrochloric acid shows absorption maxima at about 269 nm and 274 nm; absorbance at about 269 nm, about 0.73 and at about 274 nm, about 0.72.

C. Dissolve 10 mg in 1 ml of water and add 0.05 ml of copper sulphate solution and 1 ml of 5 M sodium hydroxide; a blue colour is produced. Add 1 ml of ether and shake; the ether layer remains colourless.

D. Gives the reactions of chlorides (2.3.1).

Tests

pH (2.4.24). 4.5 to 6.0, determined in a 1.0 per cent w/v solution, prepared with gentle warming if necessary.

Phenones. Absorbance of a 0.01 per cent w/v solution at about 310 nm, not more than 0.20 (2.4.7).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.6 g, dissolve in 150 ml of anhydrous glacial acetic acid, heating on a water-bath to effect dissolution. Cool and titrate with 0.1 M perchloric acid, using 1-naphtholbenzein solution as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.03378 g of C₁₈H₂₃NO₃,HCl.

Isoxsuprine Injection

Isoxsuprine Hydrochloride Injection

Isoxsuprine Injection is a sterile solution of Isoxsuprine Hydrochloride in Water for Injections.

Isoxsuprine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of isoxsuprine hydrochloride, C₁₈H₂₃NO₃,HCl.

Identification

A. To a volume containing 50 mg of Isoxsuprine Hydrochloride add 20 ml of water and 10 ml of ammonia buffer pH 10.0 and extract with three quantities, each of 15 ml, of dichloromethane. Shake the combined extracts with 5 g of anhydrous sodium sulphate, filter, evaporate the filtrate to dryness, dissolve the residue in 5 ml of 0.1 M methanolic hydrochloric acid and evaporate to dryness. Dissolve the residue in 5 ml of methanol, evaporate to dryness, redissolve the residue in 2 ml of methanol, add 15 ml of dichloromethane, again evaporate to dryness and dry the residue at 60° at a pressure of 2 kPa for 1 hour.

On the residue determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with isoxsuprine hydrochloride RS treated in the same manner or with the reference spectrum of isoxsuprine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows absorption maxima at about 269 nm and 274 nm.

Tests

pH (2.4.24). 4.9 to 6.0.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. To an accurately measured volume containing about 50 mg of Isoxsuprine Hydrochloride add sufficient 0.1 M hydrochloric acid to produce 100.0 ml. Dilute 10.0 ml to 100.0 ml with the same solvent and measure the absorbance of the resulting solution at the maximum at about 274 nm (2.4.7). Calculate the content of C₁₈H₂₃NO₃,HCl taking 73 as the specific absorbance at 274 nm.
**Isoxsuprine Tablets**

Isoxsuprine Hydrochloride Tablets

Isoxsuprine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of isoxsuprine hydrochloride, C$_{18}$H$_{23}$NO$_3$HCl.

**Identification**

A. To a quantity of the powdered tablets containing 50 mg of Isoxsuprine Hydrochloride add 50 ml of 0.1 M hydrochloric acid and heat on a water-bath for 30 minutes. Cool, filter, add 10 ml of ammonia buffer pH 10.0 and extract with three quantities, each of 15 ml, of dichloromethane. Shake the combined extracts with 5 g of anhydrous sodium sulphate, filter, evaporate the filtrate to dryness, dissolve the residue in 5 ml of 0.1 M methanolic hydrochloric acid and evaporate to dryness. Dissolve the residue in 5 ml of methanol, evaporate to dryness, redissolve the residue in 2 ml of methanol, add 15 ml of dichloromethane, again evaporate to dryness and dry the residue at 60° at a pressure of 2 kPa for 1 hour.

On the residue determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with isoxsuprine hydrochloride RS treated in the same manner or with the reference spectrum of isoxsuprine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows absorption maxima at about 269 nm and 274 nm.

**Tests**

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 20 mg of Isoxsuprine Hydrochloride, add 50 ml of 0.1 M hydrochloric acid and boil on a water-bath for 30 minutes. Cool, add sufficient 0.1 M hydrochloric acid to produce 100.0 ml, mix and filter. Dilute 25.0 ml of the filtrate to 100.0 ml with 0.1 M hydrochloric acid and measure the absorbance of the resulting solution at the maximum at about 274 nm (2.4.7). Calculate the content of C$_{18}$H$_{23}$NO$_3$HCl taking 73 as the specific absorbance at 274 nm.
K

Kanamycin Sulphate
Kanamycin Acid Sulphate
Kanamycin Injection
Heavy Kaolin
Light Kaolin
Ketamine Hydrochloride
Ketamine Injection
Ketoconazole
Ketoconazole Tablets
Ketoprofen
Ketoprofen Capsules
Kanamycin Sulphate

\[ \text{C}_{18}\text{H}_{30}\text{N}_{4}\text{O}_{11}\cdot\text{H}_{2}\text{SO}_4\cdot\text{H}_2\text{O} \]

Mol. Wt. 600.6

Kanamycin Sulphate is 6-O-(3-amino-3-deoxy-α-D-glucopyranosyl)-4-O-(6-amino-6-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine sulphate monohydrate, an antimicrobial substance produced by the growth of certain strains of *Streptomyces kanamyceticus*.

Kanamycin Sulphate has a potency of not less than 750 Units per mg, calculated on the dried basis.

**Description.** A white or almost white, crystalline powder; odourless or almost odourless.

**Identification**

A. Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of carbomer (such as Carbopol 934) with 240 ml of water and allow to stand, with moderate shaking, for 1 hour; adjust to pH 7.0 by the gradual addition, with continuous shaking, of 2 M sodium hydroxide and add 30 g of silica gel H. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

**Mobile phase.** A 7 per cent w/v solution of potassium dihydrogen phosphate.

**Test solution.** A 0.1 per cent w/v solution of kanamycin sulphate RS in water.

**Reference solution (a).** A 0.1 per cent w/v solution of kanamycin sulphate RS in water.

**Reference solution (b).** A solution containing 0.1 per cent w/v each of kanamycin sulphate RS, neomycin sulphate RS and streptomycin sulphate RS in water.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with a mixture of equal volumes of a 0.2 per cent w/v solution of 1,3-naphthalenediol in ethanol (95 per cent) and a 45 per cent w/v solution of sulphuric acid and heat at 150° for 5 to 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

B. Dissolve 0.5 g in 10 ml of water and add 10 ml of a 1 per cent w/v solution of picric acid. If necessary initiate crystallisation by scratching the walls of the container with a glass rod, allow to stand and filter. The crystals, after washing with 20 ml of water and drying at 105°, melt at about 235°, with decomposition (2.4.21).

C. Dissolve 50 mg in 2 ml of water, add 1 ml of a 1 per cent w/v solution of ninhydrin and heat for a few minutes on a water-bath; a violet colour is produced.

D. Gives the reactions of sulphates (2.3.1).

**Tests**

**pH (2.4.24).** 6.5 to 8.5, determined in a 1.0 per cent w/v solution.

**Specific optical rotation (2.4.22).** +112° to +123°, determined at 20° in a 1.0 per cent w/v solution.

**Kanamycin B.** Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of carbomer (such as Carbopol) with 240 ml of water and allow to stand, with moderate shaking, for 1 hour; adjust to pH 7.0 by the gradual addition, with continuous shaking, of 2 M sodium hydroxide and add 30 g of silica gel H. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

**Mobile phase.** A 7 per cent w/v solution of potassium dihydrogen phosphate.

**Test solution.** A 0.5 per cent w/v solution of the substance under examination in water.

**Reference solution.** A 0.02 per cent w/v solution of kanamycin B sulphate RS in water.

Apply to the plate 4 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with ninhydrin and stannous chloride reagent and heat at 110° for 15 minutes. Any spot corresponding to kanamycin B in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Sulphates.** 15.0 to 17.0 per cent of SO₄, calculated on the dried basis and determined by the following method. Dissolve 0.25 g in 100 ml of water and adjust the pH to 11 using strong ammonia solution. Add 10.0 ml of 0.1 M barium chloride and
0.5 mg of metalphthalaein. Titrate with 0.1 M disodium edetate; when the colour of the solution begins to change, add 50 ml of ethanol (95 per cent) and continue the titration until the blue colour disappears.

1 ml of 0.1 M barium chloride is equivalent to 0.009606 g of SO₄.

**Sulphated ash** (2.3.18). Not more than 0.5 per cent.

**Loss on drying** (2.4.19). Not more than 1.5 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

**Assay**. Determine by the microbiological assay of antibiotics, Method A or B (2.2.10).

**Kanamycin Sulphate** intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

**Bacterial endotoxins** (2.2.3). Not more than 0.67 Endotoxin Unit per mg of kanamycin.

**Kanamycin Sulphate** intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store protected from light and moisture. If the contents are intended for use in the manufacture of parenteral preparations, the container should be sterile and sealed so as to exclude micro-organisms.

**Labelling**. The label states (1) the number of Units per mg; (2) whether or not the material is intended for use in the manufacture of parenteral preparations.

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**Kanamycin Acid Sulphate**

Kanamycin Acid Sulphate is a form of kanamycin sulphate prepared by adding Sulphuric Acid to a solution of Kanamycin Sulphate and subsequent drying.

Kanamycin Acid Sulphate has a potency of not less than 650 Units per mg, calculated on the dried basis.

**Description**. A white or almost white powder; odourless or almost odourless; hygroscopic.

**Identification**

A. Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of carbomer (such as Carbopol 934) with 240 ml of water and allow to stand, with moderate shaking, for 1 hour; adjust to pH 7.0 by the gradual addition, with continuous shaking, of 2 M sodium hydroxide and add 30 g of silica gel H. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

**Mobile phase**. A 7 per cent w/v solution of potassium dihydrogen phosphate.

**Test solution**. A 0.1 per cent w/v solution of kanamycin sulphate RS in water.

**Reference solution (a)**. A 0.1 per cent w/v solution of kanamycin sulphate RS in water.

**Reference solution (b)**. A solution containing 0.1 per cent w/v each of kanamycin sulphate RS, neomycin sulphate RS and streptomycin sulphate RS in water.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with a mixture of equal volumes of a 0.2 per cent w/v solution of 1,3-naphthalenediol in ethanol (95 per cent) and a 45 per cent w/v of solution of sulphuric acid and heat at 150° for 5 to 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

B. Dissolve 0.5 g in 10 ml of water and add 10 ml of a 1 per cent w/v solution of picric acid. If necessary initiate crystallisation by scratching the walls of the container with a glass rod, allow to stand and filter. The melting point of the crystals, after washing with 20 ml of water and drying at 105°, is about 235°, with decomposition (2.4.21).

C. Dissolve 50 mg in 2 ml of water, add 1 ml of a 1 per cent w/v solution of ninhydrin and heat for a few minutes in a water-bath; a violet colour is produced.

D. Gives the reactions of sulphates (2.3.1).

**Tests**

**pH** (2.4.24). 5.5 to 7.5, determined in a 1.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +103° to +115°, determined at 20° in a 1.0 per cent w/v solution.

**Kanamycin B**. Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of carbomer (such as Carbopol 934) with 240 ml of water and allow to stand, with moderate shaking, for 1 hour; adjust to pH 7.0 by the gradual addition, with continuous shaking, of 2 M sodium hydroxide and add 30 g of silica gel H. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

**Mobile phase**. A 7 per cent w/v solution of potassium dihydrogen phosphate.
Test solution. A 0.5 per cent w/v solution of the substance under examination in water.

Reference solution. A 0.02 per cent w/v solution of kanamycin B sulphate RS in water.

Apply to the plate 4 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with ninhydrin and stannous chloride reagent and heat at 110° for 15 minutes. Any spot corresponding to kanamycin B in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphates. 23.0 to 26.0 per cent of SO₄, calculated on the dried basis and determined by the following method. Dissolve 0.25 g in 100 ml of water and adjust the pH to 11 using strong ammonia solution. Add 10.0 ml of 0.1 M barium chloride and 0.5 mg of metolphthalein. Titrate with 0.1 M disodium edetate; when the colour of the solution begins to change, add 50 ml of ethanol (95 per cent) and continue the titration until the blue colour disappears.

1 ml of 0.1 M barium chloride is equivalent to 0.009606 g of SO₄.

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. Determine by the microbiological assay of antibiotics, Method A or B (2.2.10).

Kanamycin Acid Sulphate intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.67 Endotoxin Unit per mg of kanamycin.

Kanamycin Sulphate intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light and moisture. If the material is intended for use in the manufacture of parenteral preparations, the container should be sterile and sealed so as to exclude micro-organisms.

Labelling. The label states (1) the number of Units per mg; (2) whether or not the material is intended for use in the manufacture of parenteral preparations.

Kanamycin Injection

Kanamycin Injection is either a sterile solution of Kanamycin Sulphate in Water for Injections containing Sulphuric Acid and suitable buffering and stabilising agents or, is a sterile material consisting of Kanamycin Acid Sulphate with buffering agents and other excipients. It is filled in a sealed container.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of carboxer (such as Carbopol 934) with 240 ml of water and allow to stand, with moderate shaking, for 1 hour; adjust the pH to 7.0 by the gradual addition, with continuous shaking, of 2 M sodium hydroxide and add 30 g of silica gel H. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

Mobile phase. A 7 per cent w/v solution of potassium dihydrogen phosphate.

Test solution. A suitable volume diluted with water to contain 800 Units per ml.

Reference solution (a). A 0.1 per cent w/v solution of kanamycin sulphate RS in water.

Reference solution (b). A solution containing 0.1 per cent w/v each of kanamycin sulphate RS, neomycin sulphate RS and streptomycin sulphate RS in water.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with a mixture of equal volumes of a 0.2 per cent w/v solution of 1,3-naphthalenediol in ethanol (95 per cent) and a 45 per cent w/v of solution of sulphuric acid and heat at 150° for 5 to 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

Tests

pH (2.4.24). 4.0 to 6.0.

Kanamycin B. Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of carboxer (such as Carbopol 934) with 240 ml of water and allow to stand, with moderate shaking, for 1 hour; adjust to pH 7.0 by the gradual addition, with continuous
shaking, of 2 M sodium hydroxide and add 130 g of silica gel H. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

Mobile phase. A 7 per cent w/v solution of potassium dihydrogen phosphate.

Test solution. A suitable volume diluted with water to contain 3750 Units per ml.

Reference solution. A 0.02 per cent w/v solution of kanamycin B sulphate RS in water.

Apply to the plate 4 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with ninhydrin and stannous chloride reagent and heat at 110° for 15 minutes. Any spot corresponding to kanamycin B in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Bacterial endotoxins (2.2.3). Not more than 0.67 Endotoxin Unit per mg of kanamycin.

Assay. Determine by the microbiological assay of antibiotics, Method A or B (2.2.10).

The upper fiducial limit of error is not less than 97.0 per cent and the lower fiducial limit of error is not more than 110.0 per cent of the stated number of Units.

B. Kanamycin Injection (Powder)

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Kanamycin Injection contains not less than 95.0 per cent and not more than 115.0 per cent of the stated number of Units of kanamycin

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of carbomer (such as Carbopol 934) with 240 ml of water and allow to stand, with moderate shaking, for 1 hour; adjust the pH to 7.0 by the gradual addition, with continuous shaking, of 2 M sodium hydroxide and add 30 g of silica gel H. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

Mobile phase. A 7 per cent w/v solution of potassium dihydrogen phosphate.

Test solution. A suitable volume diluted with water to contain 800 Units per ml.

Reference solution (a). A 0.1 per cent w/v solution of kanamycin sulphate RS in water.

Reference solution (b). A solution containing 0.1 per cent w/v each of kanamycin sulphate RS, neomycin sulphate RS and streptomycin sulphate RS in water.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with a mixture of equal volumes of a 0.2 per cent w/v solution of 1,3-napthalenediol in ethanol (95 per cent) and a 45 per cent w/v of solution of sulphuric acid and heat at 150° for 5 to 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

Tests

pH (2.2.4). 5.5 to 7.5, determined in a 1.0 per cent w/v solution.

Kanamycin B. Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of carbomer (such as Carbopol 934) with 240 ml of water and allow to stand, with moderate shaking, for 1 hour; adjust to pH 7.0 by the gradual addition, with continuous shaking, of 2 M sodium hydroxide and add 30 g of silica gel H. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

Mobile phase. A 7 per cent w/v solution of potassium dihydrogen phosphate.

Test solution. A suitable volume diluted with water to contain 3750 Units per ml.

Reference solution. A 0.02 per cent w/v solution of kanamycin B sulphate RS in water.

Apply to the plate 4 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with ninhydrin and stannous chloride reagent and heat at 110° for 15 minutes. Any spot corresponding to kanamycin B in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Bacterial endotoxins (2.2.3). Not more than 0.67 Endotoxin Unit per mg of kanamycin.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of carbomer (such as Carbopol 934) with 240 ml of water and allow to stand, with moderate shaking, for 1 hour; adjust the pH to 7.0 by the gradual addition, with continuous shaking, of 2 M sodium hydroxide and add 30 g of silica gel H. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

Mobile phase. A 7 per cent w/v solution of potassium dihydrogen phosphate.

Test solution. A suitable volume diluted with water to contain 800 Units per ml.

Reference solution (a). A 0.1 per cent w/v solution of kanamycin sulphate RS in water.

Reference solution (b). A solution containing 0.1 per cent w/v each of kanamycin sulphate RS, neomycin sulphate RS and streptomycin sulphate RS in water.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with a mixture of equal volumes of a 0.2 per cent w/v solution of 1,3-napthalenediol in ethanol (95 per cent) and a 45 per cent w/v of solution of sulphuric acid and heat at 150° for 5 to 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

Tests

pH (2.2.4). 5.5 to 7.5, determined in a 1.0 per cent w/v solution.

Kanamycin B. Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of carbomer (such as Carbopol 934) with 240 ml of water and allow to stand, with moderate shaking, for 1 hour; adjust to pH 7.0 by the gradual addition, with continuous shaking, of 2 M sodium hydroxide and add 30 g of silica gel H. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

Mobile phase. A 7 per cent w/v solution of potassium dihydrogen phosphate.

Test solution. A suitable volume diluted with water to contain 3750 Units per ml.

Reference solution. A 0.02 per cent w/v solution of kanamycin B sulphate RS in water.

Apply to the plate 4 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with ninhydrin and stannous chloride reagent and heat at 110° for 15 minutes. Any spot corresponding to kanamycin B in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Bacterial endotoxins (2.2.3). Not more than 0.67 Endotoxin Unit per mg of kanamycin.
Assay. Determine the weight of the contents of 10 containers. Using the mixed contents of the 10 containers determine by the microbiological assay of antibiotics, Method A or Method B (2.2.10).

For a container of average content weight, the upper fiducial limit of error is not less than 95.0 per cent and the lower fiducial limit of error is not more than 115.0 per cent of the stated number of Units.

Storage. Store in single dose containers protected from light and moisture.

Labelling. The label states (1) the strength in terms of the number of Units or the equivalent weight of kanamycin in a suitable dose-volume or in the sealed container; (2) the volume of Water for Injections for constituting the solution (for contents of a sealed container).

Heavy Kaolin
Heavy Kaolin is a purified, natural, hydrated aluminium silicate of variable composition.

Description. Fine, white or greyish white, soft powder; odourless.

Identification
A. To 0.5 g in a metal crucible add 1 g of potassium nitrate and 3 g of anhydrous sodium carbonate, heat until the mixture has melted and allow to cool. To the residue add 20 ml of boiling water, mix, filter and wash the residue with 50 ml of water. To the residue add 1 ml of hydrochloric acid and 5 ml of water and filter. To the filtrate add 1 ml of 10 M sodium hydroxide and filter. To the filtrate add 3 ml of ammonium chloride solution; a gelatinous, white precipitate is produced.

B. 0.25 g gives the reaction of silicates (2.3.1).

Tests

Acidity or alkalinity. To 1.0 g add 20 ml of carbon dioxide-free water, shake for 2 minutes and filter. To 10 ml of the filtrate add 0.1 ml of phenolphthalein solution. The solution is colourless and not more than 0.25 ml of 0.01 M sodium hydroxide is required to change the colour of the solution to pink.

Arsenic (2.3.10). Disperse 5.0 g in 50 ml of water and add 10 ml of stannated hydrochloric acid. The resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals (2.3.13). Boil 5.0 g with 7.5 ml of 2 M hydrochloric acid and 27.5 ml of water for 5 minutes, filter, wash the residue with water and dilute the combined filtrate and washings to 50 ml with water (solution A). To 5 ml of solution A add 5 ml of water, 10 ml of hydrochloric acid and 25 ml of 4-methylpentan-2-one, shake for 2 minutes, allow the layers to separate and evaporate the aqueous layer to dryness on a water-bath. Dissolve the residue in 1 ml of 5 M acetic acid, dilute to 25 ml with water and filter. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (50 ppm). Use lead standard solution (1 ppm Pb) to prepare the standard.

Chlorides (2.3.12). Shake 4.0 g with a mixture of 34 ml of distilled water and 6 ml of 5 M acetic acid for 1 minute and filter. 10 ml of the filtrate complies with the limit test for chlorides (250 ppm).

Sulphates (2.3.17). 2 ml of the filtrate obtained in the test for chlorides diluted to 15 ml complies with the limit test for sulphates (750 ppm).

Substances soluble in mineral acids. Not more than 1 per cent, determined by the following method. To 10 ml of solution A add 1.5 ml of 1 M sulphuric acid, evaporate to dryness on a water-bath, ignite, cool and weigh.

Organic impurities. Heat 0.3 g to redness in a calcination tube. The residue is only slightly more coloured than the original substance.

Adsorption power. In a ground-glass-stoppered test-tube shake 1.0 g with 10 ml of a 0.37 per cent w/v solution of methylene blue for 2 minutes and allow to settle. Centrifuge and dilute 1 volume of the solution to 100 volumes with water. The solution is not more intensely coloured than a 0.003 per cent w/v solution of methylene blue.

Swelling power. Triturate 2 g with 2 ml of water; the mixture does not flow.

Loss on ignition (2.4.20). Not more than 15.0 per cent, determined on 1.0 g.

Light Kaolin
Light Kaolin is a native hydrated aluminium silicate, freed from most of its impurities by elutriation and dried. It may contain a suitable dispersing agent.

Description. Light, white powder free from gritty particles; odourless; almost tasteless; unctuous to the touch.

Identification
A. Fuse 1 g with 2 g of anhydrous sodium carbonate, warm the residue with 10 ml of water, filter, wash the filter with 5 ml of water and reserve the residue. To the combined filtrate and washings add 3 ml of hydrochloric acid; a gelatinous precipitate is produced.

B. Dissolve the residue reserved in test A in 10 ml of 2 M hydrochloric acid; the solution gives reaction B of aluminium salts (2.3.1).
C. Triturate 2 g with 2 ml of water; the resulting mixture flows.

Coarse particles. Transfer 5 g to a stoppered cylinder (16 cm × 35 mm), add 60 ml of a 1 per cent w/v solution of sodium pyrophosphate, shake thoroughly and allow to stand for 5 minutes. Using a pipette, withdraw 50 ml from a point about 5 cm below the surface of the liquid. To the remaining liquid add 50 ml of water, shake, allow to stand for 5 minutes and withdraw 50 ml in the same manner as before. Repeat the operation until a total of 400 ml of suspension has been withdrawn under the prescribed conditions. Transfer the remainder to an evaporating dish and evaporate to dryness on a water-bath. The residue, after drying at 105°, weighs not more than 25 mg.

Fine particles. Disperse 5 g in 250 ml of water by shaking vigorously for 2 minutes in a stoppered flask, pour immediately into a glass cylinder, 5 cm in diameter, and transfer 20 ml to a glass dish using a pipette. Evaporate to dryness and dry to constant weight at 105°. Allow the remainder of the suspension to stand for 4 hours at 20° and withdraw a second 20-ml portion using a pipette with its tip exactly 5 cm below the surface and without disturbing the sediment. Transfer the second portion to a glass dish, evaporate to dryness and dry to constant weight at 105°. The weight of the residue from the second portion is not less than 70 per cent of the weight of the residue from the first portion.

**Tests**

**Arsenic** (2.3.10). Disperse 5 g in 50 ml of water and add 10 ml of stannated hydrochloric acid; the resulting solution complies with the limit test for arsenic (2 ppm).

**Heavy metals.** Heat 6.0 g for 15 minutes under a reflux condenser on a water-bath with a mixture of 70 ml of water and 10 ml of hydrochloric acid and filter. To 40 ml of the filtrate add 0.5 ml of nitric acid and evaporate to a low bulk. Add 20 ml of water, 2 g of ammonium chloride and 2 g of ammonium thiocyanate and extract with two quantities, each of 10 ml, of a mixture of equal volumes of amyl alcohol and ether. To the aqueous layer add 2 g of citric acid and sufficient water to produce 60 ml. 12 ml of the solution complies with the limit test for heavy metals, Method D (2.3.13) (20 ppm).

**Chlorides** (2.3.12). Boil 1.0 g with 40 ml of water and 20 ml of 2 M nitric acid under a reflux condenser for 5 minutes, cool and filter. 30 ml of the filtrate complies with the limit test for chlorides (330 ppm).

**Soluble matter.** Boil 2 g with 100 ml of 0.2 M hydrochloric acid under a reflux condenser for 5 minutes, cool, filter and evaporate 50 ml to dryness. The residue, after ignition at about 600° for 30 minutes, weighs not more than 10 mg.

**Loss on drying** (2.4.19). Not more than 1.5 per cent, determined on 1 g by drying in an oven at 105°.

**Loss on ignition** (2.4.20). Not more than 15.0 per cent, determined on 1 g by igniting at 600°.

**Storage.** Store protected from moisture.

**Ketamine Hydrochloride**

\[\text{C}_{13}\text{H}_{16}\text{ClNO},\text{HCl}\]

Mol. Wt. 274.2

Ketamine Hydrochloride is (RS)-2-(2-chlorophenyl)-2-methylaminocyclohexanone hydrochloride.

Ketamine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of C_{13}H_{16}ClNO.HCl.

**Description.** A white, crystalline powder.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ketamine hydrochloride RS or with the reference spectrum of ketamine hydrochloride.

B. A 10 per cent w/v solution gives the reactions of chlorides (2.3.1).

C. Melting range (2.4.21). 258° to 261°.

**Tests**

**Appearance of solution.** A 20.0 per cent w/v solution is clear (2.4.1), and colourless (2.4.1).

**pH** (2.4.24). 3.5 to 4.1, determined in a 10.0 per cent w/v solution.

**Foreign amines.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel.

**Mobile phase.** A mixture of 80 volumes of toluene, 19.5 volumes of 2-propanol and 0.5 volume of strong ammonia solution.

**Test solution.** Dissolve 0.6 g of the substance under examination in 10 of methanol.

**Reference solution.** A 0.03 per cent w/v solution of the substance under examination in methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with freshly prepared acid potassium iodosbismuthate solution. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.
**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel.

**Mobile phase.** A mixture of 80 volumes of toluene, 19.5 volumes of 2-propanol and 0.5 volume of strong ammonia solution.

**Test solution.** Dissolve 0.5 g of the substance under examination in 10 ml of methanol.

**Reference solution (a).** A 0.02 per cent w/v solution of the substance under examination in methanol.

**Reference solution (b).** A 0.0125 per cent w/v solution of the substance under examination in methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and expose to iodine vapour for about an hour. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Heavy metals.** (2.3.13). 1.0 g complies with limit test for heavy metals, Method A (20 ppm).

**Sulphated ash.** (2.3.18). Not more than 0.1 per cent.

**Assay.** To an accurately measured volume containing about 0.5 g of ketamine add sufficient water to produce 200.0 ml and mix. To 20.0 ml of the resulting solution add 3 ml of 0.1 M sodium hydroxide and extract with three quantities, each of 15 ml, of chloroform. Combine the chloroform extracts and shake successively with three quantities, each of 30 ml, of 0.05 M sulphuric acid. Dilute the combined acid extracts to 200.0 ml with 0.05 M sulphuric acid (saturated with chloroform), and measure the absorbance of the resulting solution at the maximum at about 269 nm (2.4.7). Calculate the content of $C_{13}H_{16}ClNO$ from the absorbance obtained by repeating the determination using a standard solution of ketamine hydrochloride RS containing the equivalent of 250 µg per ml of ketamine base in the same medium in place of the substance under examination.

**Storage.** Store protected from light at a temperature not exceeding 30°.

**Labelling.** The label states the strength in terms of the equivalent amount of ketamine in a suitable dose-volume.

---

**Ketoconazole**

C$_{26}$H$_{28}$Cl$_2$N$_4$O$_4$  Mol. Wt. 531.4

Ketoconazole is cis-1-acetyl-4-[[2RS,4RS]-2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-y1]methoxylphenylpiperazine.

Ketoconazole contains not less than 98.0 per cent and not more than 102.0 per cent of C$_{26}$H$_{28}$Cl$_2$N$_4$O$_4$, calculated on the dried basis.

**Description.** A white to off-white, crystalline powder.
Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ketoconazole RS or with the reference spectrum of ketoconazole.

B. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

C. Melting range (2.4.21). 148° to 152°.

Tests

Specific optical rotation (2.4.22). –1.0° to +1.0°, determined in a 4.0 per cent w/v solution in methanol.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 42 volumes of hexane, 40 volumes of ethyl acetate, 15 volumes of methanol, 2 volumes of water and 1 volume of glacial acetic acid.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of chloroform.

Reference solution (a). A 1 per cent w/v solution of ketoconazole RS in chloroform.

Reference solution (b). A 0.02 per cent w/v solution of ketoconazole RS in chloroform.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and expose to iodine vapour. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 80° at a pressure not exceeding 2.7 kPa for 4 hours.

Assay. Weigh accurately about 0.2 g, dissolve in 40 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02657 g of C_{26}H_{28}Cl_{2}N_{4}O_{4}.

Ketoconazole Tablets

Ketoconazole Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ketoconazole, C_{26}H_{28}Cl_{2}N_{4}O_{4}.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 42 volumes of hexane, 40 volumes of ethyl acetate, 15 volumes of methanol, 2 volumes of water and 1 volume of glacial acetic acid.

Test solution. Shake a quantity of the powdered tablets containing 200 mg of Ketoconazole with 10 ml of chloroform, dilute to 20 ml with chloroform and filter.

Reference solution (a). A 1 per cent w/v solution of ketoconazole RS in chloroform.

Reference solution (b). A 0.02 per cent w/v solution of ketoconazole RS in chloroform.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and expose to iodine vapour. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 200 mg of Ketoconazole, shake with 50.0 ml of a mixture of equal volumes of methanol and dichloromethane and centrifuge. To 5.0 ml of this solution add 5.0 ml of a 0.5 per cent w/v solution of terconazole RS (internal standard) in the methanol-dichloromethane solvent mixture and dilute to 50.0 ml with the same solvent mixture.

Reference solution. Dissolve 20 mg of ketoconazole RS in 20 ml of the methanol-dichloromethane solvent mixture, add 5.0 ml of the internal standard solution and dilute to 50.0 ml with the same solvent mixture.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilyl silica gel,
- mobile phase: a mixture of 7 volumes of 0.2 per cent w/v of di-isopropylamine in methanol and 3 volumes of a 0.5 per cent w/v solution of ammonium acetate,
- flow rate: 3 ml per minute,
- spectrophotometer set at 225 nm,
- a 20 µl loop injector.

Inject the test solution and the reference solution. The relative retention times are about 0.6 for ketoconazole and 1.0 for terconazole.

Calculate the content of C_{26}H_{28}Cl_{2}N_{4}O_{4} in the tablets.
Ketoprofen

\[
\text{C}_{16}\text{H}_{14}\text{O}_3 \quad \text{Mol. Wt. 254.3}
\]

Ketoprofen is (RS)-2-(3-benzoylphenyl)propionic acid.

Ketoprofen contains not less than 98.5 per cent and not more than 100.5 per cent of C₁₆H₁₄O₃, calculated on the dried basis.

**Description.** A white or almost white, crystalline powder; odourless or almost odourless.

**Identification**

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with ketoprofen RS or with the reference spectrum of ketoprofen.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in methanol (75 per cent) shows an absorption maximum only at about 258 nm; absorbance at about 258 nm, about 0.66.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 50 volumes of acetone, 49 volumes of dichloromethane and 1 volume of glacial acetic acid.

**Test solution.** Dissolve 0.1 g of the substance under examination in 100 ml of acetone.

**Reference solution (a).** A 0.1 per cent w/v solution of ketoprofen RS in acetone.

**Reference solution (b).** A mixture of equal volumes of 1.0 per cent w/v solution of indomethacin RS and reference solution (a) in acetone.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than three such spots are more intense than the spot in the chromatogram obtained with reference solution (b).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa.

**Assay.** Weigh accurately about 0.5 g, dissolve in 25 ml of ethanol (95 per cent) previously neutralised to phenolphthalein solution, add 25 ml of water. Titrate with 0.1 M sodium hydroxide using phenolphthalein solution as indicator.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.02543 g of C₁₆H₁₄O₃.

**Storage.** Store protected from moisture.

**Ketoprofen Capsules**

Ketoprofen Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of ketoprofen, C₁₆H₁₄O₃.

**Identification**

A. Shake a quantity of the contents of the capsules containing 0.5 g of Ketoprofen with 50 ml of chloroform for 5 minutes, filter, evaporate to dryness using a rotary evaporator, induce crystallisation by prolonged scratching of the inside wall of the container with a glass rod and separate the crystals by centrifugation or filtration.

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 70 volumes of toluene, 30 volumes of di-isopropyl ether and 1 volume of formic acid.

**Test solution.** A 10 per cent w/v solution of the substance under examination in acetone.

**Reference solution (a).** A 0.05 per cent w/v solution of the substance under examination in acetone.

**Reference solution (b).** A 0.02 per cent w/v solution of the substance under examination in acetone.

Apply rapidly to the plate 5 µl of each solution. Develop the chromatogram within 10 minutes of applying the first spot. After development, dry the plate and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than three such spots are more intense than the spot in the chromatogram obtained with reference solution (b).
B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum only at about 258 nm.

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase.* A mixture of 70 volumes of *toluene*, 30 volumes of *di-isopropyl ether* and 1 volume of *formic acid*.

*Test solution.* Shake a quantity of the contents of the capsules containing 0.3 g of Ketoprofen with 10 ml of *chloroform*, filter, wash with 5 ml of *chloroform*, evaporate the combined filtrate and washings to dryness and dissolve the residue in 3 ml of *acetone*.

*Reference solution (a).* Dilute 1 volume of *test solution* to 100 volumes with *acetone*.

*Reference solution (b).* Dilute 1 volume of test solution to 200 volumes with *acetone*.

Apply rapidly to the plate 5 µl of each solution. Develop the chromatogram within 10 minutes of applying the first spot.

After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than three such spots are more intense than the spot in the chromatogram obtained with reference solution (b).

**Other tests.** Comply with the tests stated under Capsules.

**Assay.** Weigh accurately a quantity of the mixed contents of 20 capsules containing about 25 mg of Ketoprofen, shake for 10 minutes with 150 ml of *methanol (75 per cent)*, mix and dilute to 250.0 ml with *methanol (75 per cent)*. Allow to stand, dilute 10.0 ml of the supernatant liquid to 100.0 ml with *methanol (75 per cent)* and measure the absorbance of the resulting solution at the maximum at about 258 nm (2.4.7).

Calculate the content of \( C_{16}H_{14}O_3 \) taking 662 as the specific absorbance at 258 nm.

**Storage.** Store protected from moisture.
L

Labetalol Hydrochloride
Labetalol Tablets
Lactic Acid
Lactose
Lamivudine
Lamivudine Oral Solution
Lamivudine Tablets
Lamivudine And Tenofovir Tablets
Lamivudine And Zidovudine Tablets
Lamivudine, Nevirapine And Stavudine Tablets
Lamivudine, Nevirapine and Stavudine Dispersible Tablets
Lamotrigine
Lamotrigine Dispersible Tablets
Lanatoside C
Lanatoside C Tablets
Levamisole Hydrochloride
Levamisole Tablets
Levocetirzine Dihydrochloride
Levocetirzine Tablets
Levodopa
Levodopa And Carbidopa Tablets
Levodopa Capsules
Levodopa Tablets
Levofloxacin Hemihydrate
Levofloxacin Infusion
Levofloxacin Tablets
Levonorgestrel
Levonorgestrel And Ethinyloestradiol Tablets
Lignocaine Hydrochloride
Lignocaine And Adrenaline Injection
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<td>Lignocaine Injection</td>
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<td>Lynoestrenol</td>
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Labetalol Hydrochloride

\[
\text{C}_{19}H_{24}N_2O_3\cdot\text{HCl}
\]
Mol. Wt. 364.9

Labetalol Hydrochloride is \textit{all-rac}-2-hydroxy-5-[1-hydroxy-2-(1-methyl-3-phenylpropylamino)ethyl]benzamide hydrochloride.

Labetalol Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of \(\text{C}_{19}H_{24}N_2O_3\cdot\text{HCl}\), calculated on the dried basis.

\textbf{Description.} A white or almost white powder or granules.

\textbf{Identification.}

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with \textit{labetalol hydrochloride RS} or with the reference spectrum of labetalol hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution in \(0.1\ M\ \text{hydrochloric acid}\) shows an absorption maximum only at 302 nm; absorbance at 302 nm, about 0.43.

C. Dissolve 10 mg in 5 ml of \textit{water} and add 1 ml of \textit{ferric chloride test solution}; a purple colour is produced.

D. A 1 per cent w/v solution gives the reactions of chlorides (2.3.1).

\textbf{Tests.}

\textbf{Appearance of solution.} A 1.0 per cent w/v solution is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

\textbf{pH (2.4.24).} 4.0 to 5.0, determined in a 1.0 per cent w/v solution.

\textbf{Diasterioisomer ratio.} Determine by gas chromatography (2.4.13).

\textit{Test solution.} Dissolve 2 mg of the substance under examination in 1 ml of a 1.2 per cent w/v solution of \textit{1-butaneboronic acid in anhydrous pyridine} and allow to stand for 20 minutes.

\textbf{Chromatographic system}

\begin{itemize}
  \item a stainless steel column 1.5 m \times 4 mm, packed with acid-washed, silanised diatomaceous support (125 to 150 \(\mu\)m) coated with 3 per cent w/w of \textit{polymethyl phenyl siloxane},
  \item temperature: column. 270°C, \textit{inlet port and detector at 300°C},
  \item flow rate. 20 ml per minute of the carrier gas.
\end{itemize}

Two peaks due to the two diasterioisomers appear in the chromatogram. Adjust the sensitivity of the detector so that in the chromatogram obtained, the height of the taller of the diasterioisomer peaks is about 80 per cent of the full-scale deflection. The area of each peak is not less than 45 per cent and not more than 55 per cent of the sum of the areas of the two peaks. The test is not valid unless the height of the trough separating the two diasterioisomers peaks is less than 5 per cent of the full-scale deflection.

\textbf{Related substances.} Determine by liquid chromatography (2.4.14).

\textit{Test solution.} Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 10 ml with the mobile phase.

\textit{Reference solution.} Dilute 0.5 ml of the test solution to 100 ml with the mobile phase.

\textbf{Chromatographic system}

\begin{itemize}
  \item a stainless steel column 15 cm \times 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 \(\mu\)m),
  \item column. temperature
  \item mobile phase: a degassed mixture of 150 ml of \textit{tetrahydrofuran}, 300 ml of \textit{methanol}, 550 ml of \textit{water}, 0.82 g of \textit{tetrabutylammonium hydrogen sulphate}, 1 g of \textit{sodium octyl sulphate} and 10 ml of a 10 per cent w/v solution of \textit{sulphuric acid},
  \item flow rate. 1 ml per minute,
  \item spectrophotometer set at 229 nm,
  \item a 20 \(\mu\)l loop injector.
\end{itemize}

Equilibrate the column with the mobile phase for about 30 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with the reference solution is at least 50 per cent of the full scale of the recorder. The retention time of the principal peak is 10 minutes to 15 minutes. If necessary, adjust the water content of the mobile phase ensuring that the 2:1 ratio of methanol to tetrahydrofuran is maintained.

Inject each solution. Continue the chromatography for three times the retention time of the principal peak in the chromatogram obtained with the test solution; the area of any peak other than the principal peak is not greater than 0.6 times that of the principal peak in the chromatogram obtained with the reference solution (0.3 per cent); the sum of the areas of any such peaks is not greater than the area of the principal peak.
peak in the chromatogram obtained with the reference solution (0.5 per cent). Ignore any peak due to the solvent and any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution.

**Heavy metals** (2.3.13). Dissolve 2.0 g in a mixture of 20 ml of water and 5 ml of dilute sodium hydroxide solution. The solution complies with the limit test for heavy metals, Method C (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° at a pressure not exceeding 0.7 kPa.

**Assay.** Weigh accurately about 0.2 g, dissolve in 10 ml of anhydrous formic acid and 40 ml of acetic anhydride. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.03649 g of C₁₉H₂₄N₂O₃.HCl.

**NOTE** - Mix thoroughly throughout the titration and stop the titration immediately after the end-point is reached.

**Storage.** Store protected from moisture.

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**Labetalol Tablets**

Labetalol Hydrochloride Tablets

Labetalol Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of labetalol hydrochloride, C₁₉H₂₄N₂O₃.HCl.

**Identification**

A. To a quantity of the powdered tablets containing 50 mg of Labetalol Hydrochloride add 50 ml of 0.1 M hydrochloric acid and heat on a water-bath for 30 minutes. Cool, filter, add 10 ml of ammonia buffer pH 10.0 and extract with three quantities, each of 15 ml, of dichloromethane. Shake the combined extracts with 5 g of anhydrous sodium sulphate, filter and evaporate the filtrate to dryness.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with labetalol RS or with the reference spectrum of labetalol.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.004 per cent w/v solution of the residue obtained in test A in 0.1 M sodium hydroxide shows an absorption maximum only at about 333 nm.

C. Disperse a quantity of the powdered tablets containing 10 mg of Labetalol Hydrochloride in a mixture of 2 ml of water and 2 ml of ferric chloride test solution; a purple colour is produced.

**Tests**

**Diasterioisomer ratio.** Determine by gas chromatography (2.4.13).

**Test solution.** Shake a quantity of the powdered tablets containing 0.5 g of Labetalol Hydrochloride with 10 ml of methanol, filter and evaporate the filtrate to dryness using a rotary evaporator.

**Chromatographic system**

- a stainless steel column 1.5 m × 4 mm, packed with acid-washed, silanised diatomaceous support (125 to 150 mm) coated with 3 per cent w/w of polymethyl phenyl siloxane,
- temperature: column, 270°, inlet port and detector at 300°,
- flow rate, 20 ml per minute of the carrier gas.

Two peaks due to the two diasterioisomers appear in the chromatogram. Adjust the sensitivity of the detector so that in the chromatogram obtained, the height of the taller of the diasterioisomer peaks is about 80 per cent of the full-scale deflection. The area of each peak is not less than 45 per cent and not more than 55 per cent of the sum of the areas of the two peaks. The test is not valid unless the height of the trough separating the two diasterioisomers peaks is less than 5 per cent of the full-scale deflection.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 75 volumes of dichloromethane, 25 volumes of methanol and 5 volumes of strong ammonia solution.

**Test solution.** Shake a quantity of the powdered tablets containing 0.5 g of Labetalol Hydrochloride with 10 ml of methanol, filter and use the filtrate.

**Reference solution (a).** Dilute 1 volume of the test solution to 100 volumes with methanol.

**Reference solution (b).** Dilute reference solution (a) with an equal volume of methanol.

Apply to the plate 20 µl of each solution. After development, dry the plate in a current of warm air; heat at 105° for 30 minutes, cool and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is
more intense than the spot in the chromatogram obtained with reference solution (b).

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.5 g of Labetalol Hydrochloride, shake with 200 ml of 0.05 M sulphuric acid for 30 minutes and dilute to 250.0 ml with 0.05 M sulphuric acid, mix and filter. Dilute 10.0 ml of the filtrate to 250.0 ml with the same solvent and measure the absorbance of the resulting solution at the maximum at about 302 nm (2.4.7).

Calculate the content of C_{19}H_{24}N_{2}O_{3}, HCl taking 86 as the specific absorbance at 302 nm.

**Storage.** Store protected from moisture.

---

**Lactic Acid**

\[ \text{C}_3\text{H}_6\text{O}_3 \quad \text{Mol. Wt. 90.1} \]

Lactic Acid consists of a mixture of 2-hydroxypropionic acid, its condensation products, such as lactoyl-lactic acid and other polyactic acids, and water. The equilibrium between lactic acid and polyactic acids depends on concentration and temperature.

It is usually in the form of the racemate [(RS)-lactic acid], but in some cases the (S)-isomer may predominate.

Lactic Acid contains the equivalent of not less than 88.0 per cent and not more than 92.0 per cent w/w of C_{3}H_{6}O_{3}.

**Description.** A colourless or slightly yellow, viscous liquid; almost odourless; hygroscopic.

**Identification**

A. Warm 1 g with 0.1 g of potassium permanganate; acetaldehyde is evolved.

B. Gives reaction A of lactates (2.3.1).

C. A 10 per cent w/v solution is strongly acidic.

**Tests**

**Appearance of solution.** The substance under examination is not more intensely coloured than reference solution YS6 (2.4.1).

**Arsenic** (2.3.10). Mix 10.0 g with 50 ml of water and 10 ml of stannated hydrochloric acid. The resulting solution complies with the limit test for arsenic (1 ppm).

**Heavy metals** (2.3.13). Dissolve 5.0 g in 42 ml of 1 M sodium hydroxide and dilute to 50 ml with distilled water. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (10 ppm).

**Citric, oxalic and phosphoric acids.** To 5 ml of the solution prepared in the test for Heavy metals add 6 M ammonia until slightly alkaline. Add 1 ml of calcium chloride solution and heat on a water-bath for 5 minutes. Both before and after heating, any opalescence in the solution is not more intense than that in a mixture of 5 ml of the test solution and 1 ml of water.

**Ether-insoluble substances.** Dissolve 1.0 g in 25 ml of ether; the solution is not more opalescent than the solvent used for the test.

**Volatile fatty acids.** Cautiously heat 5 g in a glass-stoppered flask at 50° for 10 minutes; no unpleasant odour resembling that of the lower fatty acids is recognisable immediately after opening the flask.

**Methanol and methyl esters.** Place 2.0 g in a ground-glass-stoppered round-bottom flask and add 10 ml of water. Cool in ice, cautiously add 30 ml of a 30 per cent w/v solution of potassium hydroxide and cool in ice for a further 10 to 15 minutes. Steam distil the mixture into a 10-ml graduated cylinder containing 1 ml of ethanol, collecting a volume of at least 9.5 ml and dilute to 10.0 ml with water. To 1.0 ml of the distillate add 5 ml of potassium permanganate and phosphoric acid solution and mix. After 15 minutes add 2 ml of oxalic acid and sulphuric acid solution, stir with a glass rod until the solution is colourless and then add 5 ml of decolourised magenta solution. After 2 hours any colour in the solution is not more intense than that of 1 ml of a reference solution containing 100 µg of methanol and 0.1 ml of ethanol treated in the same manner beginning at the words “add 5 ml of potassium permanganate and phosphoric acid solution...”.

**Reducing sugars.** Dilute 1 g with 10 ml of water, neutralise with sodium hydroxide solution, add 5 ml of potassium cupric tartrate solution, and boil; no red or greenish precipitate is produced.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Assay.** Weigh accurately about 1.0 g in a ground-glass-stoppered flask and add 10 ml of water. Add 20.0 ml of 1 M sodium hydroxide, stopper the flask and allow to stand for 30 minutes. Titrate the excess of alkali with 1 M hydrochloric acid, using dilute phenolphthalein solution as indicator until the pink colour is discharged.

1 ml of 1 M sodium hydroxide is equivalent to 0.09008 g of C_{3}H_{6}O_{3}.

**Storage.** Store protected from light.
Lactose

Lactose Monohydrate; Milk Sugar

\[
C_{12}H_{22}O_{11} \cdot H_2O \quad \text{Mol. Wt. 360.3}
\]

Lactose is \(\alpha\)-\(\beta\)-\(\beta\)-galactopyranosyl-(1 \(\rightarrow\) 4)-\(\alpha\)-\(\alpha\)-glucopyranoside monohydrate.

Description. A white or almost white, crystalline powder; odourless.

Identification

Test A may be omitted if Tests B and C are carried out. Tests B and C may be omitted if Test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with lactose RS or with the reference spectrum of lactose.

B. To 5 ml of a saturated solution add 5 ml of 1 M sodium hydroxide and gently warm the mixture; the liquid becomes yellow and then brownish-red. Cool to room temperature and add 0.2 ml of potassium cupri-tartrate solution; a red precipitate is formed.

C. Heat 5 ml of a 5 per cent w/v solution with 5 ml of 10 M ammonia in a water-bath at 80° for 10 minutes; a red colour develops.

Tests

Appearance of solution. Dissolve 1.0 g in water by heating to 50°, dilute to 10 ml with water and allow to cool. The solution is clear (2.4.1), and not more intensely coloured than reference solution BYS7 (2.4.1).

Acidity or alkalinity. Dissolve 6 g in 25 ml of carbon dioxide-free water by boiling, cool and add 0.3 ml of phenolphthalein solution. The solution is colourless and not more than 0.4 ml of 0.1 M sodium hydroxide is required to change the colour of the solution to pink.

Specific optical rotation (2.4.22). +54.4° to +55.9°, determined in a solution obtained by dissolving 10.0 g in 80 ml of water by heating to 50°, allowing to cool, adding 0.2 ml of 6 M ammonia, allowing to stand for 30 minutes and diluting to 100.0 ml with water.

Light absorption (2.4.7). Dissolve 1.0 g in boiling water and dilute to 10 ml with the same solvent (solution A). Absorbance of solution A measured at the maximum at about 400 nm, not more than 0.04.

Dilute 1 ml of solution A to 10 ml with water.

When examined in the range 210 nm to 300 nm, absorbance is not more than 0.25 in the range 210 nm to 220 nm and not more than 0.07 in the range 270 nm to 300 nm.

Arsenic (2.3.10). Dissolve 10.0 g in 50 ml of water and add 10 ml of stannated hydrochloric acid AsT; the resulting solution complies with the limit test for arsenic (1 ppm).

Heavy metals (2.3.13). Dissolve 4.0 g in 20 ml of warm water, 1.0 ml of 0.1 M hydrochloric acid and sufficient water to produce 25 ml. The solution complies with the limit test for heavy metals, Method A (5 ppm).

Microbial contamination (2.2.9). Total microbial count not more than 100 per g; 1 g is free from Escherichia coli and salmonellae.

Sulphated ash. Not more than 0.1 per cent, determined in the following manner. To 1.0 g add 1 ml of sulphuric acid, evaporate to dryness on a water-bath and ignite to constant weight.

Water (2.3.43). 4.5 to 5.5 per cent, determined on 0.5 g.

Storage. Store protected from moisture.

Lamivudine

\[
\text{C}_8\text{H}_{11}\text{N}_3\text{O}_3\text{S} \quad \text{Mol. Wt. 229.3}
\]

Lamivudine is (2R,5S)-4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone.

Lamivudine contains not less than 98.0 per cent and not more than 102.0 per cent of C\(_8\)H\(_{11}\)N\(_3\)O\(_3\)S, calculated on the anhydrous basis.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with lamivudine RS or with the reference spectrum of lamivudine.
B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to lamivudine in the chromatogram obtained with the reference solution.

C. Melting range (2.4.21). 172° to 178°.

**Tests**

**Specific optical rotation** (2.4.22). –136° to –144°, determined in a 1.0 per cent w/v solution in methanol.

**Related substances.** Determine by liquid chromatography (2.4.14), as described in the Assay but using the following solutions.

**Test solution.** Dissolve 0.1 g of the substance under examination in 100 ml mobile phase.

**Reference solution.** A solution containing 0.001 per cent w/v each of lamivudine RS and salicylic acid in the mobile phase.

Inject the reference solution and record the chromatogram for at least twice the retention time of lamivudine. The order of elution is lamivudine and then, salicylic acid. The test is not valid unless the resolution between the peaks due to lamivudine and salicylic acid is not less than 10, the column efficiency determined from lamivudine peak is not less than 5000 theoretical plates and the tailing factor is not more than 1.5.

Separately inject the test solution and the reference solution and measure the peak responses for each impurity. In the chromatogram obtained with the test solution, the area of any peak other than the principal peak is not greater than 10% of the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Water** (2.3.43). Not more than 0.5 per cent determined on 2.0 g.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 25 mg of the substance under examination in 100 ml of mobile phase.

**Reference solution.** A 0.025 per cent w/v solution of lamivudine RS in mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- temperature column 35°,
- mobile phase: a degassed mixture of 5 volumes of methanol and 95 volumes of a buffer prepared by dissolving 1.9 g of ammonium acetate in 1000 ml of water and adjusting the pH to 3.8 ± 0.2 with glacial acetic acid,
- flow rate. 1 ml per minute,
- spectrophotometer set at 270 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the column efficiency determined from the lamivudine peak is not less than 5000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternatively the test solution and the reference solution. Calculate the content of C₈H₁₁N₃O₃S.

**Storage.** Store protected from light and moisture.

**Lamivudine Tablets**

Lamivudine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lamivudine, C₈H₁₁N₃O₃S. The tablets may be coated.

**Identification**

A. When examined in the range 200 nm to 400 nm (2.4.7), a 0.001 per cent w/v solution in a mixture of 50 volumes of water and 50 volumes of acetonitrile, shows an absorption maximum at about 270 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to lamivudine in the chromatogram obtained with the reference solution.

**Tests**

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Shake a quantity of the powdered tablets with a suitable quantity of water, and disperse with the aid of ultrasound. Add a quantity of acetonitrile containing half of the final volume to get a final concentration of 0.6 per cent w/v of lamivudine. Mix with the aid of ultrasound for 10 minutes and make up the volume with water. Filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate.

**Reference solution.** A 0.024 per cent w/v solution of lamivudine RS in a mixture of 80 volumes of water and 20 volumes of acetonitrile.

Chromatographic system as described under Assay.
Inject the reference solution. The test is not valid unless the column efficiency determined from the lamivudine peak is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Separately inject the test solution. Calculate the amount of related substances by the area normalisation method. The content of any individual impurity is not more than 1.0 per cent and the sum of all impurities is not more than 2.0 per cent.

**Dissolution (2.5.2).**

Apparatus. No 2

Medium. 900 ml of 0.01 M hydrochloric acid

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Measure the absorbance of the filtrate, suitably diluted if necessary, at the maximum at about 270 nm (2.4.7). Calculate the content of C₈H₁₁N₃O₃S, in the medium from the absorbance obtained from a solution of known concentration of lamivudine RS in 0.01 M hydrochloric acid.

D. Not less than 70 per cent of the stated amount of C₈H₁₁N₃O₃S.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh and powder 20 tablets. Shake a quantity of the powder containing about 100 mg of Lamivudine with a mixture of 50 volumes of water and 50 volumes of acetonitrile to have a final concentration of 0.024 per cent of Lamivudine. Disperse with the aid of ultrasound and filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate.

*Reference solution.* A 0.024 per cent w/v solution of lamivudine RS in the same solvent mixture.

**Chromatographic system**

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a degassed mixture of 5 volumes of methanol and 95 volumes of a buffer prepared by dissolving 1.0 g of ammonium acetate and 1.0 ml of glacial acetic acid in sufficient water to make 1000 ml and adjusting the pH to 3.8 with glacial acetic acid, flow rate. 1.5 ml per minute,
- spectrophotometer set at 270 nm,
- a 10 µl loop injector.

Separately inject the test solution and the reference solution and measure the peak responses of the major peak. Calculate the content of C₈H₁₁N₃O₃S in the tablets.

**Storage.** Store protected from moisture.

**Lamivudine and Tenofovir Tablets**

Lamivudine and Tenofovir Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of lamivudine, C₈H₁₁N₃O₃S and tenofovir disoproxil fumarate, C₁₀H₁₉N₂O₁₈P₂C₄H₆O₄.

**Identification**

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

**Tests**

**Dissolution (2.5.2).**

Apparatus. No 1

Medium. 900 ml of 0.1 M hydrochloric acid.

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate. Determine by liquid chromatography (2.4.14)

*Test solution.* The filtrate obtained as given above. Dilute the filtrate if necessary, with the dissolution medium.

*Reference solution.* 5.0 ml of each of a 0.16 per cent w/v solution of lamivudine RS and of tenofovir disoproxil fumarate RS in methanol, diluted to 25 ml with the dissolution medium.

Use the chromatographic system given in the Assay.

Inject the reference solution. The resolution between the peaks due to lamivudine and tenofovir disoproxil is not less than 2.0.

Inject the test solution and the reference solution. Calculate the contents of C₈H₁₁N₃O₃S and C₁₀H₁₉N₂O₁₈P₂C₄H₆O₄.

D. Not less than 75 per cent of the stated amounts of C₈H₁₁N₃O₃S and C₁₀H₁₉N₂O₁₈P₂C₄H₆O₄.

**Related substances.** Determine by liquid chromatography (2.4.14).

*NOTE — Prepare the solutions immediately before use.*

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 100 mg of Lamivudine, disperse in 100 ml of mobile phase A and filter.
Reference solution (a). A solution containing 0.1 per cent w/v of lamivudine RS and tenofovir disoproxil fumarate RS equivalent to 0.1 per cent w/v of tenofovir disoproxil in mobile phase A.

Reference solution (b). Dilute 1 ml of the solution to 100 ml with mobile phase A.

Reference solution (c). A 0.02 per cent w/v solution of fumaric acid in mobile phase A.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed octadecylsilane bonded to porous silica (5 µm),
- column temperature 35º,
- mobile phase: A. a mixture of 95 volumes of a buffer solution prepared by dissolving 1.9 g of ammonium acetate in 1000 ml of distilled water and adjusting the pH to 3.8 with glacial acetic acid and 5 volumes of methanol,
- B. methanol,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 277 nm,
- a 10 µl loop injector.

<table>
<thead>
<tr>
<th>Time (in min.)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
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<td>50</td>
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<tr>
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<td>0</td>
</tr>
<tr>
<td>67</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Inject reference solution (a). The test is not valid unless the column efficiency determined from lamivudine and tenofovir disoproxil peaks is not less than 750 and 1500 theoretical plates respectively and the tailing factor is not more than 2.0.

Inject the test solution, reference solutions (b) and (c). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 3.5 times the area of any peak in the chromatogram obtained with the reference solution (b) (3.5 per cent) and the sum of areas of all the secondary peaks is not more than 6 times the area of any peak in the chromatogram obtained with the reference solution (b) (6.0 per cent). Ignore the peak corresponding to the peak in the chromatogram obtained with reference solution (c).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 20 mg of Lamivudine, dissolve in 100 ml of the mobile phase and filter.

Reference solution. A 0.1 per cent w/v solution each of lamivudine RS and tenofovir disoproxil fumarate RS in the mobile phase. Dilute 20.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 35º,
- mobile phase: a mixture of 50 volumes of a buffer solution prepared by dissolving 7.8 g of sodium dihydrogen orthophosphate dihydrate in 1000 ml of distilled water, adding 1 ml of triethylamine and adjusting the pH to 2.3 with orthophosphoric acid, and 50 volumes of acetonitrile,
- flow rate. 1 ml per minute,
- spectrophotometer set at 260 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the column efficiency determined from the peaks due to lamivudine and tenofovir disoproxil is not less than 750 and 1500 theoretical plates respectively, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent for each component.

Inject the test solution and the reference solution.

Calculate the content of \(C_8H_{11}N_3O_3S\) and \(C_{19}H_{30}N_5O_{10}P\) in the tablets.

Storage. Store protected from moisture, at temperature not exceeding 30º.

Lamivudine and Zidovudine Tablets
Lamivudine and Zidovudine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of lamivudine, \(C_8H_{11}N_3O_3S\), and zidovudine, \(C_{10}H_{13}N_5O_4\). The tablets may be coated.

Identification
In the Assay, the two principal peaks in the chromatogram obtained with the test solution correspond to the peaks due to lamivudine and zidovudine in the chromatogram obtained with the reference solution.
Tests

Dissolution (2.5.2).

Apparatus. No 2
Medium. 900 ml of 0.1 M hydrochloric acid
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate.

Determine by liquid chromatography (2.4.14).

Test solution. Dilute 5.0 ml of the filtrate to 50.0 ml with the mobile phase.

Reference solution. Weigh accurately about 30 mg of lamivudine RS and 60 mg of zidovudine RS, transfer into a 100-ml volumetric flask, dissolve and dilute to volume with 0.1 M hydrochloric acid. Further dilute 5.0 ml of this solution to 20.0 ml with the mobile phase.

Chromatographic system
– a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
– mobile phase: a degassed mixture of 60 volumes of a buffer solution containing 0.1 M ammonium acetate in 0.1 per cent acetic acid and 40 volumes of methanol,
– flow rate. 1 ml per minute,
– spectrophotometer set at 270 nm,
– a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections for each of the peaks corresponding to lamivudine and zidovudine is not more than 2.0 per cent.

Separately inject the test solution and the reference solution and measure the responses of the major peaks due to lamivudine and zidovudine. Calculate the contents of C8H11N3O3S and C10H13N5O4.

D. Not less than 75.0 per cent of the stated amounts of C8H11N3O3S and C10H13N5O4.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of the powdered tablets containing the average weight of one tablet, transfer to a 100-ml volumetric flask and add about 50 ml of methanol. Disperse with the aid of ultrasound for about 10 minutes with occasional shaking to obtain a uniform dispersion, cool to room temperature, dilute to volume with methanol and mix. Filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate.

Reference solution (a). A 0.015 per cent w/v solution of salicylic acid in methanol.

Reference solution (b). A solution containing 0.01 per cent w/v each of thymine and β-thymidine in methanol.

Reference solution (c). Transfer 5 ml of reference solution (a) and 15 ml of reference solution (b) to a 100-ml volumetric flask and dilute to volume with methanol.

Reference solution (d). Weigh accurately about 300 mg of zidovudine RS and about 150 mg of lamivudine RS, transfer to a 100-ml volumetric flask, add 30 ml of reference solution (b) and 20 ml of reference solution (a) and disperse with the aid of ultrasound for about 15 minutes to dissolve. Dilute to volume with methanol and filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate.

Chromatographic system
– a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
– temperature. column 35°,
– mobile phase: degassed mixtures of methanol and a buffer prepared by dissolving 1.945 g of ammonium acetate in 900 ml of water, adjusting the pH of the solution to 3.8 with glacial acetic acid and diluting to 1000.0 ml with water in the proportions and at the intervals given below
– flow rate. 1 ml per minute,
– linear gradient programme using the conditions given below,
– spectrophotometer set at 270 nm,
– a 10 µl loop injector.

<table>
<thead>
<tr>
<th>Time (in min.)</th>
<th>Buffer (pH 3.8) (%)</th>
<th>Methanol (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>25</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>40</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>45</td>
<td>90</td>
<td>10</td>
</tr>
</tbody>
</table>

Inject reference solution (d). The test is not valid unless the tailing factor of the lamivudine and zidovudine peaks is not more than 2.0 and the relative standard deviation for replicate injections for each of the peaks corresponding to lamivudine and zidovudine is not more than 2 per cent.

Separately inject the test solution and record the chromatograms for at least two times the retention time of the zidovudine peak. Separately inject reference solutions (a), (b) and (c).

Calculate the amounts of the related substances by the area normalisation method. The content of thymine is not greater.
than 2.0 per cent, of b-thymidine is not greater than 1.0 per cent, of salicylic acid is not greater than 0.2 per cent and of any unknown impurity not greater than 0.5 per cent. The sum of all the impurities is not greater than 3.0 per cent.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Transfer an accurately weighed quantity of the powder equivalent to the average weight of one tablet into a 200-ml volumetric flask. Add 100 ml of the mobile phase and disperse with the aid of ultrasound for about 15 minutes with occasional shaking to obtain a uniform dispersion. Cool to room temperature and dilute to volume with the mobile phase. Filter the solution through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate. Dilute 5.0 ml of the filtrate to 25.0 ml with the mobile phase. Further dilute 5.0 ml to 50.0 ml with the mobile phase and mix.

Reference solution. Weigh accurately about 30 mg of lamivudine RS and 60 mg of zidovudine RS, transfer to a 100-ml volumetric flask, dissolve in the mobile phase and dilute to volume with the mobile phase. Further dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecysilane chemically bonded to porous silica (5 µm),
- mobile phase: a degassed mixture of 60 volumes of a buffer solution containing 0.1 M ammonium acetate in 0.1 per cent acetic acid and 40 volumes of methanol,
- flow rate. 1 ml per minute,
- spectrophotometer set at 270 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections for each of the peaks corresponding to lamivudine and zidovudine is not more than 2.0 per cent.

Separately inject the test solution and the reference solution and measure the peak responses of the major peaks.

Calculate the contents of C₈H₁₁N₃O₃S and C₁₀H₁₂N₂O₄ in the tablets.

Storage. Store protected from moisture.

Lamivudine, Nevirapine and Stavudine Dispersible Tablets

Lamivudine, Nevirapine And Stavudine Dispersible Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of lamivudine, C₈H₁₁N₃O₃S, nevirapine, C₁₅H₁₄N₄O and stavudine, C₁₀H₁₂N₂O₄.

Identification
In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

Tests
Dissolution (2.5.2).

Apparatus. No 1
Medium. 900 ml of 0.1 M hydrochloric acid.
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of water and 50 volumes of methanol.

Test solution. The filtrate obtained as given above, diluted suitably if necessary, with the dissolution medium.

Reference solution. A solution containing 0.02 per cent w/v of stavudine RS, 0.09 per cent w/v lamivudine RS and 0.15 per cent w/v of nevirapine RS dissolved in minimum quantity of methanol and makeup with solvent mixture. Dilute 5 ml of the solution to 100 ml with the dissolution medium.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecysilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of a buffer solution prepared by dissolving 1 g of octane sulphonic acid sodium salts and 0.68 g of potassium dihydrogen phosphate in 1000 ml of water, adding 1 ml of triethylamine and adjusting the pH to 2.5 with orthophosphoric acid, and 35 volumes of methanol,
- flow rate. 1 ml per minute,
- spectrophotometer set at 266 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5 for each component, the column efficiency in not less than 2000 theoretical plates for lamivudine and the relative standard deviation for replicate injections is not more than 2.0 per cent for each component.

Inject the test solution and the reference solution.

Calculate the content of C₈H₁₁N₃O₃S, C₁₅H₁₄N₄O and C₁₀H₁₂N₂O₄.

D. Not less than 80 per cent of the stated amounts of C₈H₁₁N₃O₃S, C₁₅H₁₄N₄O and C₁₀H₁₂N₂O₄.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.
Solvent mixture. 70 volumes of 0.2 per cent v/v orthophosphoric acid and 30 volumes of methanol.

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 75 mg of Lamivudine, dissolve in 100 ml of the solvent mixture and filter.

Reference solution (a). Weigh accurately about 75 mg of lamivudine RS, 130 mg of nevirapine RS and 20 mg of stavudine RS, dissolve in 20 ml of methanol and dilute to 100 ml with the solvent mixture. Dilute 5 ml of the solution to 50 ml with the solvent mixture.

Reference solution (b). Dissolve 0.38 mg of Thymine and 0.15 mg of Carboxylic acid in 50 ml of the solvent mixture, add 10 ml of reference solution (a), dilute to 100 ml with the solvent mixture and filter.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. methanol,
  - B. a buffer solution prepared by dissolving 1.925 g of ammonium acetate in 1000 ml of water and adjusting the pH to 3.0 with trifluoroacetic acid,
- flow rate. 1.2 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 266 nm,
- a 10 µl loop injector.

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Methanol (per cent)</th>
<th>Buffer solution pH 3 (per cent)</th>
</tr>
</thead>
<tbody>
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<td>0</td>
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<td>95</td>
</tr>
<tr>
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<td>5</td>
<td>95</td>
</tr>
<tr>
<td>60</td>
<td>5</td>
<td>95</td>
</tr>
</tbody>
</table>

Inject reference solution (b). The relative retention times for carboxylic acid is 0.36, for thymine is 0.42, for lamivudine, 0.74, for stavudine, 1.0 and for nevirapine, 2.19. The test is not valid unless the tailing factor is not more than 1.5 and the resolution between carboxylic acid and thymine is not less than 2.0.

Inject the test solution. Any individual impurity each for lamivudine and nevirapine is not more than 1 per cent and for stavudine, is not more than 3 per cent and the sum of all impurities is not more than 5.0 per cent.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of water and 50 volumes of methanol.

Test solution. Weigh accurately a quantity of the powdered tablets containing 50 mg of Lamivudine, dissolve in 100.0 ml of solvent mixture. Dilute 10.0 ml of the solution to 50.0 ml with the solvent mixture and filter.

Reference solution. A solution containing 0.10 per cent w/v of lamivudine RS, 0.175 per cent w/v nevirapine RS and 0.025 per cent w/v of stavudine RS dissolved in minimum quantity of methanol and makeup with solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of a buffer solution prepared by dissolving 1 g of octane sulphonic acid and 0.68 g of potassium dihydrogen phosphate in 1000 ml of water, adding 1 ml of triethylamine and adjusting the pH to 2.5 with orthophosphoric acid, and 35 volumes of methanol,
- flow rate. 1 ml per minute.
- spectrophotometer set at 266 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5 for each component, the column efficiency in not less than 2000 theoretical plates for lamivudine and the relative standard deviation for replicate injections is not more than 2.0 per cent for each component.

Inject the test solution and the reference solution.

Calculate the content of C8H11N3O3S, C15H14N4O and C10H12N2O4 in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 25°.

Lamivudine, Nevirapine and Stavudine Tablets

Lamivudine, Nevirapine and Stavudine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of lamivudine, C8H11N3O3S, nevirapine, C15H14N4O, and stavudine, C10H12N2O4. The tablets may be coated.

Identification

In the Assay, the three principal peaks in the chromatogram obtained with the test solution have retention times similar to those of the peaks due to lamivudine, nevirapine and stavudine in the chromatogram obtained with the reference solution.
Tests

Dissolution (2.5.2).

Apparatus. No 2

Medium. 900 ml of 0.01 M hydrochloric acid

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate as the test solution.

Reference solution. Weigh accurately about 150 mg of lamivudine RS, 200 mg of nevirapine RS and 30 mg of stavudine RS (if claim of stavudine per tablet is 30 mg) or 40 mg of stavudine RS (if claim of stavudine per tablet is 40 mg) and transfer to a 100-ml volumetric flask. Add about 20 ml of methanol, disperse with the aid of ultrasound to dissolve and dilute to volume with a solvent mixture of equal volumes of methanol and water. Dilute 5.0 ml of this solution to 50.0 ml with 0.01 M hydrochloric acid.

Chromatographic system

– a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
– mobile phase: a degassed mixture of 35 volumes of methanol and 65 volumes of a buffer prepared by dissolving 0.68 g of potassium dihydrogen phosphate and 1.0 g of sodium octanesulphonate in 1000.0 ml of water to which 1 ml of triethylamine is added and the pH of which is adjusted to 2.5 with phosphoric acid,
– flow rate. 1 ml per minute,
– spectrophotometer set at 266 nm,
– a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the column efficiency determined from the lamivudine peak is not less than 2000 theoretical plates, the tailing factor for the individual lamivudine, nevirapine and stavudine peaks is not more than 1.5 and the relative standard deviation for replicate injections of all the analyte peaks is not more than 1.0 per cent.

Separately inject the test solution and measure the peak responses of the major peaks due to lamivudine, nevirapine and stavudine. Calculate the contents of C₈H₁₁N₃O₃S, C₁₅H₁₄N₄O and C₁₀H₁₂N₂O₄ respectively.

D. Not less than 70 per cent of the stated amounts of C₈H₁₁N₃O₃S, C₁₅H₁₄N₄O and C₁₀H₁₂N₂O₄.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of the powdered tablets (a minimum of 2 tablets should be powdered) containing 100 mg of nevirapine, transfer to a 200-ml volumetric flask and add about 150 ml of water. Disperse with the aid of ultrasound for 10 minutes with occasional shaking to obtain a uniform dispersion, cool to room temperature, dilute to volume with water and mix. Filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate.

Reference solution (a). A solution containing 0.15 per cent w/v of stavudine RS and 0.015 per cent w/v of thymine RS in water.

Reference solution (b). Weigh accurately about 75 mg of lamivudine RS and about 100 mg of nevirapine RS, transfer to a 200-ml volumetric flask, add 20 ml of methanol and mix with the aid of ultrasound to dissolve. Add 10 ml of the test solution to this solution and make up to volume with water and filter.

Chromatographic system

– a stainless steel column 25 cm x 4.6 mm, packed with octylsilane chemically bonded to porous silica (5 µm),
– mobile phase: degassed gradient mixtures of 0.1 M ammonium acetate and acetonitrile.
– flow rate. 1 ml per minute,
– a linear gradient programme using the conditions given below,
– spectrophotometer set at 270 nm,
– a 20 µl loop injector.

<table>
<thead>
<tr>
<th>Time (in min.)</th>
<th>0.1 M Ammonium acetate (per cent v/v)</th>
<th>Acetonitrile (per cent v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>95</td>
<td>05</td>
</tr>
<tr>
<td>05</td>
<td>95</td>
<td>05</td>
</tr>
<tr>
<td>25</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>30</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>31</td>
<td>95</td>
<td>05</td>
</tr>
<tr>
<td>35</td>
<td>95</td>
<td>05</td>
</tr>
</tbody>
</table>

Separately inject reference solutions (a) and (b). The test is not valid unless the column efficiency determined for the thymine, stavudine, lamivudine and nevirapine peaks is not less than 3000 theoretical plates and the tailing factor for the same peaks is not more than 2.0.

Separately inject the test solution and measure the peak responses of the major peaks due to lamivudine, nevirapine and stavudine. Calculate the amounts of related substances by the area normalisation method. The content of thymine is not greater than 3.0 per cent and that of any other impurity is not greater than 1.0 per cent. The sum of all the impurities is not greater than 3.5 per cent.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Transfer an accurately weighed quantity of the powder containing about
150 mg of lamivudine to a 100-ml volumetric flask, add 20 ml of methanol and about 50 ml of a mixture of equal volumes of water and methanol and disperse with the aid of ultrasound for 5 minutes. Dilute suitably with the same solvent mixture to obtain a solution containing 0.15 mg of lamivudine per ml. Filter this solution through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate.

Reference solution. A solution containing 0.015 per cent w/v of lamivudine RS and 0.02 per cent w/v of nevirapine RS and a concentration of stavudine RS similar to that of the concentration of stavudine in the test solution.

The chromatographic procedure may be carried out using the conditions described under Dissolution.

Inject the reference solution. The test is not valid unless the column efficiency determined from the lamivudine peak is not less than 2000 theoretical plates, the tailing factor for the individual peaks due to lamivudine, nevirapine and stavudine is not more than 1.5 and the relative standard deviation for replicate injections of all the analyte peaks is not more than 1.0 per cent.

Inject test solution and the reference solution.

Calculate the contents of C₈H₁₁N₃O₃S, C₁₅H₁₄N₄O and C₁₀H₁₂N₂O₄ in the tablets.

Storage. Store protected from moisture.

Lamivudine Oral Solution

Lamivudine Oral Solution is a solution of Lamivudine in a suitable flavoured vehicle.

Lamivudine Oral Solution contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lamivudine, C₈H₁₁N₃O₃S.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 40 volumes of 1-butanol, 30 volumes of heptane, 30 volumes of acetone and 10 volumes of strong ammonia solution.

Test solution. Dilute the preparation under examination with methanol to obtain a solution containing 2 mg of lamivudine per ml.

Reference solution. A 0.2 per cent w/v solution of lamivudine RS in a mixture of 75 volumes methanol and 25 volumes of water.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm.

The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to lamivudine in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 5.0 to 7.0.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Transfer an accurately measured volume of the preparation under examination containing 50 mg of lamivudine to a 50-ml volumetric flask, add about 30 ml of a solution prepared by mixing 10 volumes of acetonitrile and 90 volumes of 0.1 M potassium dihydrogen phosphate, the pH of which is adjusted to 3.0 with dilute phosphoric acid (solution A), mix with the aid of ultrasound for 5 minutes, dilute to volume with solution A and filter. Dilute 5 ml of the filtrate to 50 ml with solution A.

Reference solution. Weigh accurately about 25 mg of lamivudine RS and transfer to a 50-ml volumetric flask, dissolve and dilute to volume with solution A. Further dilute 5 ml of this solution to 250 ml with solution A.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: degassed gradient mixtures of acetonitrile and 0.05 M potassium dihydrogen phosphate, adjusting the pH of the solution to 3.0 with dilute phosphoric acid, flow rate. 0.8 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 270 nm,
- a 20 µl loop injector.

<table>
<thead>
<tr>
<th>Time (in min.)</th>
<th>Phosphate buffer (pH 3.0) (per cent v/v)</th>
<th>Acetonitrile (per cent v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>98</td>
<td>02</td>
</tr>
<tr>
<td>10</td>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td>25</td>
<td>30</td>
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<tr>
<td>40</td>
<td>98</td>
<td>02</td>
</tr>
<tr>
<td>50</td>
<td>98</td>
<td>02</td>
</tr>
</tbody>
</table>

Inject the reference solution. The test is not valid unless the column efficiency determined from the lamivudine peak is not less than 15,000 theoretical plates and the tailing factor is not more than 1.5.
Inject solution A and the test solution. Examine the chromatogram obtained with solution A for any extraneous peaks and ignore the corresponding peaks observed in the chromatogram obtained with the test solution. Ignore any peaks due to preservatives also.

Any secondary peak observed in the chromatogram obtained with the test solution should not be more than 1.0 per cent and the sum of the areas of all the secondary peaks should not be more than 2.0 per cent when calculated by percentage area normalisation.

**Other tests.** Comply with the tests stated under Oral Liquids.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh accurately a quantity of the preparation under examination containing about 100 mg of lamivudine in a 100-ml volumetric flask, add about 50 ml of water, mix with the aid of ultrasound for 10 minutes, dilute to volume with water, mix and filter. Dilute 5.0 ml of the filtrate to 50.0 ml with water.

**Reference solution.** Weigh accurately about 100 mg of lamivudine RS in a 100-ml volumetric flask, dissolve and dilute to volume with water. Dilute 5.0 ml of this solution to 50.0 ml with water.

**Chromatographic system**
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: 80 volumes of water and 20 volumes of methanol,
- flow rate. 1 ml per minute,
- spectrophotometer set at 270 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the column efficiency determined from the lamivudine peak is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject separately the test solution and the reference solution and measure the responses for the major peak.

Determine the weight per ml (2.4.29) of the oral solution and calculate the content of C₈H₁₁N₃O₃S weight in volume.

**Lamotrigine**

![Lamotrigine structure](image)

C₉H₇Cl₂N₅ Mol. Wt. 256.1

Lamotrigine is 6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine.

Lamotrigine contains not less than 98.5 per cent and not more than 101.5 per cent of C₉H₇Cl₂N₅, calculated on the dried basis.

**Description.** A white to off-white powder.

**Identification**

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with lamotrigine RS or with the reference spectrum of lamotrigine.

B. When examined in the range 210 to 360 nm (2.4.7), a 0.0025 per cent w/v solution in methanol exhibits a maximum at about 309 nm.

C. In the Assay, the chromatogram obtained with test solution corresponds to the chromatogram obtained with reference solution.

**Tests**

**Related substances.** Determine by liquid chromatography (2.4.14).

**Solvent mixture.** 70 volumes of phosphate buffer pH 7.5 and 30 volumes of acetonitrile.

**Test solution.** Dissolve 100 mg of the substance under examination in 100 ml of solvent mixture.

**Reference solution (a).** A 0.05 per cent w/v solution of lamotrigine RS in solvent mixture.

**Reference solution (b).** Dilute 1 ml of reference solution (a) to 100 ml with solvent mixture.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. 0.174 per cent w/v solution of dipotassium hydrogen phosphate adjusted to pH 7.5 with orthophosphoric acid and filter, B. acetonitrile,
- a linear gradient programme using the conditions given below,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 250 nm,
- a 20 µl loop injector.

<table>
<thead>
<tr>
<th>Time (in mins.)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>20</td>
</tr>
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<td>15</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>30</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>35</td>
<td>80</td>
<td>20</td>
</tr>
</tbody>
</table>
Inject reference solution (b). Test is not valid unless the column efficiency is not less than 5000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than 2 times the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105º, under vacuum, for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in 100 ml of mobile phase. Dilute 10.0 ml of the solution to 50.0 ml with mobile phase.

Reference solution. A 0.02 per cent w/v solution of lamotrigine RS in mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of acetonitrile and 70 volumes of 0.408 per cent potassium dihydrogen orthophosphate adjusted the pH to 7.0 with dilute potassium hydroxide solution.
- flow rate 1.2 ml per minute,
- spectrophotometer set at 308 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

D. Not less than 75 per cent of the stated amount of C9H7Cl2N5.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Accurately transfer 10 intact tablets in a suitable volumetric flask, add mobile phase, sonicate to dissolve and dilute with mobile phase to obtain a concentration of 0.02 per cent w/v of Lamotrigine.

Reference solution. A 0.02 per cent w/v solution of lamotrigine RS in mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of a 0.408 per cent w/v solution of potassium dihydrogen phosphate previously adjusted to pH 7.0 with potassium hydroxide, 30 volumes of acetonitrile
- flow rate 1.2 ml per minute,
- spectrophotometer set at 308 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Lamotrigine Dispersible Tablets

Lamotrigine Dispersible Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lamotrigine, C9H7Cl2N5.
Inject the reference solution. The test is not valid unless the column efficiency is not less than 2500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent. Inject the test solution and the reference solution.

Storage. Store protected from light and moisture.

Labelling. The label states that the tablets should be dispersed in water immediately before use.

Lanatoside C

C₉₀H₇₆O₂₁ Mol. Wt. 985.1
Lanatoside C is 3-{[(O-β-D-glucopyranosyl-(1→4))-O-3-acetyl-2,6-dideoxy-β-D-ribo-hexopyranosyl-(1→4))-O-2,6-dideoxy-β-D-ribo-hexopyranosyl-(1→4))-O-2,6-dideoxy-β-D-ribo-hexopyranosyl]-oxy}-12,14-dihydroxy-3β,5β,2β-card-20(22)-enolide.

Lanatoside C contains not less than 97.0 per cent and not more than 103.0 per cent of C₉₀H₇₆O₂₁, calculated on the dried basis.

Description. A white or slightly yellowish, crystalline powder or fine crystals; hygroscopic.

Identification

Test A may be omitted if test B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with lanatoside C RS.
B. In the test for Related substances, the principal band in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).
C. Suspend about 0.5 mg in 0.5 ml of ethanol (60 per cent) and add 0.1 ml of 3,5-dinitrobenzoic acid solution and 0.1 ml of 2 M sodium hydroxide; a violet colour develops.
D. Dissolve 5 mg in 5 ml of glacial acetic acid, add 0.1 ml of ferric chloride test solution, mix and add 2 ml of sulphuric acid slowly so as to form a lower layer; a brown ring is formed at the junction of the two liquids and the upper layer develops a green colour which becomes blue on standing.

Tests

Appearance of solution. A 2.0 per cent w/v solution in methanol is clear (2.4.1), and not more intensely coloured than reference solutions YS7 or BYS7 (2.4.1).

Specific optical rotation (2.4.22). +31.5° to +35.5°, determined in a 2.0 per cent w/v solution in methanol.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 60 volumes of toluene, 30 volumes of ethanol (95 per cent), 20 volumes of dichloromethane and 1 volume of water.

Test solution (a). Dissolve 0.2 g of the substance under examination in 10 ml of methanol.
Test solution (b). Dissolve 0.2 g of the substance under examination in 100 ml of methanol.

Reference solution (a). A 0.2 per cent w/v solution of lanatoside C RS in methanol.
Reference solution (b). A 0.03 per cent w/v solution of lanatoside C RS in methanol.
Reference solution (c). A 0.02 per cent w/v solution of lanatoside C RS in methanol.
Reference solution (d). A 0.01 per cent w/v solution of lanatoside C RS in methanol.

Apply to the plate 5 µl of each solution as bands. After development, dry the plate in a current of cool air for 5 minutes and carry out a second development in the same direction. After development, dry the plate in a current of cool air for 5 minutes, spray with ethanolic sulphuric acid (5 per cent), heat at 140° for 15 minutes and examine in daylight. In the chromatogram obtained with test solution (a) any secondary band is not more intense than the band in the chromatogram.
obtained with reference solution (b), not more than three such bands are more intense than the band in the chromatogram obtained with reference solution (d) and not more than one of these bands is more intense than the band in the chromatogram obtained with reference solution (c).

**Sulphated ash (2.3.18).** Not more than 0.1 per cent, determined on the residue from the test for Loss on drying.

**Loss on drying (2.4.19).** Not more than 7.5 per cent, determined on 0.5 g by drying over phosphorus pentoxide at 105° at a pressure of 1.5 to 2.5 kPa.

**Assay.** Before carrying out the Assay, allow both the substance under examination and the reference substance to stand in a desiccator containing a saturated solution of potassium thiocyanate for 24 hours. Weigh accurately about 50 mg, dissolve in sufficient ethanol (95 per cent) to produce 50.0 ml and dilute 5.0 ml to 100.0 ml with the same solvent. To 5.0 ml of this solution add 3 ml of alkaline sodium picrate solution and allow to stand protected from light in a water-bath at 19° to 21° for 40 minutes. Measure the absorbance of the resulting solution at the maximum at about 484 nm (2.4.7), using as the blank a mixture of 5.0 ml of ethanol (95 per cent) and 3 ml of alkaline sodium picrate solution prepared at the same time.

Calculate the content of C_{49}H_{76}O_{20} from the absorbance obtained by repeating the operation using lanatoside C RS in place of the substance under examination.

**Storage.** Store protected from light and moisture, in well-filled glass containers in a cold place.

### Lanatoside C Tablets

Lanatoside Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lanatoside C, C_{49}H_{76}O_{20}.

**Identification**

In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 80 volumes of hexane, 10 volumes of ether and 10 volumes of glacial acetic acid.

**Test solution.** Shake a quantity of the powdered tablets containing 2.5 mg of Lanatoside C with 10 ml of a mixture of 10 volumes of chloroform, 5 volumes of methanol and 1 volume of water, filter, evaporate the filtrate to dryness and dissolve the residue in 5 ml of methanol.

**References solution (a).** A 0.05 per cent w/v solution of lanatoside C RS in methanol.

**Reference solution (b).** Dilute 1 ml of the test solution to 10 ml with methanol.

Apply to the plate 2 μl of each solution. Allow the mobile phase to rise 13 cm. Dry the plate at 100° for 15 minutes, cool and develop once again with a mixture of 40 volumes of 1-propanol, 40 volumes of water, 10 volumes of ethyl acetate and 10 volumes of strong ammonia solution as the mobile phase. Allow the mobile phase to rise 13 cm. Dry the plate at 120° for 1 hour, cool and spray with a 5 per cent w/v solution of potassium dichromate in a 40 per cent w/w solution of sulphuric acid. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

**Uniformity of content.** Comply with the test stated under Tablets.

Powder one tablet, shake with 10.0 ml of ethanol (95 per cent) for 15 minutes and centrifuge, protecting the mixture from direct sunlight. To 5.0 ml of the clear supernatant liquid, add 3 ml of alkaline sodium picrate solution and allow to stand protected from light in a water-bath at 19° to 21° for 40 minutes. Measure the absorbance of the resulting solution at the maximum at about 484 nm (2.4.7), using as the blank a mixture of 5.0 ml of ethanol (95 per cent) and 3 ml of alkaline sodium picrate solution prepared at the same time. Calculate the content of C_{49}H_{76}O_{20} from the absorbance obtained by repeating the operation using lanatoside C RS in place of the substance under examination.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 5 mg of Lanatoside C and shake with 50 ml of ethanol (95 per cent) in a mechanical shaker for 15 minutes, protecting the mixture from direct sunlight. Dilute to 100.0 ml with ethanol (95 per cent), mix and filter, discarding the first 20 ml of the filtrate. To 5.0 ml of the filtrate add 3 ml of alkaline sodium picrate solution and allow to stand protected from light in a water-bath at 19° to 21° for 40 minutes. Measure the absorbance of the resulting solution at the maximum at about 484 nm (2.4.7), using as the blank a mixture of 5.0 ml of ethanol (95 per cent) and 3 ml of alkaline sodium picrate solution prepared at the same time. Calculate the content of C_{49}H_{76}O_{20} from the absorbance obtained by repeating the operation using lanatoside C RS in place of the substance under examination.

**Storage.** Store protected from light and moisture.
**Levamisole Hydrochloride**

![Chemical Structure]

C\(_{11}H_{12}N_{2}S\), HCl  
Mol. Wt. 240.8

Levamisole Hydrochloride is \((S)-2,3,5,6\)-tetrahydro-6-phenylimidazo[2,1-b]thiazole hydrochloride.

Levamisole Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of C\(_{11}H_{12}N_{2}S\), HCl, calculated on the dried basis.

**Description.** A white or almost white, crystalline powder.

**Identification**

Test A may be omitted if tests B, C, D and E are carried out. Tests B and D may be omitted if tests A, C and E are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with levamisole RS or with the reference spectrum of levamisole.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) examined in ultraviolet light at 254 nm corresponds to that in the chromatogram obtained with reference solution (b).

C. Complies with the test for Specific optical rotation.

D. Dissolve 0.5 g in 20 ml of water and add 6 ml of 1 M sodium hydroxide. Extract with 20 ml of dichloromethane, wash the lower layer with two quantities, each of 10 ml, of water, dry over anhydrous sodium sulphate, filter and evaporate the solvent at a temperature not exceeding 40° under reduced pressure. The residue melts at 58° to 61° (2.4.21).

E. Gives reaction A of chlorides (2.3.1).

**Tests**

**Appearance of solution.** A 5.0 per cent w/v solution in carbon dioxide-free water is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

**pH** (2.4.24). 3.0 to 4.5, determined in a 5.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). –121° to –128°, determined in a 5.0 per cent w/v solution.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel HF254.

Mobile phase. A mixture of 60 volumes of toluene, 40 volumes of acetone and 1 volume of strong ammonia solution.

**Test solution (a).** Dissolve 0.5 g of the substance under examination in 10 ml of methanol.

**Test solution (b).** Dissolve 0.5 g of the substance under examination in 100 ml of methanol.

**Reference solution (a).** Dissolve 0.25 g of the substance under examination in 100 ml of methanol.

**Reference solution (b).** A 0.5 per cent w/v solution of levamisole hydrochloride RS in methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate at 105° for 15 minutes and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). Expose the plate to iodine vapour in an airtight tank for 15 minutes. Any secondary spot in the chromatogram obtained with test solution (a), other than any spot with a very low R\(_f\) value, is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

**Assay.** Weigh accurately about 0.2 g, dissolve in 30 ml of ethanol (95 per cent), add 5 ml of 0.01 M hydrochloric acid. Titrate with 0.1 M sodium hydroxide determining the end-point potentiometrically (2.4.25). Record the volume added between the two inflections.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.02408 g of C\(_{11}H_{12}N_{2}S\), HCl.

**Storage.** Store protected from light and moisture.

**Levamisole Tablets**

Levamisole Hydrochloride Tablets

Levamisole Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of levamisole, C\(_{11}H_{12}N_{2}S\).

**Identification**

In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel HF254.
Mobile phase. A mixture of 60 volumes of toluene, 40 volumes of acetone and 1 volume of strong ammonia solution.

Test solution (a). Shake a quantity of the powdered tablets containing 100 mg of levamisole with 5 ml of methanol for 2 minutes and filter.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with methanol.

Reference solution (a). A 0.24 per cent w/v solution of levamisole hydrochloride RS in methanol.

Reference solution (b). Dilute 1 ml of reference solution (a) to 20 ml with methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate at 105° for 15 minutes and examine in ultraviolet light at 254 nm as well as after exposure to iodine vapour. Any secondary spot the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 300 mg of levamisole, add 30 ml of water and shake for 10 minutes. Filter, wash the filter with 20 ml of water and add the washings to the filtrate. To the combined filtrate add dilute ammonia solution to make it alkaline and extract with three quantities each of 25 ml, 15 ml and 15 ml, of chloroform, filter through cotton wool covered with a layer of anhydrous sodium sulphate. Combine the chloroform extracts and evaporate to dryness. Dissolve the residue in 50 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, using crystal violet solution as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02043 g of C11H12N2S.

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the equivalent amount of levamisole.

**Levocetirizine Hydrochloride**

\[
\text{Cl} \quad \text{N} \quad \text{N} \quad \text{O} \quad \text{H} \\
\text{C} \quad \text{H} \quad \text{C} \\
\text{C}_2\text{H}_2\text{N}_2\text{O}_3\text{Cl}_2\text{HCl} \quad \text{Mol. Wt. 461.8}
\]

Levocetirizine dihydrochloride is \((R)-2-[2-[4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid dihydrochloride.

Levocetirizine Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of C\(_{21}\)H\(_{25}\)N\(_2\)O\(_3\)Cl\(_2\)HCl calculated on the dried basis.

**Description.** A white or almost white powder.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with levocetirizine dihydrochloride RS or with the reference spectrum of levocetirizine dihydrochloride.

B. When examined in the range 200 nm and 350 nm (2.4.7), a 0.001 per cent w/v solution in methanol shows an absorption maximum at about 230 nm.

**Tests**

**Specific optical rotation** (2.4.22). +10° to +14°, determined in a 1 per cent w/v solution in carbon dioxide-free water at 365 nm.

**Heavy metals** (2.3.13). The residue obtained from Sulphated ash complies with limit test for heavy metals, Method D (20 ppm).

**Enantiomeric purity.** Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 2 ml of methanol and dilute to 50 ml with ethanol (95 per cent) and filter.

Reference solution. Dissolve 25 mg of the racemic cetirizine dihydrochloride RS in 2 ml of methanol and dilute to 50 ml with ethanol (95 per cent).

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with chiral Pak AD-H (5 µm),
- mobile phase: a mixture of 70 volumes of n-hexane, 15 volumes of isopropyl alcohol, 15 volumes of ethanol (95 per cent) and 0.02 volume of tri-fluoro acetic acid,
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- a 20 µl loop injector.

Inject the reference solution and the test solution. The relative retention time of levocetirizine isomer is about 2 with respect to levocetirizine peak.

Calculate the chiral purity of levocetirizine dihydrochloride by area normalization method, the enantiomeric purity is not less than 98 per cent.
Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in 100 ml of mobile phase and filter.

Reference solution (a). A 0.02 per cent w/v solution of levocetirizine dihydrochloride RS in mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecysilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 0.4 volume of 5.5 per cent v/v solution of sulphuric acid, 6.6 volumes of water and 93 volumes of acetonitrile,
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- a 20 µl loop injector.

Inject reference solution (b). Test is not valid unless the column efficiency is not less than 5000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1 g by drying in an oven at 100° at a pressure not exceeding 0.7 kPa.

Assay. Weigh accurately about 0.1 g, dissolve in 70 ml of a mixture of 30 ml of water and 70 ml of acetone. Titrate with 0.1 M sodium hydroxide up to the second point of inflection. Determine the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.01539 g of C₂₁H₂₅Cl₂N₂O₃,2HCl.

Storage. Store protected from moisture.

Levocetirizine Tablets

Levocetirizine Dihydrochloride Tablets

Levocetirizine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of levocetirizine hydrochloride, C₂₁H₂₇Cl₃N₂O₃,2HCl.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1
Medium. 900 ml of phosphate buffer pH 6.8
Speed and time. 50 rpm for 30 minutes.
Withdraw a suitable volume of the medium and filter.
Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate.

Reference solution (a). A 0.1 per cent w/v solution of lamotrigine RS in mobile phase. Dilute 1 ml of the solution to 50 ml with dissolution medium.

Chromatographic system as described under Assay.

Calculate the content of C₂₁H₂₇Cl₃N₂O₃,2HCl.

D. Not less than 75 per cent of the stated amount of C₂₁H₂₇Cl₃N₂O₃,2HCl.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablet containing 25 mg of levocetirizine dihydrochloride, dissolve in 100 ml of mobile phase.

Reference solution (a). A 0.1 per cent w/v solution of levocetirizine dihydrochloride RS in mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with mobile phase.

Chromatographic system as described under Assay.

Inject reference solution (a). Test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

Uniformity of content. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay.
Test solution. Transfer 1 tablet in a suitable volumetric flask. Add about 20 per cent v/v water to disperse with shaking. Further dilute with methanol to obtain a final concentration of 0.0025 per cent w/v.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablet containing 25 mg of Levocetirizine Dihydrochloride, disperse in 100.0 ml of mobile phase and filter. Dilute 5.0 ml of the solution to 25.0 ml with mobile phase.

Reference solution. A 0.005 per cent w/v solution of levocetirizine dihydrochloride RS in mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecysilane chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of 0.05 M potassium dihydrogen phosphate and 40 volumes of acetonitrile, adjust the pH to 6.0 with 10 per cent w/v of sodium hydroxide,
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0. The column efficiency in not less than 1500 theoretical plates. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution. Calculate the content of C21H27Cl3N2O3,2HCl.

Storage. Store protected from light and moisture.

Labelling. The label states the strength of Levocetirizine Dihydrochloride.

Levodopa

L-Dopa

\[
\text{HO} \quad \text{H} \quad \text{NH}_2 \quad \text{COOH}
\]

\[
\text{C}_9\text{H}_{11}\text{NO}_4 \quad \text{Mol. Wt. 197.2}
\]

Levodopa is 3-(3,4-dihydroxyphenyl)-L-alanine.

Levodopa contains not less than 98.5 per cent and not more than 101.0 per cent of \(\text{C}_9\text{H}_{11}\text{NO}_4\), calculated on the dried basis.

Description. A white or slightly cream, crystalline powder; odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with levodopa RS or with the reference spectrum of levodopa.

B. Dissolve about 2 mg in 2 ml of water and add 0.2 ml of ferric chloride solution; a green colour develops which changes to bluish violet on the addition of 0.1 g of hexamine.

C. Dissolve about 5 mg in a mixture of 5 ml of 1 M hydrochloric acid and 5 ml of water. Add 0.1 ml of sodium nitrite solution containing 10 per cent w/v of ammonium molybdate; a yellow colour develops which changes to red on the addition of 10 M sodium hydroxide.

D. Mix about 5 mg with 1 ml of water, 1 ml of pyridine and about 5 mg of 4-nitrobenzoyl chloride and allow to stand for 3 minutes; a violet colour develops which changes to pale yellow on boiling. Add, while shaking, 0.2 ml of sodium carbonate solution; the violet colour reappears.

Tests

Appearance of solution. A 4.0 per cent w/v solution in 1 M hydrochloric acid is not more intensely coloured than reference solution BY6 (2.4.1).

\(\text{pH (2.4.24). 4.5 to 7.0, determined in a suspension prepared by shaking 0.1 g with 10 ml of carbon dioxide-free water for 15 minutes.}\)

Optical rotation (2.4.22). \(-1.27^\circ\) to \(-1.34^\circ\), determined at 20° in a solution prepared in the following manner. Dissolve a quantity containing 0.2 g of the substance on the dried basis and 5 g of hexamine in 10 ml of 1 M hydrochloric acid, add sufficient 1 M hydrochloric acid to produce 25 ml and allow to stand for 3 hours, protected from light.

Light absorption. Dissolve 30 mg in sufficient 0.1 M hydrochloric acid to produce 100.0 ml and dilute 10.0 ml to 100.0 ml with 0.1 M hydrochloric acid.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum only at about 280 nm; absorbance at 280 nm, 0.41 to 0.44.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with microcrystalline cellulose.

Mobile phase. A mixture of 50 volumes of 1-butanol, 25 volumes of glacial acetic acid and 25 volumes of water.

Test solution. Dissolve 0.1 g of the substance under examination in 5 ml of anhydrous formic acid and add sufficient methanol to produce 10 ml; prepare immediately before use.
Reference solution (a). Dilute 1 volume of the test solution to 200 volumes with methanol.

Reference solution (b). A mixture of equal volumes of the test solution and a solution prepared by dissolving 30 mg of L-tyrosine in 1 ml of anhydrous formic acid and diluting to 100 ml with methanol.

Apply to the plate 10 µl each of the test solution and reference solution (a) and 20 µl of reference solution (b) as bands. Allow the mobile phase to rise 20 cm. Dry the plate in a current of air. After development, dry the plate in a current of warm air, spray with a freshly prepared mixture of equal volumes of a 10 per cent w/v solution of ferric chloride and a 5 per cent w/v solution of potassium ferricyanide and examine the plate immediately. Any secondary band in the chromatogram obtained with the test solution is not more intense than the band in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows a distinct band, at a higher Rf value than the principal band, which is more intense than the band in the chromatogram obtained with reference solution (a).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°C.

Assay. Weigh accurately about 0.3 g, dissolve, heating if necessary, in 5 ml of anhydrous formic acid and add 25 ml of anhydrous glacial acetic acid and 25 ml of dioxan. Titrate with 0.1 M perchloric acid, using 0.1 ml of crystal violet solution as indicator and titrating to a green end-point. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.01972 g of C9H11NO4.

Storage. Store protected from light and moisture.

**Levodopa and Carbidopa Tablets**

Co-careldopa Tablets

Levodopa and Carbidopa Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous carbidopa, C10H14N2O4, and not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of levodopa, C9H11NO4.

**Identification**

A. In the Assay, the chromatogram obtained with the test solution shows two principal peaks that correspond to those due to carbidopa and levodopa in the chromatogram obtained with the reference solution.

B. Shake a quantity of the powdered tablets containing 50 mg of Levodopa with 4 ml of ethanol (95 per cent) and 1 ml of 1 M sulphuric acid. Add 2 ml of cinnamaldehyde, allow to stand for 20 minutes, add 50 ml of 0.1 M hydrochloric acid, shake for 2 minutes and allow to stand. Filter the aqueous layer and to 5 ml of the filtrate add 0.1 ml of ferric chloride test solution. To half of the solution add an excess of dilute ammonia solution; a purple colour is produced. To the remainder add an excess of sodium hydroxide solution; a deep red colour is produced.

C. Shake a quantity of the powdered tablets containing 1 mg of anhydrous carbidopa with 5 ml of 0.05 M sulphuric acid and filter. Add 5 ml of dimethylaminobenzaldehyde reagent to the filtrate; a yellow colour is produced.

**Tests**

**Uniformity of Content.** For tablets containing 10 mg or less of Carbidopa.

Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

**Test solution.** Shake one tablet with 20 ml of 0.1 M phosphoric acid for 30 minutes, add sufficient water to produce 200.0 ml, mix and filter.

**Reference solution.** Weigh accurately about 10 mg of carbidopa RS, dissolve in 20 ml of 0.1 M phosphoric acid by gentle warming and add sufficient water to produce 200.0 ml. Carry out the chromatographic procedure described under Assay.

Calculate the content of C10H14N2O4 in the tablet.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh and powder 20 tablets. Shake a quantity of the powder containing about 250 mg of Levodopa and 25 mg of Carbidopa, with 50 ml of 0.1 M phosphoric acid for 30 minutes, add sufficient water to produce 500.0 ml, mix and filter.

**Reference solution.** Weigh accurately about 250 mg of levodopa RS and about 25 mg of carbidopa RS, dissolve in 2 ml of 0.1 M phosphoric acid by gentle warming and add sufficient water to produce 500.0 ml.

**Chromatographic system**

- a stainless steel column 30 cm x 3.9 mm, packed with octysilane chemically bonded to porous silica (5 µm),
- mobile phase: mix 950 ml of sodium dihydrogen phosphate solution (1.162 per cent w/v) with 1.3 ml of...
sodium 1-decanesulfonate solution (0.024 per cent w/v), adjust to a pH of about 2.8 with phosphoric acid and dilute with water to produce 1000 ml, – flow rate. 2 ml per minute, – spectrophotometer set at 280 nm, – a 20 µl loop injector.

Inject alternately the test solution and the reference solution. The retention times are about 4 minutes and 11 minutes for levodopa and carbidopa respectively.

Calculate the content of C16H16N2O4 and C9H11NO4 in the tablets.

Storage. Store protected from light and moisture.

Labelling. The label states the quantity of Carbidopa in terms of the equivalent amount of anhydrous carbidopa, and the quantity of Levodopa in each tablet.

Levodopa Capsules

L-Dopa Capsules

Levodopa Capsules contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of levodopa, C8H11NO4.

Identification

A. Dissolve as completely as possible a quantity of the contents of the capsules containing 0.5 g of Levodopa in 25 ml of 1 M hydrochloric acid and filter. Adjust the pH to 3 with 5 M ammonia, added dropwise with stirring, and allow to stand for several hours, protected from light. Filter, wash the precipitate and dry it at 105\(^\circ\).

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with levodopa RS or with the reference spectrum of levodopa.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with microcrystalline cellulose.

Mobile phase. A mixture of 50 volumes of 1-butanol, 25 volumes of glacial acetic acid and 25 volumes of water.

Test solution. A solution prepared immediately before use by shaking a quantity of the contents of the capsules containing 0.1 g of Levodopa with 10 ml of a mixture of equal volumes of anhydrous formic acid and methanol.

Reference solution (a). Dilute 1 volume of the test solution to 200 volumes with methanol.

Reference solution (b). A mixture of equal volumes of the test solution and a solution prepared by dissolving 30 mg of L-tyrosine in 1 ml of anhydrous formic acid and diluting to 100 ml with methanol.

Apply to the plate 10 µl of each of the test solution and reference solution (a) and 20 µl of reference solution (b) as bands. Allow the mobile phase to rise 20 cm. After development, dry the plate in a current of warm air, spray with a freshly prepared mixture of equal volumes of a 10 per cent w/v solution of ferric chloride and a 5 per cent w/v solution of potassium ferricyanide. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). –38.5\(^\circ\) to –41.5\(^\circ\), determined in the following manner. Weigh accurately a quantity of the contents of the capsules containing 1.25 g of Levodopa, shake with 25.0 ml of 0.5 M hydrochloric acid for 30 minutes, centrifuge and filter the supernatant liquid. To 10.0 ml of the filtrate add 10 ml of a 21.5 per cent w/v solution of aluminium sulphate, 20 ml of a 21.8 per cent w/v solution of sodium acetate and sufficient water to produce 50.0 ml and measure the optical rotation of the resulting solution at 20\(^\circ\). Separately dilute 5.0 ml of the filtrate to 200.0 ml with 0.1 M hydrochloric acid, mix well and dilute 10.0 ml to 200.0 ml with 0.1 M hydrochloric acid. Measure the absorbance of the resulting solution at the maximum at about 280 nm (2.4.7). Calculate the content of C9H11NO4 in the filtrate taking 142 as the specific absorbance at 280 nm and from the result so obtained calculate the specific optical rotation.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with microcrystalline cellulose.

Mobile phase. A mixture of 50 volumes of 1-butanol, 25 volumes of glacial acetic acid and 25 volumes of water.

Test solution. A solution prepared immediately before use by shaking a quantity of the contents of the capsules containing 0.1 g of Levodopa with 10 ml of a mixture of equal volumes of anhydrous formic acid and methanol.

Reference solution (a). Dilute 1 volume of the test solution to 200 volumes with methanol.

Reference solution (b). A mixture of equal volumes of the test solution and a solution prepared by dissolving 30 mg of L-tyrosine in 1 ml of anhydrous formic acid and diluting to 100 ml with methanol.

Apply to the plate 10 µl of each of the test solution and reference solution (a) and 20 µl of reference solution (b) as bands. Allow the mobile phase to rise 20 cm. After development, dry the plate in a current of warm air, spray with a freshly prepared mixture of equal volumes of a 10 per cent w/v solution of ferric chloride and a 5 per cent w/v solution of potassium ferricyanide and examine the plate immediately. Any secondary band in the chromatogram obtained with the test solution is not more intense than the band in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows a distinct band, at a higher Rf value than the principal band, which is more intense than the band in the chromatogram obtained with reference solution (a).
**Dissolution** (2.5.2).

**Apparatus.** No 2

**Medium.** 900 ml of 0.1 M hydrochloric acid

**Speed and time.** 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the resulting solution at the maximum at about 280 nm (2.4.7). Calculate the content of C₉H₁₁NO₄ taking 141 as the specific absorbance at 280 nm.

D. Not less than 75 per cent of the stated amount of C₉H₁₁NO₄.

**Other tests.** Comply with the tests stated under Capsules.

**Assay.** Weigh accurately a quantity of the mixed contents of 20 capsules containing about 0.4 g of Levodopa, dissolve as completely as possible in 10 ml of anhydrous formic acid, add 80 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, using oracet blue B solution as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.01972 g of C₉H₁₁NO₄.

**Storage.** Store protected from light and moisture.

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**Levodopa Tablets**

L-Dopa Tablets

Levodopa Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of levodopa, C₉H₁₁NO₄.

**Identification**

A. Dissolve as completely as possible a quantity of the tablet containing 0.5 g of Levodopa in 25 ml of 1 M hydrochloric acid and filter. Adjust the pH to 3 with 5 M ammonia, added dropwise with stirring, and allow to stand for several hours, protected from light. Filter, wash the precipitate and dry it at 105°.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with levodopa RS or with the reference spectrum of levodopa.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with microcrystalline cellulose.

**Mobile phase.** A mixture of 50 volumes of 1-butanol, 25 volumes of glacial acetic acid and 25 volumes of water.

**Test solution.** Shake a quantity of the powdered tablets containing 0.1 g of Levodopa with 10 ml of 1 M hydrochloric acid and filter.

**Reference solution.** A 1 per cent w/v solution of levodopa RS in 1 M hydrochloric acid.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of warm air and spray with a freshly prepared mixture of equal volumes of a 10 per cent w/v solution of ferric chloride and a 5 per cent w/v solution of potassium ferricyanide. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

**Tests**

**Specific optical rotation** (2.4.22). –38.5° to –41.5°, determined in the following manner. Weigh accurately a quantity of the powdered tablets containing 1.25 g of Levodopa, shake with 25.0 ml of 0.5 M hydrochloric acid for 30 minutes, centrifuge and filter the supernatant liquid. To 10.0 ml of the filtrate add 10 ml of a 21.5 per cent w/v solution of aluminium sulphate, 20 ml of a 21.8 per cent w/v solution of sodium acetate and sufficient water to produce 50.0 ml and measure the optical rotation at 20°. Separately dilute 5.0 ml of the filtrate to 200.0 ml with 0.1 M hydrochloric acid, mix well and dilute 10.0 ml to 200.0 ml with 0.1 M hydrochloric acid. Measure the absorbance of the resulting solution at the maximum at about 280 nm (2.4.7). Calculate the content of C₉H₁₁NO₄ in the filtrate taking 142 as the specific absorbance at 280 nm and from the result so obtained calculate the specific optical rotation.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with microcrystalline cellulose.

**Mobile phase.** A mixture of 50 volumes of 1-butanol, 25 volumes of glacial acetic acid and 25 volumes of water.

**Test solution.** Shake a quantity of the powdered tablets containing 0.1 g of Levodopa with 10 ml of a mixture of equal volumes of anhydrous formic acid and methanol.

**Reference solution (a).** Dilute 1 volume of the test solution to 200 volumes with methanol.

**Reference solution (b).** A mixture of equal volumes of the test solution and a solution prepared by dissolving 30 mg of l-tyrosine in 1 ml of anhydrous formic acid and diluting to 100 ml with methanol.

Apply to the plate 10 µl of each of the test solution and reference solution (a) and 20 µl of reference solution (b) as bands. Allow the mobile phase to rise 20 cm. Dry the plate in a current of warm air, spray with a freshly prepared mixture of equal volumes of a 10 per cent w/v solution of ferric chloride and a 5 per cent w/v solution of potassium ferricyanide and examine the plate immediately. Any secondary band in the chromatogram obtained with the test solution is not more intense than the band in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained...
with reference solution (b) shows a distinct band, at a higher Rf value than the principal band, which is more intense than the band in the chromatogram obtained with reference solution (a).

**Dissolution (2.5.2).**

Apparatus. No 2

Medium. 900 ml of 0.1 M hydrochloric acid

Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the resulting solution at the maximum at about 280 nm (2.4.7). Calculate the content of C9H11NO4 taking 141 as the specific absorbance at 280 nm.

D. Not less than 75 per cent of the stated amount of C9H11NO4.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 4.0 g of Levodopa, dissolve as completely as possible in 10 ml of anhydrous formic acid, add 80 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, using oracet blue B solution as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.01972 g of C9H11NO4.

**Storage.** Store protected from light and moisture.

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**Levofloxacin Hemihydrate**

![Image of Levofloxacin Hemihydrate]

C18H20FN3O4·½H2O  Mol. Wt. 370.4

Levofloxacin Hemihydrate is (-)-(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid hemihydrate.

Levofloxacin Hemihydrate contains not less than 98.5 per cent and not more than 101.0 per cent of C18H20FN3O4, calculated on the anhydrous basis.

**Description.** A yellowish white to yellow powder.

**Identification**

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with levofloxacin hemihydrate RS.

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**Tests**

**D-ofloxacin.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 10 mg of the substance under examination in 50 ml of mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 40°C,
- mobile phase: dissolve 1.25 g of copper(II)sulphate pentahydrate and 1.31 g of L-isolucine in 113 ml of methanol and make 1000 ml with water,
- flow rate. 1.8 ml per minute,
- spectrophotometer set at 330 nm,
- a 10 µl loop injector.

Inject the test solution. The relative retention times are about 1.0 for levofloxacin and 1.2 for D-ofloxacin. The area of peak corresponds to D-ofloxacin in the chromatogram obtained with the test solution is not more than 2.0 per cent of the area of the principal peak.

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent, determined on 1.0 g.

**Water** (2.3.43). Not more than 3.0 per cent, determined on 0.1 g.

**Assay.** Weigh accurately about 0.18 g, dissolve in 50 ml of anhydrous acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml 0.1 M perchloric acid is equivalent of 0.03614 g of C18H20FN3O4.

**Storage.** Store protected from light.
Other tests. Complies with the tests stated under Parenteral Preparation (Infusions).

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared solutions and carry out the test protected from light.

Test solution. Measure accurately a volume containing 50 mg of levofloxacin, dilute to 50.0 ml with 0.1 M hydrochloric acid. Dilute 5.0 ml of the solution to 25.0 ml with water.

Reference solution. A 0.1 per cent w/v solution of levofloxacin hemihydrate RS in 0.1 M hydrochloric acid. Dilute 5.0 ml of the solution to 25.0 ml with a water.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecysilane bonded to porous silica (5µm),
- mobile phase: a mixture of 85 volumes of buffer solution prepared by mixing 84 volumes of 0.05 M citric acid monohydrate and 1 volume of 1 M ammonium acetate and 15 volumes of acetonitrile,
- flow rate. 1 ml per minute,
- spectrophotometer set at 293 nm,
- 10 µl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and column efficiency is not less than 3000 theoretical plates and the relative standard deviation is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of C₁₈H₂₀FN₃O₄ in the infusion.

Storage. Store protected from light and moisture.

Levofloxacin Tablets

Levofloxacin Tablets contain Levofloxacin hemihydrate.

Levofloxacin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of levofloxacin, C₁₈H₂₀FN₃O₄.

Identification

In the Assay, the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2

Medium. 900 ml of 0.01 M hydrochloric acid.

Speed and time. 100 rpm for 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 326 nm (2.4.7). Calculate the content of C₁₈H₂₀FN₃O₄ in the medium from the absorbance obtained from a solution of known concentration of levofloxacin hemihydrate RS in the same medium.

D. Not less than 70.0 per cent of the stated amount of C₁₈H₂₀FN₃O₄.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared solutions and carry out the test protected from light.

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of powdered tablet containing 100 mg of Levofloxacin, disperse in 100 ml of 0.1 M hydrochloric acid and filter. Dilute 5 ml of the solution to 10 ml with a water.

Reference solutionn (a). A 0.1 per cent w/v solution of levofloxacin hemihydrate RS in 0.1 M hydrochloric acid. Dilute 5 ml of the solution to 10 ml with a water.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with water.

Chromatographic system as described under Assay.

Inject reference solution (a). Test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

Loss on drying (2.4.19). Not more than 6.0 per cent, determined on 1 g by drying at 105°, under vaccume, for 3 hours.

Other tests. Comply with the tests stated under the Tablets.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared solutions and carry out the test protected from light.

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of powdered tablet containing 100 mg of levofloxacin, disperse in 100.0 ml of 0.1 M hydrochloric acid and filter. Dilute 5.0 ml of the solution to 50.0 ml with water.

Reference solutionn. A 0.1 per cent w/v solution of levofloxacin hemihydrate RS in 0.1 M hydrochloric acid. Dilute 5 ml of the solution to 50 ml with a water.
Chromatographic system
– a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm) (such as Cosmosil C18 MS II),
– mobile phase: a mixture of 85 volume of buffer solution prepared by dissolving 84 volumes of 0.05 M citric acid monohydrate and 1 volume of 1 M ammonium acetate, filter and 15 volumes of acetonitrile,
– flow rate. 1 ml per minute,
– spectrophotometer set at 293 nm,
– 10 µl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, column efficiency is not less than 3000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.
Calculate the content of C18H20FN3O4 in the tablets.

Storage. Store protected from light and moisture.

Levonorgestrel

\[
\begin{align*}
\text{Levonorgestrel is } & 13\beta\text{-ethyl-17β-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one.} \\
\text{Levonorgestrel contains not less than 98.0 per cent and not more than 102.0 per cent of } C_{21}H_{28}O_2, \text{ calculated on the dried basis.}
\end{align*}
\]

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Identification
A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with levonorgestrel RS or with the reference spectrum of levonorgestrel.
B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in methanol shows an absorption maximum only at about 240 nm.
C. Melts at about 237° (2.4.21).

Tests
Specific optical rotation (2.4.22). –30.0° to –35.0°, determined in a 2.0 per cent w/v solution in chloroform.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 80 volumes of dichloromethane and 20 volumes of ethyl acetate.

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of chloroform.

Reference solution (a). A 0.01 per cent w/v solution of the substance under examination in chloroform.

Reference solution (b). A 0.004 per cent w/v solution of the substance under examination in chloroform.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with phosphomolybdic acid solution. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.3 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.1 g, dissolve in sufficient ethanol (95 per cent) to produce 100.0 ml, dilute stepwise with ethanol (95 per cent) to obtain a solution containing 0.001 per cent w/v of Levonorgestrel and measure the absorbance of the resulting solution at the maximum at about 241 nm (2.4.7).

Calculate the content of C21H28O2 from the absorbance obtained with a 0.001 per cent w/v solution of levonorgestrel RS in ethanol (95 per cent).

Storage. Store protected from light and moisture, at a temperature not exceeding 15°.

Levonorgestrel And Ethinyloestradiol Tablets

Levonorgestrel and Ethinyloestradiol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of levonorgestrel, C21H28O2, and ethinyloestradiol, C20H24O2. The tablets may be film-coated.

Identification
Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.
**Mobile phase.** A mixture of 96 volumes of dichloromethane and 4 volumes of ethanol (95 per cent).

**Test solution.** Powder 20 tablets finely, triturate with 20 ml of dichloromethane, allow the solids to sediment and use the clear supernatant liquid.

**Reference solution.** A solution containing 0.06 per cent w/v of levonorgestrel RS and 0.006 per cent w/v of ethinyloestradiol RS in water.

Apply to the plate 40 µl of each solution. After development, dry the plate in air, spray with ethanolic sulphuric acid (80 per cent v/v), heat at 110° for 10 minutes and examine in ultraviolet light at 365 nm. The principal spots in the chromatogram obtained with the test solution correspond to the spots for levonorgestrel (red fluorescence) and ethinyloestradiol (orange-yellow fluorescence) in the chromatogram obtained with the reference solution.

**Tests**

**Uniformity of content.** Comply with the test stated under Tablets.

Carry out the procedure described under Assay but using the following solutions.

**Test solution.** Add 2.0 ml of methanol (70 per cent) and 2.0 ml of a 0.00002 per cent w/v solution of diphenyl in methanol (70 per cent) (internal standard) to one tablet, shake for 20 minutes, centrifuge, filter the supernatant liquid through a membrane filter with a pore size of not more than 0.2 µm and use the filtrate.

**Reference solution (a).** Weigh accurately a quantity of norgestrel RS containing 100 times the stated amount of Levonorgestrel per tablet, dissolve in sufficient methanol (70 per cent) to produce 200.0 ml. Take 2.0 ml of this solution, add 2.0 ml of the internal standard solution and use the resulting solution.

**Reference solution (b).** Weigh accurately a quantity of ethinyloestradiol RS containing 100 times the stated amount of Ethinyloestradiol per tablet, dissolve in sufficient methanol (70 per cent) to produce 200.0 ml. Take 2.0 ml of the solution, add 2.0 ml of the internal standard solution and use the resulting solution.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh and finely powder 20 tablets. To a quantity of the powdered tablets containing about 3 mg of Levonorgestrel in a 200-ml volumetric flask, add the mobile phase and 10 ml of a 0.0025 per cent w/v solution of diphenyl (internal standard) in the mobile phase. Shake by mechanical means for 20 minutes and dilute to volume with the mobile phase. Centrifuge and use the clear supernatant liquid.

**Reference solution.** Transfer 15.0 ml of a solution of levonorgestrel RS in the mobile phase and 3.0 ml of a solution of ethinyloestradiol RS in the mobile phase, each solution having a concentration of about 0.1 mg per ml, into a 100-ml volumetric flask. Add 10 ml of a 0.0025 per cent w/v solution of diphenyl (internal standard) in the mobile phase. Dilute to volume with the mobile phase and mix. Each ml of this reference solution has a known concentration of about 15 µg and 3 µg of levonorgestrel and ethinyl oestradiol per ml respectively.

**Chromatographic system**
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of acetonitrile, 15 volumes of methanol and 45 volumes of water,
- flow rate. 1 to 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- a 50 µl loop injector.

Inject the reference solution and record the peak response. The test is not valid unless the resolution between the two major peaks is not less than 2.5, and the relative standard deviation for replicate injections is not more than 2.0.

Inject separately the test solution and the reference solution and measure the responses for the major peaks. The relative retention times are about 0.7 for ethinyloestradiol and 1.0 for levonorgestrel.

Calculate the contents of C₂₁H₂₈O₂ and C₂₈H₃₀O₂ in the tablets.

**Storage.** Store protected from moisture.

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**Lignocaine Hydrochloride**

**Lidocaine Hydrochloride**

![Chemical Structure](image)

\[
\text{C}_{14}\text{H}_{22}\text{N}_{2}\text{O}\cdot\text{HCl}, \text{H}_{2}\text{O}
\]

Mol. Wt. 288.8

Lignocaine Hydrochloride is 2-diethylaminoaceto-2’,6’-xylidide hydrochloride monohydrate.

Lignocaine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of C₁₄H₂₂N₂O.HCl, calculated on the anhydrous basis.

**Description.** A white, crystalline powder; odourless or practically odourless.
Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B, C, D and E may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with lignocaine hydrochloride RS or with the reference spectrum of lignocaine hydrochloride.

B. To 10 ml of a 2.5 per cent w/v solution add sodium hydroxide solution till alkaline and filter. Wash the residue with water, dissolve half of the residue in 1 ml of ethanol (95 per cent) and add 0.5 ml of a 10 per cent w/v solution of cobalt chloride; a bluish-green precipitate is produced.

C. To 5 mg add 0.5 ml of fuming nitric acid, evaporate to dryness on a water-bath, cool, dissolve the residue in 5 ml of acetone and add 1 ml of 0.1 M ethanolic potassium hydroxide; a green colour is produced.

D. Dissolve 0.2 g in 10 ml of water and add 10 ml of picric acid solution. The precipitate, after washing with water and drying, melts at about 229° (2.4.21).

E. Gives reaction A of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in carbon dioxide-free water is clear (2.4.1), and colourless (2.4.1).

pH (2.4.24). 4.0 to 5.5, determined in a 0.5 per cent w/v solution.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

Sulphates. Dissolve 0.2 g in 20 ml of water, add 2 ml of 3 M hydrochloric acid, mix and divide into two parts. To one part add 1 ml of barium chloride solution; no more opalescence is produced than in the remaining portion of the solution to which nothing has been added.

2,6-Dimethylaniline. To 2 ml of a 2.5 per cent w/v solution in methanol (solution A), add 1 ml of a 1 per cent w/v solution of 4-dimethylaminobenzaldehyde in methanol and 2 ml of glacial acetic acid and allow to stand for 10 minutes at room temperature. Any yellow colour produced is more intense than that obtained by repeating the test using 2 ml of methanol in place of solution A and less intense than the colour produced using a mixture of 1 ml of a solution of 2,6-dimethylaniline in methanol containing 5 µg per ml and 1 ml of methanol in place of solution A (100 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 5.0 to 7.5 per cent, determined on 0.25 g.

Assay. Weigh accurately about 0.5 g, dissolve in 30 ml of anhydrous glacial acetic acid, add 6 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid, using crystal violet solution as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02708 g of C₁₄H₂₂N₂O.HCl.

Storage. Store protected from moisture.

Lignocaine and Adrenaline Injection

Lignocaine Hydrochloride and Adrenaline Bitartrate Injection; Lignocaine and Adrenaline Injection

Lignocaine and Adrenaline Injection is a sterile solution of Lignocaine Hydrochloride and Adrenaline Bitartrate in Water for Injections.

Lignocaine and Adrenaline Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of lignocaine hydrochloride, C₁₄H₂₂N₂O.HCl,H₂O and not less than 87.5 per cent and not more than 112.5 per cent of the stated amount of adrenaline, C₈H₁₄N₂O₃.

Description. A clear colourless solution.

Identification

A. To 5 ml add 1 ml of hydrochloric acid, cool to 0°, add 5 ml of a 1 per cent w/v solution of sodium nitrite and pour the mixture into 2 ml of 2-naphthol solution containing 1 g of sodium acetate; no red colour is produced.

B. To 10 ml add 4 ml of disodium hydrogen phosphate solution and sufficient 0.1 M iodine to produce a distinct brown colour. Add 0.01 M sodium thiosulphate to remove the excess of iodine; a pink colour is produced.

C. To 3 ml add 3 ml of water and 6 ml of picric acid solution, shake gently and allow to stand until the precipitate becomes crystalline; the precipitate, after washing with water and drying at 105°, melts at about 229° (2.4.21).

Tests

pH (2.4.24). 3.0 to 4.5.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. For lignocaine hydrochloride — Make an accurately measured volume containing 0.1 g of Lignocaine Hydrochloride alkaline with 2 M sodium hydroxide and extract with three quantities, each of 20 ml, of chloroform, washing each extract with the same 10 ml of water. Filter the washed extracts through a filter paper moistened with chloroform, wash the filter with 10 ml of chloroform, combine the washings with the
filtrate. Titrate with 0.02 M perchloric acid, using crystal violet solution as indicator.

1 ml of 0.02 M perchloric acid is equivalent to 0.005776 g of C₆H₂N₂O.HCl.H₂O.

For adrenaline — To an accurately measured volume containing 0.1 mg of adrenaline add 20 mg of sodium metabisulphite, 0.1 ml of ferrous sulphate-citrate solution, 1 ml of glycine buffer solution and mix. Allow to stand for 10 minutes, extract with 10 ml of ether, allow to separate, reject the ether and measure the absorbance of a 4-cm layer of the solution at about 540 nm (2.4.7). Calculate the content of adrenaline, C₉H₁₃NO₃, from a reference curve prepared by treating suitable aliquots of a solution of adrenaline bitartrate RS in the same manner.

1 mg of adrenaline bitartrate is equivalent to 0.0005497 g of C₉H₁₃NO₃.

Storage. Store protected from light.

Labelling. The label states the strength of Adrenaline Bitartrate in terms of the equivalent amount of adrenaline.

Lignocaine and Dextrose Injection

Lignocaine Hydrochloride and Dextrose Injection; Lidocaine Hydrochloride and Dextrose Injection; Lidocaine and Dextrose Injection

Lignocaine and Dextrose Injection is a sterile solution of Lignocaine Hydrochloride and Dextrose in Water for Injections.

Lignocaine and Dextrose Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of lignocaine hydrochloride, C₁₄H₂₂N₂O,HCl,H₂O, and dextrose, C₆H₁₂O₆,H₂O.

Description. A clear colourless or slightly yellow solution.

Identification

A. To a volume containing about 0.5 g of Lignocaine Hydrochloride in a separator add 2 ml of 2 M sodium hydroxide and extract with four quantities, each of 15 ml, of chloroform. Combine the chloroform extracts and evaporate the solution to dryness with the aid of a current of air. Dissolve the residue in 2 ml of hexane, evaporate with the aid of warm air and dry the residue over silica gel for 24 hours at a pressure not exceeding 0.7 kPa.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with lignocaine hydrochloride RS treated in the same manner.

B. To a volume containing 0.1 g of Dextrose add 10 ml of water and 3 ml of potassium cupri-tartrate solution and heat; a red precipitate is produced.

Tests

pH (2.4.24). 3.0 to 7.0.

Other tests. Complies with the tests described under Parenteral Preparations (Injections).

Assay. For lignocaine hydrochloride - Make an accurately measured volume containing about 0.1 g of Lignocaine Hydrochloride alkaline with 2 M sodium hydroxide and extract with three quantities, each of 20 ml, of chloroform, washing each extract with the same 10 ml of water. Filter the washed extracts through a filter paper moistened with chloroform, wash the filter with 10 ml of chloroform, combine the washings with the filtrate. Titrate with 0.02 M perchloric acid, using crystal violet solution as indicator.

1 ml of 0.02 M perchloric acid is equivalent to 0.005776 g of C₁₄H₂₂N₂O,HCl,H₂O.

For dextrose — To an accurately measured volume containing 2 g to 5 g of Dextrose add sufficient water to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 1.0425 represents the weight, in g, of dextrose, C₆H₁₂O₆,H₂O in the volume taken for assay.

Storage. Store in single dose containers in a cool place.

Lignocaine Gel

Lignocaine Hydrochloride Gel; Lidocaine Hydrochloride Gel

Lignocaine Gel is a sterile solution of Lignocaine Hydrochloride in a suitable water-miscible base. It may contain suitable antioxidants, stabilisers and antimicrobial preservatives.

Lignocaine Gel contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of anhydrous lignocaine hydrochloride, C₁₄H₂₂N₂O,HCl.

Identification

To a quantity of the gel containing 80 mg of anhydrous lignocaine hydrochloride add 4 ml of hydrochloric acid and heat on a water-bath for 10 minutes. Allow to cool, transfer to a separating funnel with the aid of 20 ml of water, add 5 M sodium hydroxide until precipitation is complete and extract with two quantities, each of 20 ml, of chloroform. Filter the chloroform extracts through anhydrous sodium sulphate and evaporate the filtrate to dryness on a water-bath using a stream of nitrogen. The residue complies with the following tests.
A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with lignocaine hydrochloride RS or with the reference spectrum of lignocaine hydrochloride.

B. Dissolve 20 mg in 1 ml of ethanol (95 per cent), add 0.5 ml of a 10 per cent w/v solution of cobalt chloride and 0.5 ml of 5 M sodium hydroxide and shake for 2 minutes; a bluish green precipitate is produced.

C. Dissolve 40 mg in 5 ml of 1 per cent w/v solution of cetrimide, add 1 ml of 5 M sodium hydroxide and 1 ml of bromine water; a yellow colour is produced.

**Tests**

**pH** (2.4.24). 6.0 to 7.0.

2,6-Dimethylaniline. Mix a quantity of the gel containing 15 mg of anhydrous lignocaine hydrochloride with sufficient water to produce 3 ml, using a rotary mixer. To 2 ml of the resulting solution, add 1 ml of a freshly prepared 1 per cent w/v solution of 4-dimethylaminobenzaldehyde in methanol. Mix thoroughly using a rotary mixer. Add 2 ml of glacial acetic acid and allow to stand for 10 minutes. Any yellow colour produced is not more intense than that obtained by using a mixture of 2 ml of a solution of 2,6-dimethylaniline in methanol containing 2 µg per ml in place of the solution of the gel (20 ppm).

Sterility (2.2.11). Complies with the test for sterility.

Other tests. Complies with the tests stated under Gels.

**Assay.** Weigh accurately a quantity containing about 10 mg of anhydrous lignocaine hydrochloride and disperse in 20 ml of water. Add 5 ml of acetate buffer pH 2.8, 120 ml of chloroform and 5 ml of dimethyl yellow-oracet blue B solution and titrate with 0.005 M dioctyl sodium sulphosuccinate swirling vigorously, until near the end-point, then add the titrant dropwise and, after each addition, swirl vigorously, allow to separate and swirl gently for 5 seconds. The end-point is indicated when the colour of the chloroform layer changes from green to pinkish-grey. Carry out a blank titration.

1 ml of 0.005 M dioctyl sodium sulphosuccinate is equivalent to 0.001354 g of C₁₄H₂₂N₂O.HCl.

Determine the weight per ml of the gel (2.4.29), and calculate the percentage of C₁₄H₂₂N₂O.HCl, weight in volume.

**Storage.** Store in suitable tamper-proof containers holding sufficient of the gel for use on one occasion, and at a temperature not exceeding 30°. The gel should not be frozen.

**Labelling.** The label states (1) that the contents are sterile; (2) the strength in terms of the equivalent amount of anhydrous lignocaine hydrochloride; (3) that any of the gel not used in a single application should be discarded.

**Lignocaine Injection**

Lignocaine Hydrochloride Injection; Lidocaine Hydrochloride Injection

Lignocaine Injection is a sterile solution of Lignocaine Hydrochloride in Water for Injections.

Lignocaine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of lignocaine hydrochloride, C₁₄H₂₂N₂O.HCl.H₂O.

**Description.** A clear colourless solution.

**Identification**

A. To a volume containing 0.1 g of Lignocaine Hydrochloride add sufficient sodium hydroxide solution to make alkaline, filter, wash the residue with water, dissolve in 1 ml of ethanol (95 per cent), add 0.5 ml of a 10 per cent w/v solution of cobalt chloride and shake for 2 minutes; a bluish-green precipitate is formed.

B. To a volume containing 0.1 g of Lignocaine Hydrochloride add 10 ml of picric acid solution; the precipitate, after washing with water and drying at 105°, melts at about 229° (2.4.21).

C. Gives the reactions of chlorides (2.3.1).

**Tests**

**pH** (2.4.24). 5.0 to 7.0.

2,6-Dimethylaniline. To a volume containing 25 mg of Lignocaine Hydrochloride add water if necessary to produce 10 ml, add 2 M sodium hydroxide until the solution is just alkaline and extract with three quantities, each of 5 ml, of chloroform. Dry the combined chloroform extracts over anhydrous sodium sulphate, filter, wash with a further 5 ml of chloroform and evaporate the filtrate to dryness at a pressure of 2 kPa. Dissolve the residue in 2 ml of methanol, add 1 ml of a 1 per cent w/v solution of 4-dimethylamino-benzaldehyde in methanol and 2 ml of glacial acetic acid and allow to stand at room temperature for 10 minutes. Any yellow colour produced is not more intense than the colour produced by repeating the operation using 10 ml of a solution in water containing 1 µg per ml of 2,6-dimethylaniline in place of the preparation under examination (400 ppm).

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** Make an accurately measured volume containing about 0.1 g of Lignocaine Hydrochloride alkaline with 2 M sodium hydroxide and extract with three quantities, each of 20 ml, of chloroform, washing each extract with the same 10 ml of water. Filter the washed extracts through a filter paper moistened with chloroform, wash the filter with 10 ml of chloroform,
combine the washings with the filtrate. Titrate with 0.02 M perchloric acid, using crystal violet solution as indicator.

1 ml of 0.02 M perchloric acid is equivalent to 0.005776 g of C₈H₂₂N₂O·HCl·H₂O.

**Lincomycin Hydrochloride**

![Lincomycin Hydrochloride structure](attachment:image)

**Lincomycin Hydrochloride**

C₁₈H₃₄N₂O₆S·HCl·H₂O  
Mol. Wt. 461.0

Lincomycin Hydrochloride consists mainly of methyl 6-amino-6,8-dideoxy-N-{(2S,4R)-1-methyl-4-propylprolyl}-1-thio-D-erythro-α-D-galacto-octopyranoside hydrochloride monohydrate, an antimicrobial substance produced by *Streptomyces lincolnensis* var. *lincolnensis* or by any other means.

Lincomycin Hydrochloride contains not less than 82.5 per cent and not more than 93.0 per cent of C₁₈H₃₄N₂O₆S, calculated on the anhydrous basis.

**Description.** A white or almost white, crystalline powder.

**Identification**

**Test A** may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with lincomycin hydrochloride RS or with the reference spectrum of lincomycin hydrochloride.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** The upper layer obtained by shaking a mixture of 45 volumes of ethyl acetate, 40 volumes of a 15 per cent w/v solution of ammonium acetate previously adjusted to pH 9.6 with 10 M ammonium carbonate and 20 volumes of 2-propanol.

**Test solution.** Dissolve 0.1 g of the substance under examination in 100 ml of methanol.

**Reference solution (a).** A 0.1 per cent w/v solution of lincomycin hydrochloride RS in methanol.

**Reference solution (b).** A solution containing 0.1 per cent w/v each of lincomycin hydrochloride RS and clindamycin hydrochloride RS in methanol.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and spray with a 0.1 per cent w/v solution of potassium permanganate. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. Dissolve 10 mg in 2 ml of 2 M hydrochloric acid and heat in a water-bath for 3 minutes. Add 3 ml of a 10.5 per cent w/v solution of anhydrous sodium carbonate and 1 ml of a 2 per cent w/v solution of sodium nitroprusside; a violet-red colour is produced.

D. A 1 per cent w/v solution gives reaction A of chlorides (2.3.1).

**Tests**

**Appearance of solution.** A 10.0 per cent w/v solution in carbon dioxide-free water is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

**pH** (2.4.24). 3.5 to 5.5, determined in a 10.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +135° to +150°, determined in a 4.0 per cent w/v solution.

**Lincomycin B.** In the Assay, the chromatogram obtained with reference solution (a) shows a peak derived from lincomycin B which is eluted just before lincomycin. The area of the peak derived from lincomycin B is not more than 5 per cent of the area of the peak derived from lincomycin.

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.5 per cent.

**Water** (2.3.43). 3.0 to 4.6 per cent, determined on 0.5 g.

**Assay.** Determine by gas chromatography (2.4.13).

**Test solution.** Add 10.0 ml of a 0.8 per cent w/w solution of dotriacontane (internal standard) in chloroform to 0.1 g of lincomycin hydrochloride RS, dilute to 100.0 ml with a 2 per cent w/v solution of imidazole in chloroform and shake to dissolve. Place 4.0 ml of the resulting solution in a 15-ml glass-stoppered centrifuge tube, add 1.0 ml of a mixture of 99 volumes of N,O-bis (trimethylsilyl)-acetamide and 1 volume of trimethylchlorosilane and mix gently. Loosen the glass stopper and heat at 65° for 30 minutes.

**Reference solution (a).** Prepare in the same manner as the test solution but omitting the internal standard and using 0.1 g of the substance under examination in place of lincomycin hydrochloride RS.
Reference solution (b). Prepare in the same manner as the test solution but using 0.1 g of the substance under examination in place of lincomycin hydrochloride RS.

Chromatographic system
- a glass column 1.5 m x 3 mm, packed with acid-washed, silanised diatomaceous support impregnated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl),
- temperature: column 260°,
inlet port and detector at 260° to 290°,
- flow rate. 45 ml per minute using helium as carrier gas.

Inject 1 µl of the test solution, reference solution (a) and reference solution (b).

Calculate the content of C₁₈H₃₄N₂O₆S.

Lincomycin Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per mg.

Lincomycin Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture at a temperature not exceeding 30°. If the contents are sterile, the container should be sterile, tamper-evident and sealed so as to exclude microorganisms.

Labelling. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

Lincomycin Capsules

Lincomycin Hydrochloride Capsules

Lincomycin Capsules contain Lincomycin Hydrochloride equivalent to not less than 90.0 per cent and not more than 110 per cent of the stated amount of lincomycin, C₁₈H₃₄N₂O₆S.

Identification

In the Assay, the retention time of the principal peak derived from lincomycin hydrochloride relative to that of the internal standard in reference solution (b) is the same as the retention time of the principal peak derived from lincomycin hydrochloride RS relative to that of the internal standard in the test solution.

Tests

Lincomycin B. Examine reference solution (b) as described under the Assay but increase the sensitivity by 8 to 10 times while recording the peak derived from lincomycin B, which is eluted immediately before that derived from lincomycin. The area of the peak derived from lincomycin B, when corrected for the sensitivity factor, is not more than 5 per cent of the area of the peak derived from lincomycin.

Water (2.3.43). Not more than 7.0 per cent, determined on 0.3 g of the contents of the capsules.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by gas chromatography (2.4.13).

Test solution. Add 10.0 ml of a 0.8 per cent w/w solution of dotriacontane (internal standard) in chloroform to 0.1 g of lincomycin hydrochloride RS, dilute to 100.0 ml with a 2 per cent w/v solution of imidazole in chloroform and shake to dissolve. Place 4.0 ml of the resulting solution in a 15-ml glass-stoppered centrifuge tube, add 1.0 ml of a mixture of 99 volumes of N,O-bis(trimethylsilyl)-acetamide and 1 volume of trimethylchlorosilane and mix gently. Loosen the glass stopper and heat at 65° for 30 minutes.

Reference solution (a). Prepare in the same manner as the test solution but omitting the internal standard and using a quantity of the mixed contents of 20 capsules containing about 90 mg of lincomycin in place of lincomycin hydrochloride RS.

Reference solution (b). Prepare in the same manner as the test solution but using a quantity of the mixed contents of 20 capsules containing about 90 mg of lincomycin in place of lincomycin hydrochloride RS.

Chromatographic system
- a glass column 1.5 m x 3 mm, packed with acid-washed, silanised diatomaceous support impregnated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl),
- temperature: column 260°,
inlet port and detector at 260° to 290°,
- flow rate. 45 ml per minute using helium as carrier gas.

Inject 1 µl of the test solution, reference solution (a) and reference solution (b).

Calculate the content of C₁₈H₃₄N₂O₆S in the capsules.

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the equivalent amount of lincomycin.
Lindane

Gamma Benzene Hexachloride

\[ \text{C}_6\text{H}_6\text{Cl}_6 \quad \text{Mol.Wt.290.8} \]

Lindane is 1\(\alpha\),2\(\alpha\),3\(\beta\),4\(\alpha\),5,6\(\beta\)-hexachlorocyclohexane.

Lindane contains not less 99.0 per cent and not more than 100.5 per cent of \(\text{C}_6\text{H}_6\text{Cl}_6\).

**Description.** A white or almost white crystalline powder.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with lindane RS.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. Dissolve about 5 mg in 4 ml of ethanol (95 per cent). Add 1 ml of 0.5 M ethanolic potassium hydroxide and allow to stand for 10 minutes. The solution gives reaction A of chlorides (2.3.1).

D. Melts at 112\(^\circ\) to 115\(^\circ\) (2.4.21).

**Tests**

**Appearance of solution.** A 5.0 per cent w/v solution in acetone is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

**Related substances.** Determine by thin-layer chromatography (2.4.17) coating the plate with silica gel G.

**Mobile phase.** A mixture of 90 volumes of cyclohexane and 10 volumes of chloroform.

**Test solution (a).** Dissolve 1 g of the substance under examination in 10 ml of chloroform.

**Test solution (b).** Dilute 1 ml of test solution (a) to 10 ml with chloroform.

**Reference solution (a).** A 1 per cent w/v solution of lindane RS in chloroform.

**Reference solution (b).** Dilute 1 ml of test solution (b) to 10 ml with chloroform.

**Reference solution (c).** Dissolve 10 mg of \(\alpha\)-hexachlorocyclohexane RS in sufficient of the test solution (a) to produce 5 ml.

Apply separately to the plate 1 \(\mu\)l of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of dry air and irradiate with ultraviolet light at 254 nm for 15 minutes. Spray with a 0.6 per cent w/v solution of dicarboxidine hydrochloride in ethanol (90 per cent) and examine the spots in daylight. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

**Chlorides** (2.3.12). To 0.75 g, finely powdered, add 15 ml of water and boil for 1 minute. Allow to cool, shaking frequently, and filter. To 10 ml of the filtrate add 3 ml of water and 2 ml of ethanol (95 per cent). The solution complies with the limit test for chlorides (100 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Assay.** Weigh accurately about 0.2 g, add 10 ml of ethanol (95 per cent) and warm on a water-bath until dissolved. Cool, add 20 ml of 0.5 M ethanolic potassium hydroxide and allow to stand for 10 minutes, swirling frequently. Add 50 ml of water; 20 ml of 2 \(M\) nitric acid, 25 ml of 0.1 \(M\) silver nitrate and 5 ml of ferric ammonium sulphate solution. Titrate with 0.1 \(M\) ammonium thiocyanate until a reddish-yellow colour is obtained. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of silver nitrate required.

1 ml of 0.1 \(M\) silver nitrate is equivalent to 0.009694 g of \(\text{C}_6\text{H}_6\text{Cl}_6\).

**Storage.** Store protected from light.

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**Absorbent Lint**

Lint; Cotton Lint; Unmedicated Lint

Absorbent Lint is a cotton cloth of plain weave, on one side of which a nep has been raised from either warp or weft yarns. It absorbs water readily but its absorbency may be considerably reduced by medication, the absorbency of the product depending upon the medicament incorporated.

**Description.** Cotton cloth of plain weave, reasonably free from weaving defects, readily tearable in both directions and bleached to a good white having on one side a nep raised from either the warp or weft yarns and reasonably free from neps; it is clean and reasonably free from leaf, shell and other foreign substances. It is made of yarn that is reasonably free from slubs, snarls and other defects.

Absorbent Lint has not less than 98.0 per cent of the dimensions stated on the label.
Tests

Threads per cm. Warp not less than 16 and weft not less than 10.

Weight per unit area. 25 g has a superficial area of 1350 to 1370 sq. cm.

Absorbency. A piece 10 cm square, placed lightly by means of forceps, unreased side downwards, on the surface of water at 20°C, becomes saturated within 10 seconds.

Fluorescence. Not more than a few points of fluorescence are visible under screened ultraviolet light.

Storage. Store protected from moisture in well-closed packages in a dry place, free from dust.

Labelling. The label states the dimensions viz. the length and width in cm.

Lisinopril

\[
\text{HOOC} \quad \text{NH} \quad \text{O} \quad \text{COOH}, \ 2\text{H}_2\text{O}
\]

\[
\text{C}_{21}\text{H}_{31}\text{N}_{3}\text{O}_{5}, \ 2\text{H}_2\text{O} \quad \text{Mol. Wt.} \ 441.5
\]

Lisinopril is \((S)-1-\left[N^2-(1\text{-carboxy-3-phenylpropyl})\text{-L-lysyl}\right]-\text{L-proline dihydrate}.

Lisinopril contains not less than 98.0 per cent and not more than 102.0 per cent of \( \text{C}_{21}\text{H}_{31}\text{N}_{3}\text{O}_{5} \), calculated on the anhydrous basis.

Description. A white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with lisinopril RS or with the reference spectrum of lisinopril.

B. In the Assay, the chromatogram obtained with the test solution corresponds to the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). -115.0° to -123.0°, determined on 1.0 per cent w/v solution in 0.25 M zinc acetate at 405 nm.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in 10 ml of mobile phase A

Reference solution (a). A 0.2 per cent w/v solution of lisinopril RS in mobile phase A.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octylsilane bonded to porous silica (5 μm) (such as Symmetry C-8),
- column temperature 50°C,
- mobile phase: A. a mixture of 30 volumes of acetonitrile and 970 volumes of 0.02 M sodium dihydrogen phosphate, adjust the pH to 5.0 with a 5 per cent w/v solution of sodium hydroxide and filter.
- B. a mixture of 200 volumes of acetonitrile and 800 volumes of 0.02 M sodium dihydrogen phosphate, adjust the pH to 5.0 with 5 per cent w/v solution of sodium hydroxide and filter.
- a linear gradient programme using the conditions given below,
- flow rate. 1.8 ml per minute,
- spectrophotometer set at 210 nm,
- a 20 μl loop injector.

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Mobile phase A (per cent)</th>
<th>Mobile phase B (per cent)</th>
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<tr>
<td>0</td>
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<tr>
<td>75</td>
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<td>0</td>
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</tbody>
</table>

Inject reference solution (b). Test is not valid unless the tailing factor is not more than 3.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 8.0 to 9.5, determined on 0.1 g.

Assay. Determine by liquid chromatography (2.4.14).
Test solution. Dissolve 30 mg of the substance under examination in 100 ml of water and filter.

Reference solution. A 0.03 per cent w/v solution of lisinopril RS in water.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with dimethyloctylsilane (C8 alkyl chain) fully endcapped stationary phase (5 µm) (such as Hypersil MOS),
- column temperature 50º,
- mobile phase: a mixture of 96 volumes of buffer pH 5.0 prepared by dissolving 2.76 g of monobasic sodium phosphate in 1000 ml of water: adjust the pH to 5.0 with 1 M sodium hydroxide and 4 volumes of acetonitrile,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 180 theoretical plates. The tailing factor is not more than 2.0. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.
Calculate the content of C₂₁H₃₁N₃O₅.

Storage. Store protected from moisture, at a temperature not exceeding 25º.

Lisinopril Tablets
Lisinopril Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lisinopril, C₂₁H₃₁N₃O₅.

Identification
In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests
Dissolution (2.5.2).
Apparatus. No 1
Medium. 900 ml of 0.1 M hydrochloric acid.
Speed and time. 50 rpm for 30 minutes.
Withdraw a suitable volume of the medium and filter.
Determine by liquid chromatography (2.4.14).
Test solution. The filtrate obtained as given above.
Reference solution. A 0.001 per cent w/v solution of lisinopril RS in dissolution medium.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octylsilane bonded to porous silica (5 µm) (such as Symmetry C8),
- column temperature 50º,
- mobile phase: dissolve 1.0 g of hexane sulphonic acid sodium salt in 800 volumes of phosphate solution prepared by dissolving 4.1 g of monobasic potassium phosphate in 900 ml water, adjust pH to 2.0 with orthophosphoric acid, dilute to 1000 ml with water and 200 volumes of acetonitrile, mix, filter and degas.
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- a 50 µl loop injector.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 850 theoretical plates, the tailing factor is not more than 2.0. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.
D. Not less than 75 per cent of the stated amount of C₂₁H₃₁N₃O₅.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a powdered tablet containing 100 mg of Lisinopril to a 50-ml volumetric flask. Add about 25 ml of mobile phase A and sonicate for 10 minutes with intermittent shaking. Make up the volume with the mobile phase A and filter.

Reference solution (a). A 0.2 per cent w/v solution of lisinopril RS in the mobile phase A.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with mobile phase A.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with packed with dimethyloctylsilane (C8 alkyl chain) fully endcapped stationary phase (5 µm) (such as Hypersil MOS/ Symmetry C8),
- column temperature 50º,
- mobile phase: A. a mixture of 3 volumes of acetonitrile and 97 volumes of 0.02 M sodium dihydrogen phosphate, adjust the pH to 5.0 with 5 per cent w/v solution of sodium hydroxide and filter,
B. a mixture of 20 volumes of acetonitrile and 80 volumes of 0.02 M sodium dihydrogen phosphate, adjust the pH to 5.0 with 5 per cent w/v solution of sodium hydroxide and filter,
- a linear gradient programme using the conditions given below,
- flow rate. 1.8 ml per minute,
- spectrophotometer set at 210 nm,
**Lithium Carbonate**

**Li₂CO₃**  
Mol.Wt. 73.9

Lithium Carbonate contains not less than 98.5 per cent and not more than 100.5 per cent of Li₂CO₃.

**Description.** A white, crystalline powder; odourless.

**Identification**

A. When moistened with hydrochloric acid and introduced on a platinum wire, it imparts a red colour to a non-luminous flame.

B. Dissolve 0.2 g in 1 ml of hydrochloric acid and evaporate to dryness on a water-bath; the residue is soluble in 3 ml of ethanol (95 per cent).

C. Gives reaction A of carbonates (2.3.1).

**Tests**

**Appearance of solution.** Suspend 10.0 g in 30 ml of distilled water and dissolve by adding 22 ml of nitric acid. Neutralise with 2 M sodium hydroxide and dilute to 100.0 ml with distilled water (solution A). The solution is clear (2.4.1), and colourless (2.4.1).

**Arsenic** (2.3.10). Dissolve 5.0 g in 15 ml of brominated hydrochloric acid, add 45 ml of water and remove the excess of bromine with a few drops of stannous chloride solution asT. The resulting solution complies with the limit test for arsenic (2 ppm).

**Calcium and magnesium**. Dissolve 1.0 g in 30 ml of 1 M hydrochloric acid and neutralise with dilute ammonia solution, filter, if necessary, and divide into two portions; to one portion add 1 ml of ammonium oxalate solution; no turbidity or precipitate is produced on standing for 5 minutes. To the other portion add 1 ml of disodium hydrogen phosphate solution; no turbidity or precipitate is produced on standing for 5 minutes.

**Heavy metals** (2.3.13). Mix 1.0 g with 5 ml of water and 15 ml of dilute hydrochloric acid, heat to boiling and maintain that temperature for 1 minute. Add 1 drop of phenolphthalein solution and sufficient ammonia solution to give the solution a faint pink colour. Cool and dilute to 25 ml with water. The resulting solution complies with the limit test for heavy metals, Method A (20 ppm).

**Iron** (2.3.14). 20 ml of solution A complies with the limit test for iron (20 ppm).

**Storage.** Store protect from moisture, at a temperature between 20° to 25°.

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**Solvent mixture.** A mixture of 4 volumes of water and 1 volume of methanol.

**Test solution.** Transfer to a suitable size volumetric flask 10 tablets, add the solvent mixture to fill about half of the volumetric flask, shake the flask by mechanical means for 20 minutes and dilute with solvent mixture which will yield a solution having a concentration of about 0.02 per cent w/v and filter.

**Reference solution.** A 0.02 per cent w/v solution of lisinopril RS in solvent mixture.

**Chromatographic system**
- a stainless steel column 25 cm x 4.6 mm packed with octylsilane bonded to porous silica (5 µm), (such as Symmetry C-8),
- column temperature 50°,
- mobile phase: dissolve 1.0 g of hexane sulphonic acid sodium salt in 800 volumes of phosphate solution prepared by dissolving 4.1 g of monobasic potassium phosphate in 900 ml water; adjust the pH to 2.0 with orthophosphoric acid, dilute to 1000 ml with water and 200 volumes of acetonitrile, mix, filter and degas.
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- a 20 µl loop injector.

**Calcium and magnesium.** Dissolve 1.0 g in 30 ml of 1 M hydrochloric acid and neutralise with dilute ammonia solution, filter, if necessary, and divide into two portions; to one portion add 1 ml of ammonium oxalate solution; no turbidity or precipitate is produced on standing for 5 minutes. To the other portion add 1 ml of disodium hydrogen phosphate solution; no turbidity or precipitate is produced on standing for 5 minutes.

**Heavy metals** (2.3.13). Mix 1.0 g with 5 ml of water and 15 ml of dilute hydrochloric acid, heat to boiling and maintain that temperature for 1 minute. Add 1 drop of phenolphthalein solution and sufficient ammonia solution to give the solution a faint pink colour. Cool and dilute to 25 ml with water. The resulting solution complies with the limit test for heavy metals, Method A (20 ppm).

**Iron** (2.3.14). 20 ml of solution A complies with the limit test for iron (20 ppm).
Potassium. Dissolve 1.0 g in 10 ml of 7 M hydrochloric acid, add sufficient water to produce 50 ml and determine by flame photometry (2.4.4), measuring at 766.5 nm, using potassium solution FP, suitably diluted with water, to prepare the standard solutions (500 ppm).

Sodium. Dissolve 1.0 g in 10 ml of 7 M hydrochloric acid, add sufficient water to produce 50 ml and determine by flame photometry (2.4.4), measuring at 589 nm, using sodium solution FP, suitably diluted with water, to prepare the standard solutions (500 ppm).

Chlorides (2.3.12). 10 ml of solution A diluted to 15 ml with water complies with the limit test for chlorides (250 ppm).

Sulphates (2.3.17). Disperse 0.75 g in 5 ml of distilled water and dissolve by adding 5 ml of 7 M hydrochloric acid. Boil for 2 minutes, cool, neutralise with 2 M sodium hydroxide and dilute to 25 ml with distilled water. The resulting solution complies with the limit test for sulphates (200 ppm).

Assay. Weigh accurately about 0.5 g, dissolve in 25.0 ml of 1 M hydrochloric acid and titrate with 1 M sodium hydroxide using methyl orange solution as indicator.

1 ml of 1 M hydrochloric acid is equivalent to 0.03695 g of Li₂CO₃.

Storage. Store protected from moisture.

Lithium Carbonate Tablets

Lithium Carbonate Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of lithium carbonate, Li₂CO₃.

Identification

A small quantity of the powdered tablets, when moistened with hydrochloric acid and introduced on a platinum wire, imparts a red colour to a non-luminous flame.

Tests

Dissolution (2.5.2).

Apparatus. No 1
Medium. 900 ml of water
Speed and time. 100 rpm and 30 minutes.

Withdraw 90.0 ml of the medium, add a drop of hydrochloric acid and dilute to 100.0 ml with water. Determine by flame photometry Method A (2.4.4), or by atomic absorption spectrophotometry, Method A (2.4.2), measuring at 671 nm and using lithium solution FP, or lithium solution AAS, as appropriate, suitably diluted with water, for the standard solution.

D. Not less than 60 per cent of the stated amount of Li₂CO₃.

Other tests. Comply with the tests stated under Tablets.

Lomustine

\[
\text{C}_9\text{H}_{16}\text{ClN}_3\text{O}_2 \quad \text{Mol. Wt. 233.7}
\]

Lomustine is 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea.

Lomustine contains not less than 98.5 per cent and not more than 100.5 per cent of C₉H₁₆ClN₃O₂, calculated on the dried basis.

Description. A yellow, crystalline powder.

Carry out the tests protected from light and prepare the solutions immediately before use.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with lomustine RS or with the reference spectrum of lomustine.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution in ethanol (95 per cent) shows an absorption maximum at about 230 nm; absorbance at about 230 nm, about 0.52.

C. In the test A for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).

D. Dissolve about 25 mg in 1 ml of methanol, add 0.1 ml of 2 M sodium hydroxide and 2 ml of water and acidify by adding, dropwise, 1 M nitric acid. The resulting solution gives the reactions of chlorides (2.3.1).
**Tests**

**Related substances.** A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase.* A mixture of 80 volumes of *toluene* and 20 volumes of *glacial acetic acid*.

*Test solution (a).* Dissolve 0.25 g of the substance under examination in 10 ml of *methanol*.

*Test solution (b).* Dissolve 0.1 g of the substance under examination in 100 ml of *methanol*.

*Reference solution (a).* A 0.01 per cent w/v solution of the substance under examination in methanol.

*Reference solution (b).* A 0.005 per cent w/v solution of the substance under examination in methanol.

*Reference solution (c).* A 0.1 per cent w/v solution of *lomustine RS* in methanol.

*Reference solution (d).* A solution containing 0.1 per cent w/v each of *lomustine RS* and 1,3-dicyclohexylurea in methanol.

Apply to the plate 5 µl of each solution. After development, dry the plate, heat it at 110° for 1 hour, exposing the hot plate in a closed tank containing chlorine, produced by adding *dilute hydrochloric acid* to a 5 per cent w/v solution of *potassium permanganate* contained in a beaker placed at the bottom of tank. Allow the plate to stand in contact with the chlorine vapours for 5 minutes. Remove the plate from the tank and dry it in a current of cold air until the excess of chlorine is removed and an area of the plate below the line of application produces at most a very faint blue colour with 0.05 ml of *potassium iodide and starch solution*; avoid prolonged exposure to cold air. Spray the plate with *potassium iodide and starch solution*.

Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (d) shows two clearly separated principal spots.

B. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 0.25 g of the substance under examination in 10 ml of *methanol*.

*Reference solution.* A 0.025 per cent w/v solution of the substance under examination in methanol.

**Chromatographic system**
- a stainless steel column 20 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: equal volumes of *methanol* and *water*;
- flow rate, 2 ml per minute,
- spectrophotometer set at 230 nm,
- a 20 µl loop injector.

Inject the reference solution. The retention time of lomustine is about 25 minutes. When using a recorder, adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with the reference solution is not less than 50 per cent of the full scale of the recorder.

Inject separately each solution. In the chromatogram obtained with the test solution the sum of areas of any secondary peaks is not greater than the area of the peak in the chromatogram obtained with the reference solution. Ignore any peak with an area less than one-twentieth of that of the principal peak in the chromatogram obtained with the reference solution.

**Chlorides** (2.3.12). Dissolve 0.24 g in 4 ml of *methanol*, add 20 ml of *water*, allow to stand for 20 minutes and filter. To 10 ml of the filtrate add 5 ml of *methanol*. The resulting solution complies with the limit test for chlorides, replacing the 5 ml of water in the standard solution with 5 ml of *methanol* (0.25 per cent).

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 24 hours.

**Assay.** Weigh accurately about 0.2 g, add 20 ml of a 20 per cent w/v solution of *potassium hydroxide* and boil under a reflux condenser for 2 hours. Add 75 ml of *water* and 4 ml of *nitric acid*, cool. Titrate with 0.05 M *silver nitrate*, determining the end-point potentiometrically (2.4.25). Repeat the operation without the substance under examination. The difference between the titrations represents the amount of *silver nitrate* required.

1 ml of 0.05 M *silver nitrate* is equivalent to 0.01168 g of *C9H16ClN3O2*.

**Storage.** Store protected from light and moisture.

**Lomustine Capsules**

Lomustine Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lomustine, *C9H16ClN3O2*.

Carry out the tests protected from light and prepare the solutions immediately before use.

**Identification**

Shake a quantity of the contents of the capsules containing 0.2 g of Lomustine with 10 ml of *methanol*, filter and evaporate the filtrate to dryness using a rotary evaporator on a water-
bath maintained at not more than 60°. The residue, after drying at a pressure not exceeding 0.7 kPa at 60° for 30 minutes, complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with lomustine RS or with the reference spectrum of lomustine.

B. Melting range. 89° to 91° (2.4.21).

Tests

Related substances. A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*

**Mobile phase.** A mixture of 80 volumes of *toluene* and 20 volumes of *glacial acetic acid.*

**Test solution (a).** Shake a quantity of the contents of the capsules containing 0.25 g of Lomustine with 10 ml of *methanol* and filter.

**Test solution (b).** Dilute 1 volume of test solution (a) to 250 volumes with *methanol.*

**Reference solution (a).** Dilute 1 volume of test solution (b) to 2 volumes with *methanol.*

**Reference solution (b).** A 0.005 per cent w/v solution of the substance under examination in *methanol.*

**Reference solution (c).** A 0.1 per cent w/v solution of lomustine RS in *methanol.*

**Reference solution (d).** A solution containing 0.1 per cent w/v each of lomustine RS and 1,3-dicyclohexylurea in *methanol.*

Apply to the plate 5 µl of each solution. After development, dry the plate, heat it at 110° for 1 hour, exposing the hot plate in a closed tank containing chlorine, produced by adding *dilute hydrochloric acid* to a 5 per cent w/v solution of potassium permanganate contained in a beaker placed at the bottom of tank. Allow the plate to stand in contact with the chlorine vapours for 5 minutes. Remove the plate and dry it in a current of cold air until the excess of chlorine is removed and an area of the plate below the line of application produces at most a very faint blue colour with 0.05 ml of potassium iodide and starch solution; avoid prolonged exposure to cold air. Spray the plate with potassium iodide and starch solution. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (d) shows two clearly separated principal spots.

B. Determine by liquid chromatography (2.4.14).

**Test solution.** Shake a quantity of the contents of the capsules containing 0.25 g of Lomustine with 10 ml of *methanol* and filter.

**Reference solution.** Dilute 1 volume of the test solution to 50 volumes with *methanol.*

Chromatographic system

- a stainless steel column 20 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: equal volumes of *methanol* and *water,*
- flow rate, 2 ml per minute,
- spectrophotometer set at 230 nm,
- a 20 µl loop injector.

Inject the reference solution. The retention time of lomustine is about 25 minutes. When using a recorder, adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with the reference solution is not less than 50 per cent of the full scale of the recorder. Inject separately each solution. In the chromatogram obtained with the test solution the sum of areas of any secondary peaks is not greater than the area of the peak in the chromatogram obtained with the reference solution. Ignore any peak with an area less than one-twentieth of that of the principal peak in the chromatogram obtained with the reference solution.

Uniformity of content. For capsules containing 10 mg or less.

Comply with the test stated under Capsules.

Transfer the contents of a capsule quantitatively to a 100-ml volumetric flask with the aid of 20 ml of ethanol (95 per cent), shake well, make up to the volume with ethanol (95 per cent) and filter. Dilute suitably with the same solvent and measure the absorbance of the resulting solution at the maximum at about 230 nm (2.4.7). Calculate the content of C₉H₁₆ClN₃O₂ in the capsule taking 260 as the specific absorbance at 230 nm.

Other tests. Comply with the tests stated under Capsules.

**Assay.** Weigh accurately a quantity of the mixed contents of 20 capsules containing about 40 mg of Lomustine and shake with 70 ml of ethanol (95 per cent) for 20 minutes, dilute to 100.0 ml with ethanol (95 per cent) and filter. Dilute 5.0 ml of the filtrate to 100.0 ml with ethanol (95 per cent) and measure the absorbance of the resulting solution at the maximum at about 230 nm (2.4.7). Calculate the content of C₉H₁₆ClN₃O₂ taking 260 as the specific absorbance at 230 nm.

**Storage.** Store protected from light and moisture.
Loperamide Hydrochloride

\[
\text{C}_{29}\text{H}_{33}\text{ClN}_{2}\text{O}_{2}\text{HCl} \quad \text{Mol. Wt. 513.5}
\]

Loperamide Hydrochloride is 4-(4-chlorophenyl)-4-hydroxy-\(N,\text{N}\)-dimethyl-2,2-diphenylbutyramide hydrochloride.

Loperamide Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of \(\text{C}_{29}\text{H}_{33}\text{ClN}_{2}\text{O}_{2}\text{HCl}\), calculated on the dried basis.

**Description.** A white or almost white powder.

**Identification**

*Test B* may be omitted if tests *A* and *C* are carried out. *Test A* may be omitted if tests *B* and *C* are carried out.

**A.** Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with loperamide hydrochloride *RS*.

**B.** Determine by thin-layer chromatography (2.4.17), coating the plate with *octadecylsilyl silica gel*.

*Mobile phase.* A mixture of 40 volumes of *dioxan*, 40 volumes of *methanol* and 20 volumes of *ammonium acetate*.

*Test solution.* Dissolve 0.6 g of the substance under examination in 100 ml of the mobile phase.

*Reference solution (a).* A 0.6 per cent w/v solution of loperamide hydrochloride *RS* in the mobile phase.

*Reference solution (b).* A solution containing 0.6 per cent w/v each of loperamide hydrochloride *RS* and *ketaconazole RS* in the mobile phase.

Apply to the plate 5 \(\mu\)l of each solution. After development, dry the plate in air for 15 minutes and expose it to iodine vapours until the spots appear. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

**C.** Dissolve 50 mg in a mixture of 0.4 ml of *strong ammonia solution* and 2 ml of water. Mix, allow to stand for 5 minutes and filter. Acidify the filtrate with 2 \(M\) *nitric acid*. It gives reaction A of chlorides (2.3.1).

**Tests**

**Appearance of solution.** A 10.0 per cent w/v solution in methanol is clear (2.4.1), and not more intensely coloured than reference solution BYS7 (2.4.1).

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve 0.1 g of the substance under examination in 10 ml of methanol.

*Reference solution (a).* A solution containing 0.0025 per cent w/v each of loperamide hydrochloride *RS* and haloperidol *RS* in methanol.

*Reference solution (b).* Dilute 1 ml of the test solution to 100 ml with methanol. Dilute 5 ml of this solution to 20 ml with methanol.

**Chromatographic system**

- a stainless steel column 10 cm x 4.6 mm, packed with octadecysilane bonded to porous silica (5 \(\mu\)m),
- *mobile phase.* A. a 1.7 per cent w/v solution of tetrabutylammoniumhydrogen sulphate,
  B. *acetonitrile*,
- flow rate. 2 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 254 nm,
- a 10 \(\mu\)l loop injector.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>70</td>
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<td>15</td>
<td>30</td>
<td>70</td>
</tr>
</tbody>
</table>

Equilibrate the column for at least 30 minutes with acetonitrile and then equilibrate at the initial eluent composition for at least 5 minutes.

Adjust the sensitivity of the detector so that the height of the principal peak in the chromatogram obtained with reference solution (b) is 70 per cent to 90 per cent of the full scale of the recorder.

Inject reference solution (a) and record the peak responses. The retention times are: haloperidol, about 3 minutes and loperamide hydrochloride, about 4.5 minutes. The test is not valid unless the resolution between the peaks corresponding to haloperidol and loperamide hydrochloride is at least 8.0.

Inject separately methanol as a blank, the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peaks is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any
peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.4 g, dissolve in 25 ml of anhydrous glacial acetic acid, add 10 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid, using alphアナnaphtolbenzein solution as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.05135 g of C29H33CIN2O2,HCl.

Storage. Store protected from light and moisture.

Loperamide Capsules

Loperamide Hydrochloride Capsules

Loperamide Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the labeled amount of loperamide hydrochloride C29H33ClN2O2,HCl.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 85 volumes of chloroform, 10 volumes of methanol, and 5 volumes of formic acid.

Test solution. To a quantity of the contents of the capsules, containing about 10 mg of Loperamide Hydrochloride, add 10 ml of methanol, shake for 5 minutes, and filter.

Reference solution. A 0.1 per cent w/v solution of loperamide hydrochloride RS in methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and expose to iodine vapour. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2)

Apparatus No. 2

Medium. 500 ml of pH 4.7 acetate buffer, prepared by mixing 200 ml of 1 M acetic acid with 600 ml of water, adjusting with 1 M sodium hydroxide to a pH of 4.70 ± 0.05, diluting with water to 1000 ml and mixing

Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Test solution. The filtrate from the dissolution medium.

Reference solution. A solution of loperamide hydrochloride RS in the dissolution medium containing the same concentration of loperamide hydrochloride as that expected in the dissolution medium in the vessel.

Determine by liquid chromatography (2.4.14) using the chromatographic system described under Assay.

Calculate the content of C29H33ClN2O2,HCl.

D. Not less than 80 per cent of the stated amount of C29H33ClN2O2,HCl.

Uniformity of content. Comply with the test stated under Tablets.

Transfer the contents of one capsule to a 200-ml volumetric flask. Add 35 ml of 0.5 M hydrochloric acid and mix with the aid of ultrasound for 15 minutes. Add 35 ml of acetonitrile and mix with the aid of ultrasound for another 15 minutes. Dilute to volume with a mixture of equal volumes of 0.5 M hydrochloric acid and acetonitrile mix and filter.

Determine by liquid chromatography (2.4.14) using the chromatographic system and the reference solution described under Assay.

Calculate the content of C29H33ClN2O2,HCl in the capsule.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Transfer an accurately weighed portion of the mixed contents of 20 capsules containing about 20 mg of Loperamide Hydrochloride, to a 100-ml volumetric flask. Add about 35 m of 0.5 M hydrochloric acid and mix with the aid of ultrasound for 15 minutes. Add 35 ml of acetonitrile and mix with the aid of ultrasound for an additional 15 minutes. Dilute with a mixture of equal volumes of acetonitrile and 0.5 M hydrochloric acid, mix, and filter. Transfer 5.0 ml of this solution to a 100-ml volumetric flask, dilute to volume with the same solvent mixture and mix.

Reference solution. A 0.001 per cent w/v solution of loperamide hydrochloride RS in a mixture of equal volumes of acetonitrile and water.

Chromatographic system

– a stainless steel column 25 cm x 4 mm, packed with nitrile groups chemically bonded to porous silica particles (10 µm),
– mobile phase: dilute 500 ml of acetonitrile to 1000.0 ml with water, add 20 drops of phosphoric acid, mix, and filter,
– flow rate. 2 ml per minute,
– spectrophotometer set at 220 nm,
– a 50 µl loop injector.

Inject the reference solution. The column efficiency, determined from the analyte peak is not less than 1900 theoretical plates, the capacity factor, is not less than 3.5, and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of C₂₉H₃₃ClN₂O₂,HCl in the capsules.

Loperamide Tablets

Loperamide Hydrochloride Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the labeled amount of loperamide hydrochloride C₂₉H₃₃ClN₂O₂,HCl.

Identification

A. Transfer a quantity of finely powdered tablets containing about 10 mg of Loperamide Hydrochloride, to a test-tube, add 20.0 ml of isopropyl alcohol. shake by mechanical means for one minute, and allow to settle. Dilute 9.0 ml of the supernatant to 10 ml with 0.1 M hydrochloric acid. The solution so obtained shows absorption maxima and minima at the same wavelengths as that of a similar preparation of loperamide hydrochloride RS.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1

Medium. 900 of 0.01 M hydrochloric acid

Speed and time. 50 rpm and 30 minutes.

Withdraw 10 ml of the medium from each vessel, mix and filter.

Test solution. The mixed filtrate from the dissolution medium.

Reference solution. A solution of loperamide hydrochloride RS in methanol to obtain a solution having a known concentration of about 2 mg per ml. Quantitatively dilute this solution with water to obtain a solution having a known concentration of about 0.2 mg per ml.

Reference solution. Dissolve an accurately weighed quantity of loperamide hydrochloride RS in methanol to obtain a solution having a known concentration of about 2 mg per ml. Quantitatively dilute this solution with water to obtain a solution having a known concentration of about 0.2 mg per ml.

Calculate the content of C₂₉H₃₃ClN₂O₂,HCl in the medium.

Uniformity of content. Comply with the test stated under Tablets.

Test solution. Crush 1 tablet and transfer to a 200-ml volumetric flask. Add 4 ml of a 5 per cent w/v solution of phosphoric acid and 20 ml of methanol shake and dilute to volume with water.

Reference solution. Dissolve an accurately weighed quantity of loperamide hydrochloride RS in methanol to obtain a solution having a known concentration of about 2 mg per ml. Quantitatively dilute this solution with water to obtain a solution having a known concentration of about 0.2 mg per ml.

Determine by liquid chromatography (2.4.14) using the chromatographic system described under Assay.

Calculate the content of C₂₉H₃₃ClN₂O₂,HCl in the tablet.

Assay. Determine by liquid chromatography (2.4.14).

Buffer solution. To 3.0 g of triethylamine hydrochloride and 1.0 ml of phosphoric acid add 550 ml of water and mix.

Test solution. Weigh and finely powder 20 Tablets. Transfer an accurately weighed quantity of the powder containing about 16 mg of Loperamide Hydrochloride, to a 200-ml volumetric flask. Add 4 ml of a 5 per cent w/v solution of phosphoric acid and 20 ml of methanol, dilute with water to volume, and mix.

Reference solution. Dissolve an accurately weighed quantity of loperamide hydrochloride RS in methanol to obtain a solution having a known concentration of about 2 mg per ml. Quantitatively dilute this solution with water to obtain a solution having a known concentration of about 0.2 mg per ml.

Transfer 10.0 ml of this solution to a 250-ml volumetric flask, add 5.0 ml of 5 per cent phosphoric acid solution and 25 ml of methanol, dilute with water to 200.0 ml and mix.

Chromatographic system

– a stainless steel column 8 cm x 4 mm, packed with octylsilane chemically bonded to totally porous silica particles (5 µm),
– mobile phase: a mixture of 45 volumes of acetonitrile and 55 volumes of buffer solution,
– flow rate. 2 ml per minute,
– spectrophotometer set at 214 nm,
– a 20 µl loop injector.

Inject the reference solution. The tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of C₂₉H₃₃ClN₂O₂,HCl in the tablets.
**Lopinavir**

![Chemical Structure of Lopinavir](image)

C<sub>37</sub>H<sub>48</sub>N<sub>4</sub>O<sub>5</sub>   Mol. Wt. 628.8

Lopinavir is \((\alpha S)-N\-[(1S,3S,4S)-4-[[2,6-

Lopinavir contains not less than 98.0 per cent and not more than 102.0 per cent of C<sub>37</sub>H<sub>48</sub>N<sub>4</sub>O<sub>5</sub>, calculated on the anhydrous basis.

**Description.** A white or almost white powder.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with lopinavir RS or with the reference spectrum of lopinavir.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to lopinavir in the chromatogram obtained with the reference solution.

**Tests**

**Specific optical rotation** (2.4.22). –22.0° to –26.0°, determined in a 0.4 per cent w/v solution in methanol and calculated on the anhydrous basis.

**Related substances.** Determine by liquid chromatography (2.4.14).

**Solvent mixture.** 70 volumes of a buffer and 30 volumes of acetonitrile, the buffer being prepared by dissolving 2.72 g of potassium dihydrogen phosphate in 900 ml of water, pH of which is adjusted to 2.5 with phosphoric acid, and diluting to 1000 ml with water (solution A).

**Test solution.** Dissolve 0.1 g of the substance under examination in 100 ml of solvent mixture.

**Reference solution.** A 0.01 per cent w/v solution of lopinavir RS in solvent mixture.

**Chromatographic system**

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: gradient mixtures of acetonitrile and the buffer (pH 2.5),
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 210 nm,
- a 20 µl loop injector.

<table>
<thead>
<tr>
<th>Time (in min.)</th>
<th>Buffer (pH 2.5) (per cent v/v)</th>
<th>Acetonitrile (per cent v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>70</td>
<td>30</td>
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<tr>
<td>30</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>40</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>50</td>
<td>20</td>
<td>80</td>
</tr>
</tbody>
</table>

Inject the reference solution. The test is not valid unless the column efficiency determined from the lopinavir peak is not less than 5000 theoretical plates and the tailing factor is not more than 1.5.

Inject separately the test solution and the reference solution. Any secondary peak should not be more than 0.3 per cent and the sum of the areas of all the secondary peaks should not be more than 1.0 per cent when calculated by percentage area normalisation.

**Heavy metals** (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Water** (2.3.43). Not more than 4.0 per cent, determined on 0.1 g.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Dissolve about 50 mg, accurately weighed, of the substance under examination in a suitable quantity of solution A in a 50-ml volumetric flask and dilute to volume with solution A. Dilute 10.0 ml of this solution to 50.0 with solution A.

**Reference solution.** Dissolve about 50 mg, accurately weighed, of lopinavir RS in a suitable quantity of solution A in a 50-ml volumetric flask and dilute to volume with solution A. Dilute 10.0 ml of this solution to 50.0 ml with solution A.

**Chromatographic system**

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: 55 volumes of solution B prepared by mixing 80 volumes of acetonitrile and 20 volumes of methanol and 45 volumes of 0.05 M potassium dihydrogen phosphate, the pH of which is adjusted to 3.0 with dilute phosphoric acid,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- a 20 µl loop injector.
Inject the reference solution. The test is not valid unless the column efficiency determined from the lopinavir peak is not less than 1500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject separately the test solution and the reference solution and measure the responses for the principal peak.

Calculate the content of C_{37}H_{48}N_{4}O_{5}.

**Storage.** Store protected from light and moisture.

**Lopinavir and Ritonavir Capsules**

Lopinavir and Ritonavir Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of lopinavir, C_{37}H_{48}N_{4}O_{5} and ritonavir, C_{37}H_{48}N_{6}O_{5}S_{2}.

**Identification**

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

**Tests**

**Dissolution (2.5.2).**

**Apparatus.** No 1

**Medium.** 900 ml of a solution prepared by dissolving 15.7 g of polyoxyethylene 10-lauryl ether in 1000 ml of a 0.85 per cent v/v solution of hydrochloric acid.

**Speed and time.** 75 rpm and 120 minutes

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14)

**Test solution.** Weigh accurately a quantity of the contents of the capsules containing 100 mg of Lopinavir, disperse in 100 ml of the solvent mixture and filter.

**Reference solution (a).** A 0.1 per cent w/v solution of lopinavir RS in the solvent mixture.

**Reference solution (b).** Dilute 1 ml of reference solution (a) to 100 ml with the solvent mixture.

**Chromatographic system**

- a stainless steel column 25 cm x 4.6 mm, packed with octadecysilane bonded to porous silica (5µm),
- mobile phase: A. a mixture of 45 volumes of acetonitrile and 55 volumes of a buffer solution prepared by dissolving 1.36 g of potassium dihydrogen orthophosphate dihydrate in 1000 ml of water and adjusting the pH to 4.0 with orthophosphoric acid,
- B. a mixture of 80 volumes of acetonitrile and 20 volumes of the buffer solution, flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 210 nm,
- a 20 µl loop injector.

<table>
<thead>
<tr>
<th>Time (in min)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
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<tbody>
<tr>
<td>0</td>
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<tr>
<td>140</td>
<td>100</td>
<td>0</td>
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</tbody>
</table>

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

**For Ritonavir**

**Solvent mixture.** 40 volumes of the buffer solution and 60 volumes of acetonitrile.

**Test solution.** Weigh accurately a quantity of the contents of the capsules containing 50 mg of Ritonavir, disperse in 100 ml of the solvent mixture and filter.

**Reference solution (a).** A 0.05 per cent w/v solution of ritonavir RS in the solvent mixture.
Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with silica gel consisting of porous spherical particles with chemically bonded butyl group (3µm) (such as YMC C4),
- column temperature 60º,
- mobile phase: A. a mixture of 69 volumes of buffer solution prepared by dissolving 4.1 g of monobasic potassium phosphate in 1000 ml of distilled water and filtering, and 18 volumes of acetonitrile, 8 volumes of tetrahydrofuran and 5 volumes of n-Butanol.
  B. a mixture of 40 volumes of the buffer solution, 47 volumes of acetonitrile, 8 volumes of tetrahydrofuran and 5 volumes of n-Butanol,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 240 nm,
- a 50 µl loop injector.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
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<tr>
<td>155</td>
<td>100</td>
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</tbody>
</table>

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 5000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 2.5 times the area of the peak in the chromatogram obtained with the reference solution (b) (2.5 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the peak in the chromatogram obtained with the reference solution (b) (5.0 per cent).

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Mix the contents of 20 capsules. Weigh accurately a quantity of the mixed contents containing 70 mg of Lopinavir and disperse in 100.0 ml of methanol and filter. Dilute 5.0 ml of the filtrate to 50.0 ml with the mobile phase.

Reference solution. A solution containing 0.070 per cent w/v of lopinavir RS and 0.0175 per cent w/v of ritonavir RS in methanol. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 45 volumes of buffer solution prepared by dissolving 6.8 g of potassium dihydrogen orthophosphate dihydrate in 1000 ml with water and adjusting the pH to 3.0 with orthophosphoric acid, 42.5 volumes of acetonitrile and 12.5 volumes of methanol,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the resolution between lopinavir and ritonavir peak is not less than 2.5, the column efficiency for each component is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the contents of C_{37}H_{48}N_{4}O_{5} and C_{37}H_{48}N_{6}O_{5}S_{2} in the capsules.

Storage. Store protected from moisture in a refrigerator (2º to 8º).

Lopinavir and Ritonavir Tablets

Lopinavir and Ritonavir Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of lopinavir, C_{37}H_{48}N_{4}O_{5} and ritonavir, C_{37}H_{48}N_{6}O_{5}S_{2}.

Identification

In the Assay, the principal peaks in the chromatogram obtained with test solution correspond to the principal peaks in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus. No 1

Medium. 900 ml of a solution prepared by dissolving 15.7 g of polyoxyethylene 10 lauryl ether in 1000 ml of a 0.85 per cent v/v solution of hydrochloric acid.

Speed and time. 75 rpm and 120 minutes

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14)

Test solution. The filtrate obtained as given above. Dilute the filtrate if necessary, with the dissolution medium.

Reference solution. A solution containing 0.22 per cent w/v of lopinavir RS and 0.055 per cent w/v of ritonavir RS in
methanol. Dilute 5 ml of the solution to 50 ml with the
dissolution medium.

Use the chromatographic system described under Assay.

Inject the reference solution. The test is not valid unless the
relative standard deviation for replicate injections is not more
than 2.0 per cent.

Inject the test solution and the reference solution.

D. Not less than 70 per cent of the stated amounts of
C_{37}H_{48}N_{4}O_{5} and C_{37}H_{48}N_{6}O_{5}S_{2}.

Related substances. Determine by liquid chromatography
(2.4.14).

For Lopinavir

Test solution. Disperse accurately a quantity of the powdered
tablets containing 100 mg of Lopinavir in 100 ml of the mobile
phase.

Reference solution (a). A 0.1 per cent w/v solution of
lopinavir RS in the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to
100 ml with the mobile phase.

Chromatographic system
– a stainless steel column 25 cm x 4.6 mm, packed with
  octadecylsilane bonded to porous silica (5 mm),
– mobile phase: a mixture of 55 volumes of a buffer solution
  prepared by dissolving 1.36 g of potassium dihydrogen
  orthophosphate in 1000 ml of water and adjusting the
  pH to 4.0 with orthophosphoric acid, and 45 volumes
  of acetonitrile,
– flow rate.1.5 ml per minute,
– spectrophotometer set at 210 nm,
– a 20 µl loop injector.

Inject reference solution (a). The test is not valid unless the
column efficiency is not less than 3000 theoretical plates and
the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the
chromatogram obtained with the test solution, the area of any
secondary peak is not more than the area of the peak in the
chromatogram obtained with the reference solution (b) (1.0
per cent) and the sum of areas of all the secondary peaks is
not more than twice the area of the peak in the chromatogram
obtained with the reference solution (b) (2.0 per cent).

For Ritonavir

Solvent mixture. A mixture of 40 volumes of a buffer solution
prepared by dissolving 4.1 g of potassium dihydrogen
phosphate in 1000 ml of water, and 60 volumes of acetonitrile,

Test solution. Disperse an accurately weighed quantity of the
powdered tablets containing 100 mg of Ritonavir in 100 ml of the
solvent mixture.

Reference solution (a). A 0.1 per cent w/v solution of ritonavir
RS in the solvent mixture.

Reference solution (b). Dilute 1 ml of reference solution (a) to
100 ml with the solvent mixture.

Chromatographic system
– a stainless steel column 15 cm x 4.6 mm, packed with
  silica gel consisting of porous spherical particles with
  chemically bonded with butyl group (3µm) (such as YMC
  C4),
– column temperature 60º,
– mobile phase: A. a mixture of 69 volumes of a buffer
  solution prepared by dissolving 4.1 g of potassium
dihydrogen phosphate in 1000 ml of water, 18 volumes
  of acetonitrile, 8 volumes of tetrahydrofuran and
  5 volumes of n-butanol,
  B. a mixture of 40 volumes of buffer
  solution, 47 volumes of acetonitrile, 8 volumes of
tetrahydrofuran and
  5 volumes of n-butanol,
– flow rate.1 ml per minute,
– a linear gradient programme using the conditions given
  below,
– spectrophotometer set at 240 nm,
– a 50 µl loop injector.

<table>
<thead>
<tr>
<th>Time (in min.)</th>
<th>Mobile phase A (per cent w/v)</th>
<th>Mobile phase B (per cent w/v)</th>
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<tr>
<td>135</td>
<td>100</td>
<td>0</td>
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</tbody>
</table>

Inject reference solution (a). The test is not valid unless the
column efficiency is not less than 3000 theoretical plates and
the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the
chromatogram obtained with the test solution, the area of any
secondary peak is not more than 2.5 times the area of the peak
in the chromatogram obtained with the reference solution (b)
(2.5 per cent) and the sum of areas of all the secondary peaks
is not more than 5 times the area of the peak in the
chromatogram obtained with the reference solution (b)
(5.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Water (2.3.43). Not more than 6.0 per cent, determined on
0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Dissolve an
accurately weighed quantity of the powder containing 200 mg
of Lopinovir in 250.0 ml of methanol. Dilute 5.0 ml of the resulting solution to 50.0 ml with the mobile phase.

Reference solution. 5.0 ml of each of a 0.08 per cent w/v solution of lopinavir RS and a 0.02 per cent w/v solution of ritonavir RS in methanol, diluted to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octysilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 80 volumes of acetonitrile and 20 volumes of methanol,
  B. a mixture of 45 volumes of a buffer solution prepared by dissolving 6.8 g of potassium dihydrogen orthophosphate anhydrous in 1000 ml of water and adjusting the pH to 3.0 with orthophosphoric acid, and 55 volumes of mobile phase A.
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the resolution between the peak due to lopinavir (retention time, about 6 minutes) and the peak due to ritonavir (retention time, about 5 minutes) is less than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the contents of C_{37}H_{48}N_{4}O_{5} and C_{37}H_{48}N_{6}O_{5}S_{2} in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30º.

Losartan Potassium contains not less than 98.0 per cent and not more than 102.0 per cent of C_{22}H_{22}ClKN_{6}O, calculated on the anhydrous basis.

Description. A white to off-white crystalline powder.

Identification
A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with losartan potassium RS or with the reference spectrum of losartan potassium.
B. When examined in the range 200 to 400 nm (2.4.7), a 0.001 per cent w/v solution of methanol and compares with the absorbance obtained with a solution of losartan potassium RS prepared in a similar manner.
C. Gives reaction A of potassium (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 30 mg of the substance under examination in 100 ml of methanol.

Reference solution (a). A 0.03 per cent w/v solution of losartan potassium RS and 0.0002 per cent of triphenylmethanol in methanol.

Reference solution (b). A 0.0003 per cent w/v solution of losartan potassium RS in methanol.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. 0.1 per cent solution of orthophosphoric acid in water and filter,
  B. acetonitrile,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- a 10 µl loop injector.

<table>
<thead>
<tr>
<th>Time (in min.)</th>
<th>Orthophosphoric acid (per cent v/v)</th>
<th>Acetonitrile (per cent v/v)</th>
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<td>25</td>
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<td>35</td>
<td>10</td>
<td>90</td>
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<td>50</td>
<td>75</td>
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</table>

Inject reference solution (a). The relative retention times are about 1.0 for losartan and 1.9 for triphenylmethanol and the tailing factor for losartan is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any Losartan Potassium is monopotassium salt of 4-butyl-4-chloro-1-[[2′-(1H-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]-1H-imidazole-5-methanol.
secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

**Heavy metals** (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

**Water** (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

**Assay.** Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 25 mg of the substance under examination in 100.0 ml of methanol.

*Reference solution.* A 0.025 per cent w/v solution of losartan potassium RS in methanol.

**Chromatographic system**
- a stainless steel column 25 cm x 4.0 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. 0.1 per cent w/v solution of orthophosphoric acid in water and filter,
  B. acetonitrile,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 10 µl loop injector.

<table>
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<tr>
<th>Time (in min.)</th>
<th>Orthophosphoric acid ( per cent v/v)</th>
<th>Acetonitrile ( per cent v/v)</th>
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<td>90</td>
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<td>50</td>
<td>75</td>
<td>25</td>
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</tbody>
</table>

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5, the column efficiency in not less than 5000 theoretical plates. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of C₂₂H₂₂ClKN₆O.

**Storage.** Store protected from moisture.

**Losartan Tablets**

Losartan Potassium Tablets

Losartan Tablets contain Losartan Potassium.

Losartan Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of losartan potassium, C₂₂H₂₂ClKN₆O.

**Identification**

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

**Tests**

**Dissolution** (2.5.2).

Apparatus. No 1

Medium. 900 ml of water.

Speed and time. 50 rpm for 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 250 nm (2.4.7). Calculate the content of C₂₂H₂₂ClKN₆O in the medium from the absorbance obtained from a solution of known concentration of losartan potassium RS in the same medium.

D. Not less than 75 per cent of the stated amount of C₂₂H₂₂ClKN₆O.

**Related substances.** Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablet containing 100 mg of Losartan Potassium, disperse in 100 ml of water and filter.

*Reference solution (a).* A 0.1 per cent w/v solution of losartan potassium RS in water.

*Reference solution (b).* Dilute 1 ml of reference solution (a) to 100 ml with water.

**Chromatographic system**
- a stainless steel column 25 cm x 4.0 mm packed with octasilyl chemically bonded to porous silica (5 µm), (such as Lichrosphere RP8e),
- mobile phase: a mixture of 75 volumes of buffer solution prepared by mixing 770 mg of ammonium acetate in 1000 ml of water; add 2.0 ml of triethylamine, adjust pH to 6.5 with glacial acetic acid and 25 volumes of acetonitrile,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 235 nm,
- a 10 µl loop injector.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 3.0 and the column efficiency in not less than 1000 theoretical plates.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not
more than 2 times the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Transfer intact tablets in a suitable volumetric flask, dissolve in mobile phase and disperse completely. Dilute with mobile phase to obtain a final concentration of 0.0125 per cent w/v.

Reference solution. A 0.125 per cent w/v solution of losartan potassium RS in mobile phase. Dilute 10.0 ml of the solution to 100.0 ml with mobile phase.

Chromatographic system
– a stainless steel column 25 cm x 4.0 mm packed with octasilyl chemically bonded to porous silica (5 µm), (such as Lichrosphere RP8e)
– mobile phase: a mixture of 65 volumes of 0.005 M ammonium acetate, 30 volumes of acetonitrile, 5 volumes of methanol and 0.2 volumes of triethylamine, adjust the pH to 6.6 with glacial acetic acid and filter,
– flow rate. 1 ml per minute,
– spectrophotometer set at 237 nm,
– a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency is not less than 5000 theoretical plates and the relative standard deviation for replicate injections is not more than 2 per cent.

Inject the test solution and the reference solution.

Calculate the content of C22H22ClKN6O.

Storage. Store protected from light and moisture.

Labelling. The label states the strength of Losartan Potassium.

Lyneoestrenol

Lyneoestrenol contains not less than 98.0 per cent and not more than 102.0 per cent of C20H28O, calculated on the dried basis.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with lyneoestrenol RS.

B. In the test of Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Melting range. 161° to 165° (2.4.21).

Tests

Appearance of solution. A 2.0 per cent w/v solution in ethanol (95 per cent) is clear (2.4.1), and colourless (2.4.1).

Specific optical rotation (2.4.22). –9.5° to –11.0°, determined in a 3.6 w/v solution in ethanol (95 per cent).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 80 volumes of n-heptane and 20 volumes of acetone.

Test solution (a). Dissolve 0.5 g of the substance under examination in 100 ml of chloroform.

Test solution (b). Dissolve 0.25 g of the substance under examination in 100 ml of chloroform.

Reference solution (a). A 0.0025 per cent w/v solution of the substance under examination in chloroform.

Reference solution (b). A 0.25 per cent w/v solution of lyneoestrenol RS in chloroform.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with 0.25 M ethanolic sulphuric acid, heat at 105° for 10 minutes and examine in ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 0.5 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.15 g, dissolve in 40 ml of tetrahydrofuran, add 5 ml of a 10 per cent w/v solution of silver nitrate. Titrate with 0.1 M sodium hydroxide, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.
1 ml of \(0.1 \text{ M} \) sodium hydroxide is equivalent to 0.02844 g of \(\text{C}_9\text{H}_{28}\text{O} \).

**Storage.** Store protected from light and moisture.
Magaldrate
Magaldrate Oral Suspension
Magaldrate Tablets
Heavy Magnesium Carbonate
Light Magnesium Carbonate
Magnesium Chloride
Magnesium Hydroxide
Magnesium Hydroxide Oral Suspension
Heavy Magnesium Oxide
Light Magnesium Oxide
Magnesium Stearate
Magnesium Sulphate
Magnesium Trisilicate
Mannitol
Mannitol Injection
Mebendazole
Mebendazole Tablets
Mebeverine Hydrochloride
Mebeverine Tablets
Meclizine Hydrochloride
Meclizine Tablets
Medroxyprogesterone Acetate
Mefenamic Acid
Mefenamic Acid Capsules
Megestrol Acetate
Megestrol Tablets
Melphalan
Melphalan Injection
Melphalan Tablets
Menadione
Menthol
Mephentermine Sulphate
Mephentermine Injection
Mepyramine Maleate
Mepyramine Tablets
Meropenem
Meropenem Injection
Mercaptopurine
Mercaptopurine Tablets
Mestranol
Metformin Hydrochloride
Metformin Tablets
Methadone Hydrochloride
Methadone Injection
Methadone Tablets
Methdilazine Hydrochloride
Methdilazine Tablets
Methotrexate
Methotrexate Injection
Methotrexate Tablets
Methoxamine Hydrochloride
Methoxamine Injection
Methyl Salicylate
Industrial Methylated Spirit
Methylcellulose
Methyldopa
Methyldopa Tablets
Methylergometrine Maleate
Methylergometrine Injection
Methylergometrine Tablets
Methylparaben
Methylprednisolone
Methylprednisolone Tablets
Methylprednisolone Acetate
Methylprednisolone Acetate Injection
Metoclopramide Hydrochloride
Metoclopramide Injection
Metoclopramide Syrup
Metoclopramide Tablets
Metoprolol Tartrate
Metoprolol Tablets
Metronidazole
Metronidazole Benzoate
Metronidazole Benzoate Oral Suspension
Metronidazole Injection
Metronidazole Tablets
Mexiletine Hydrochloride
Mexiletine Capsules
Mexiletine Injection
Mianserin Hydrochloride
Mianserin Tablets
Miconazole Nitrate
Miconazole Cream
Miconazole Pessaries
Microcrystalline Cellulose
Microcrystalline Wax
Minoxidil
Minoxidil Tablets
Monothioglycerol
Morphine Sulphate
Morphine And Atropine Injection
Morphine Injection
Multiple Electrolytes And Dextrose Injection Type I
Multiple Electrolytes And Dextrose Injection Type II
Multiple Electrolytes And Dextrose Injection Type III
Multiple Electrolytes And Dextrose Injection Type IV
Multiple Electrolytes And Dextrose Injection Type V  ....
Multiple Electrolytes Injection Type VI  ....
Mustine Hydrochloride  ....
Mustine Injection  ....
Magaldrate

\[ \text{Al}_5\text{Mg}_{10}(\text{OH})_{31}(\text{SO}_4)_2 \cdot x\text{H}_2\text{O} \quad \text{Mol. Wt. 1097.4 (anhydrous)} \]

Magaldrate is a chemical combination of aluminium and magnesium hydroxides and sulphates corresponding approximately to the formula \( \text{Al}_5\text{Mg}_{10}(\text{OH})_{31}(\text{SO}_4)_2 \cdot x\text{H}_2\text{O} \). Magaldrate contains not less than 90.0 per cent and not more than 105.0 per cent of \( \text{Al}_5\text{Mg}_{10}(\text{OH})_{31}(\text{SO}_4)_2 \), calculated on the dried basis.

**Description.** A white or almost white, crystalline powder; odourless.

**Identification**

A. Dissolve 0.8 g in 20 ml of \( 3 \text{ M hydrochloric acid} \), dilute with \( \text{water} \) to 50 ml, add 3 drops of methyl red solution and heat to boiling. Add dilute ammonia solution until the colour changes to just yellow, continue boiling for 2 minutes and filter; the filtrate gives the reactions of magnesium salts (2.3.1).

B. Wash the precipitate obtained in test A with 50 ml of a hot 2 per cent w/v solution of ammonium chloride, then dissolve the precipitate in 15 ml of \( 3 \text{ M hydrochloric acid} \); the solution gives the reactions of aluminium salts (2.3.1).

**Tests**

**Arsenic** (2.3.10). To 1.0 g add 15 ml of \( \text{hydrochloric acid} \), 0.1 ml of stannous chloride solution \( \text{As}T \) and 5 ml of potassium iodide solution and allow to stand for 15 minutes. The resulting solution complies with the limit test for arsenic (10 ppm).

**Heavy metals** (2.3.13). Dissolve 0.33 g in 10 ml of \( 3 \text{ M hydrochloric acid} \), filtering to get a clear solution and diluting to 25 ml with \( \text{water} \). The solution complies with the limit test for heavy metals, Method A (60 ppm).

**Soluble chloride.** Boil 1.0 g, accurately weighed, with 50.0 ml of \( \text{water} \) for 5 minutes, cool, add \( \text{water} \) to the original volume, mix and filter. To 25.0 ml of the filtrate add 0.1 ml of potassium chromate solution and titrate with 0.1 M silver nitrate until a persistent pink colour is obtained; Not more than 5.0 ml of 0.1 M silver nitrate is required (3.5 per cent).

**Soluble sulphate.** Dilute 2.5 ml of the filtrate obtained in the test for Soluble chloride to 40 ml with \( \text{water} \) in a Nessler cylinder, add 1 ml of \( 3 \text{ M hydrochloric acid} \) and 3 ml of barium chloride solution, dilute to 50 ml, mix and allow to stand for 10 minutes; any turbidity produced is not greater than that produced by treating 1.0 ml of 0.01 M sulphuric acid in the same manner (1.9 per cent).

**Sodium.** Transfer 2.0 g, accurately weighed, to a 100-ml volumetric flask, place in an ice-bath, add 5 ml of nitric acid and swirl to dissolve. Allow to warm to room temperature, dilute with \( \text{water} \) to volume and mix. Filter, if necessary, to obtain a clear solution. Dilute 10.0 ml of the filtrate with \( \text{water} \) to 100.0 ml. The emission intensity of this solution, determined by flame photometry (2.4.4), at about 589 nm and corrected for background transmission at about 580 nm, is not greater than that produced by treating similarly a standard solution containing 2.2 \( \mu \text{g} \) of Na per ml.

**Aluminium hydroxide.** 32.1 to 45.9 per cent of \( \text{Al(OH)}_3 \), calculated on the dried basis and determined by the following method. Dissolve about 100 mg, accurately weighed, in 3 ml of \( \text{dilute hydrochloric acid} \) and dilute to 30 ml with \( \text{water} \). Add with stirring 25.0 ml of 0.05 M disodium edetate, mix and allow to stand for 5 minutes. Add 20 ml of acetic acid-ammonium acetate buffer, 60 ml ethanol (95 per cent) and 2 ml of dithizone solution and titrate with 0.05 M zinc sulphate to a bright rose-pink colour. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of disodium edetate required.

1 ml of 0.05 M disodium edetate is equivalent to 0.0039 g of \( \text{Al(OH)}_3 \).

**Magnesium hydroxide.** 49.2 to 66.6 per cent of \( \text{Mg(OH)}_2 \), calculated on the dried basis and determined by the following method. Dissolve about 100 mg, accurately weighed, in 3 ml of \( \text{dilute hydrochloric acid} \) and dilute with \( \text{water} \) to about 200 ml. Add with stirring 1 g of ammonium chloride, 20 ml of triethanolamine, 10 ml of ammonia-ammonium chloride buffer and 0.1 ml of eriochrome black \( T \) solution and titrate with 0.05 M disodium edetate to a blue colour. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of disodium edetate required.

1 ml of 0.05 M disodium edetate is equivalent to 0.002916 g of \( \text{Mg(OH)}_2 \).

**Sulphates.** 16.0 to 21.0 per cent, calculated on the dried basis and determined by the following method. Weigh accurately about 0.875 g, transfer to a 25-ml volumetric flask, dissolve in 10 ml of \( \text{water} \) and 5 ml of glacial acetic acid, dilute to volume with \( \text{water} \) and mix. Transfer 5.0 ml of this solution to a glass chromatographic column, 1 cm in internal diameter, prepared by filling with 15 ml of a strongly acidic styrene-divinylbenzene cation exchange resin (50 to 100 mesh) (such as Dowex 50W-X8 or Amberlite 120) and washing the resin with 30 ml of \( \text{water} \). Elute the column with 15 ml of \( \text{water} \) and collect the eluate in a 125-ml conical flask. To the eluate add 5 ml of a 5.38 per cent w/v solution of sodium alizarin sulphonate as the indicator and adding about 5 ml of the titrant in the beginning and continuing the titration slowly thereafter until the yellow colour disappears and a pink tinge is visible.

1 ml of 0.05 M barium chloride is equivalent to 0.004803 g of \( \text{SO}_4 \_2^\text{\textsuperscript{\textminus}} \).
Microbial contamination (2.2.9). 1 g is free from *Escherichia coli*.

Loss on drying (2.4.19). 10.0 to 20.0 per cent, determined on 0.5 g by drying in an oven at 200° for 4 hours.

Assay. Weigh accurately about 3.0 g and transfer to a 250-ml conical flask. Add 100.0 ml of 1 M hydrochloric acid and stir well until a clear solution is obtained. Titrinate the excess acid with 1 M sodium hydroxide to a pH of 3.0, determined potentiometrically (2.4.25). Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of hydrochloric acid required.

1 ml of 1 M hydrochloric acid is equivalent to 0.0354 g of Al$_5$Mg$_{10}$(OH)$_{31}$(SO$_4$)$_2$.

Storage. Store protected from moisture.

Magaldrate Oral Suspension

Magaldrate Suspension

Magaldrate Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous magaldrate, Al$_5$Mg$_{10}$(OH)$_{31}$(SO$_4$)$_2$.

Identification

A. Dissolve an amount of the suspension containing about 0.8 g of anhydrous magaldrate in 20 ml of 3 M hydrochloric acid, dilute with water to 50 ml, add 3 drops of methyl red solution and heat to boiling. Add dilute ammonia solution until the colour changes to just yellow, continue boiling for 2 minutes and filter; the filtrate gives the reactions of magnesium salts (2.3.1).

B. Wash the precipitate obtained in test A with 50 ml of a hot 2 per cent w/v solution of ammonium chloride, then dissolve the precipitate in 15 ml of 3 M hydrochloric acid; the solution gives the reactions of aluminium salts (2.3.1).

Tests

Aluminium hydroxide. 32.1 to 45.9 per cent of the stated content of anhydrous magaldrate, determined by the following method. To an accurately measured quantity containing about 1.0 g of magaldrate add 30 ml of dilute hydrochloric acid, shake to dissolve, dilute to 100.0 ml with water and mix (solution A). To 10.0 ml of this solution add 20 ml of water and add with stirring 25.0 ml of 0.05 M disodium edetate, mix and allow to stand for 5 minutes. Add 20 ml of acetic acid-ammonium acetate buffer, 60 ml ethanol (95 per cent) and 2 ml of dithizone solution and titrate with 0.05 M zinc sulphate to a bright rose-pink colour. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of disodium edetate required.

1 ml of 0.05 M disodium edetate is equivalent to 0.0039 g of Al(OH)$_3$.

Magnesium hydroxide. 49.2 to 66.6 per cent of the stated content of anhydrous magaldrate, determined by the following method. Take 10.0 ml of solution A and dilute with water to about 200 ml. Add with stirring 1 g of ammonium chloride, 20 ml of triethanolamine, 10 ml of ammonia-ammonium chloride buffer and 0.1 ml of eriochrome black T solution and titrate with 0.05 M disodium edetate to a blue colour. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of disodium edetate required.

1 ml of 0.05 M disodium edetate is equivalent to 0.002916 g of Mg(OH)$_2$.

Neutralising capacity. To an accurately weighed quantity of the well-shaken suspension containing 0.8 g of anhydrous magaldrate in a 250-ml beaker add water to make a total volume of about 70 ml, heat to 37° and stir continuously, maintaining the temperature at 37°. Add 30.0 ml of 1 M hydrochloric acid previously heated to 37° and maintain at 37° for 15 minutes, stirring continuously. Titrate the excess acid with 1 M sodium hydroxide to a pH of 3.5. Not more than 12 ml of 1 M sodium hydroxide is required.

Microbial contamination (2.2.9). Total microbial count, not more than 100 per ml; 0.01 ml is free from *Escherichia coli*.

Other tests. Complies with the tests stated under Oral Liquids.

Assay. To an accurately measured volume containing about 3.0 g of anhydrous magaldrate in a beaker add 100.0 ml of 1 M hydrochloric acid and stir well until a solution is obtained. Titrating the excess acid with 1 M sodium hydroxide to a pH of 3.0, determined potentiometrically (2.4.25). Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of hydrochloric acid required.

1 ml of 1 M hydrochloric acid is equivalent to 0.0354 g of Al$_5$Mg$_{10}$(OH)$_{31}$(SO$_4$)$_2$.

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of mg of anhydrous magaldrate per 5 ml.

Magaldrate Tablets

Magaldrate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous magaldrate, Al$_5$Mg$_{10}$(OH)$_{31}$(SO$_4$)$_2$.

Identification

To a quantity of the powdered tablets containing about 2 g of anhydrous magaldrate, add about 60 ml of water, shake for
3 minutes, centrifuge and discard the supernatant solution. Repeat the washing with three more quantities, each of 60 ml, of water. Transfer the residue to a beaker and heat on a water-bath to dryness.

A. Dissolve 0.8 g of the residue in 20 ml of 3 M hydrochloric acid, dilute with water to 50 ml, add 3 drops of methyl red solution and heat to boiling. Add dilute ammonia solution until the colour changes to just yellow, continue boiling for 2 minutes and filter; the filtrate gives the reactions of aluminium salts (2.3.1).

B. Wash the precipitate obtained in test A with 50 ml of a hot 2 per cent w/v solution of ammonium chloride, then dissolve the precipitate in 15 ml of 3 M hydrochloric acid; the solution gives the reactions of aluminium salts (2.3.1).

Tests

Aluminium hydroxide. 32.1 to 45.9 per cent of the stated content of anhydrous magaldrate, determined by the following method. Weigh and finely powder 20 tablets. To an accurately weighed quantity of the powder containing about 1.0 g of anhydrous magaldrate add 30 ml of dilute hydrochloric acid, shake well to dissolve, dilute to 100.0 ml with water and mix (solution A). To 10.0 ml of this solution add 20 ml of water and add with stirring 25.0 ml of 0.05 M disodium edetate, mix and allow to stand for 5 minutes. Add 20 ml of acetic acid-ammonium acetate buffer, 60 ml ethanol (95 per cent) and 2 ml of dithizone solution and titrate with 0.05 M zinc sulphate to a bright rose-pink colour. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of disodium edetate required.

1 ml of 0.05 M disodium edetate is equivalent to 0.0039 g of Al(OH)₃.

Magnesium hydroxide. 49.2 to 66.6 per cent of the stated content of anhydrous magaldrate, determined by the following method. Take 10.0 ml of solution A and dilute with water to about 200 ml. Add with stirring 1 g of ammonium chloride, 20 ml of triethanolamine, 10 ml of ammonia-ammonium chloride buffer and 0.1 ml of eriochrome black T solution and titrate with 0.05 M disodium edetate to a blue colour. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of disodium edetate required.

1 ml of 0.05 M disodium edetate is equivalent to 0.002916 g of Mg(OH)₂.

Neutralising capacity. To an accurately weighed quantity of the powdered tablets containing 0.8 g of anhydrous magaldrate in a 250-ml beaker add about 75 ml of water heat to 37° and stir continuously, maintaining the temperature at 37°. Add 30.0 ml of 1 M hydrochloric acid previously heated to 37° and maintain at 37° for 15 minutes, stirring continuously. Titrate the excess acid with 1 M sodium hydroxide to a pH of 3.5 determined potentiometrically (2.4.25). Not more than 12 ml of 1 M sodium hydroxide is required.

Disintegration (2.5.1). 2 minutes for tablets labelled to be swallowed.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and finely powder 20 tablets. To an accurately weighed quantity of the powder containing about 3.0 g of anhydrous magaldrate in a 200-ml volumetric flask add 100.0 ml of 1 M hydrochloric acid, shake well for 30 minutes. Dilute to volume and filter. Transfer 100.0 ml to a conical flask. Titrate the excess acid with 1 M sodium hydroxide to a pH of 3.0, determined potentiometrically (2.4.25). Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of hydrochloric acid required.

1 ml of 1 M hydrochloric acid is equivalent to 0.03540 g of Al₅Mg₁₀(OH)₁₆(SO₄)₂.

Storage. Store protected from moisture.

Labelling. The label states (1) the strength in terms of the equivalent amount of anhydrous magaldrate; (2) whether the tablets are to be swallowed or chewed.

Heavy Magnesium Carbonate

Heavy Magnesium Carbonate is a hydrated basic magnesium carbonate.

Heavy Magnesium Carbonate contains the equivalent of not less than 40.0 per cent and not more than 45.0 per cent of MgO.

Description. A white powder; odourless. 15 g occupies a volume of about 30 ml.

Identification

A. Gives reaction A of carbonates (2.3.1).

B. Dissolve about 15 mg in 2 ml of 2 M nitric acid and neutralise with 2 M sodium hydroxide. The resulting solution gives reaction A of magnesium salts (2.3.1).

Tests

Appearance of solution. Dissolve 5.0 g in a mixture of 70 ml of 5 M acetic acid and 30 ml of water. Filter, if necessary, through a previously ignited and weighed porcelain or silica crucible of a suitable porosity to give a clear filtrate (solution A). Reserve any residue (residue R) for the test for Substances insoluble in acetic acid. Solution A is not more intensely coloured than reference solution BS3 (2.4.1).
**Arsenic** (2.3.10). Dissolve 5.0 g in 15 ml of brominated hydrochloric acid and 45 ml of water and remove the excess of bromine with a few drops of stannous chloride solution \( \text{AsT} \). The resulting solution complies with the limit test for arsenic (2 ppm).

**Heavy metals** (2.3.13). To 20 ml of solution A add 15 ml of 7 \( M \) hydrochloric acid and shake with 25 ml of 4-methylpentan-2-one for 2 minutes. Separate the layers, evaporate the aqueous layer to dryness, dissolve the residue in 1 ml of 5 \( M \) acetic acid and dilute to 20 ml with water. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (20 ppm). Use lead standard solution (1 ppm Pb) for standard preparation.

**Iron** (2.3.14). Dissolve 0.1 g in 3 ml of 2 \( M \) hydrochloric acid and dilute to 10 ml with water. The resulting solution complies with the limit test for iron (400 ppm).

**Chlorides** (2.3.12). 5.0 ml of solution A complies with the limit test for chlorides (0.1 per cent).

**Sulphates** (2.3.17). 0.5 ml of solution A diluted to 15 ml with distilled water complies with the limit test for sulphates (0.6 per cent).

**Calcium.** To 0.2 ml of ethanolic calcium standard solution (100 ppm Ca) add 1 ml of ammonium oxalate solution. After 1 minute add a mixture of 1 ml of 2 \( M \) acetic acid and 15 ml of a solution prepared by diluting 2.6 ml of solution A to 150 ml with distilled water and shake. After 15 minutes any opalescence produced is not more intense than that of a standard prepared in the same manner using a mixture of 10 ml of calcium standard solution (10 ppm Ca) and 5 ml of distilled water in place of solution A (0.75 per cent).

**Copper.** Dissolve 1 g in 5 ml of hydrochloric acid and 25 ml of water, boil to remove carbon dioxide, make alkaline with dilute ammonia solution; no blue colour is produced.

**Soluble substances.** Mix 2.0 g with 100 ml of water, boil for 5 minutes, filter whilst hot through a sintered-glass filter (porosity No. 3), allow to cool and dilute to 100.0 ml with water. Evaporate 50.0 ml of the filtrate to dryness and dry the residue at 105\(^\circ\); the residue weighs not more than 10 mg (1.0 per cent).

**Substances insoluble in acetic acid.** Residue R when washed, dried and ignited at 600\(^\circ\), weighs not more than 2.5 mg (0.05 per cent).

**Assay.** Weigh accurately about 0.15 g, dissolve in a mixture of 20 ml of water and 2 ml of 2 \( M \) hydrochloric acid and add 50 ml of water, 10 ml of strong ammonia-ammonium chloride solution and titrate with 0.05 \( M \) disodium edetate, using 0.1 g of mordant black II mixture as indicator, until a blue colour is obtained.

1 ml of 0.05 \( M \) disodium edetate is equivalent to 0.002015 g of MgO.

**Storage.** Store protected from moisture.

### Light Magnesium Carbonate

Light Magnesium Carbonate is a hydrated basic magnesium carbonate.

Light Magnesium Carbonate contains the equivalent of not less than 40.0 per cent and not more than 45.0 per cent of MgO.

**Description.** A very light, white powder. 15 g occupies a volume of about 125 ml.

**Identification**

A. Gives reaction A of carbonates (2.3.1).

B. Dissolve about 15 mg in 2 ml of 2 \( M \) nitric acid and neutralise with 2 \( M \) sodium hydroxide. The resulting solution gives reaction A of magnesium salts (2.3.1).

**Tests**

**Appearance of solution.** Dissolve 5.0 g in a mixture of 70 ml of 5 \( M \) acetic acid and 30 ml of water. Filter, if necessary, through a previously ignited and weighed porcelain or silica crucible of a suitable porosity to give a clear filtrate (solution A). Reserve any residue (residue R) for the test for Substances insoluble in acetic acid. Solution A is not more intensely coloured than reference solution BS3 (2.4.1).

**Arsenic** (2.3.10). Dissolve 5.0 g in 15 ml of brominated hydrochloric acid and 45 ml of water and remove the excess of bromine with a few drops of stannous chloride solution \( \text{AsT} \). The resulting solution complies with the limit test for arsenic (2 ppm).

**Heavy metals** (2.3.13). To 20 ml of solution A add 15 ml of 7 \( M \) hydrochloric acid and shake with 25 ml of 4-methylpentan-2-one for 2 minutes. Separate the layers, evaporate the aqueous layer to dryness, dissolve the residue in 1 ml of 5 \( M \) acetic acid and dilute to 20 ml with water. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (20 ppm). Use lead standard solution (1 ppm Pb) to prepare the standard.

**Iron** (2.3.14). Dissolve 0.1 g in 3 ml of 2 \( M \) hydrochloric acid and dilute to 10 ml with water. The resulting solution complies with the limit test for iron (400 ppm).

**Chlorides** (2.3.12). 5.0 ml of solution A complies with the limit test for chlorides (0.1 per cent).

**Calcium**. To 0.2 ml of ethanolic calcium standard solution (100 ppm Ca) add 1 ml of ammonium oxalate solution. After 1 minute add a mixture of 1 ml of 2 \( M \) acetic acid and 15 ml of a solution prepared by diluting 2.6 ml of solution A to 150 ml with distilled water and shake. After 15 minutes any opalescence produced is not more intense than that of a standard prepared in the same manner using a mixture of 10 ml of calcium standard solution (10 ppm Ca) and 5 ml of distilled water in place of solution A (0.75 per cent).

**Copper**. Dissolve 1 g in 5 ml of hydrochloric acid and 25 ml of water, boil to remove carbon dioxide, make alkaline with dilute ammonia solution; no blue colour is produced.

**Soluble substances.** Mix 2.0 g with 100 ml of water, boil for 5 minutes, filter whilst hot through a sintered-glass filter (porosity No. 3), allow to cool and dilute to 100.0 ml with water. Evaporate 50.0 ml of the filtrate to dryness and dry the residue at 105\(^\circ\); the residue weighs not more than 10 mg (1.0 per cent).

**Substances insoluble in acetic acid.** Residue R when washed, dried and ignited at 600\(^\circ\), weighs not more than 2.5 mg (0.05 per cent).

**Assay.** Weigh accurately about 0.15 g, dissolve in a mixture of 20 ml of water and 2 ml of 2 \( M \) hydrochloric acid and add 50 ml of water, 10 ml of strong ammonia-ammonium chloride solution and titrate with 0.05 \( M \) disodium edetate, using 0.1 g of mordant black II mixture as indicator, until a blue colour is obtained.

1 ml of 0.05 \( M \) disodium edetate is equivalent to 0.002015 g of MgO.

**Storage.** Store protected from moisture.
1 minute add a mixture of 1 ml of 2 M acetic acid and 15 ml of a solution prepared by diluting 2.6 ml of solution A to 150 ml with distilled water and shake. After 15 minutes any opalescence produced is not more intense than that of a standard prepared in the same manner using a mixture of 10 ml of calcium standard solution (10 ppm Ca) and 5 ml of distilled water in place of solution A (0.75 per cent).

**Copper.** Dissolve 1 g in 5 ml of hydrochloric acid and 25 ml of water, boil to remove carbon dioxide, make alkaline with dilute ammonia solution; no blue colour is produced.

**Soluble substances.** Mix 2.0 g with 100 ml of water, boil for 5 minutes, filter whilst hot through a sintered-glass filter (porosity No. 3), allow to cool and dilute to 100.0 ml with water. Evaporate 50.0 ml of the filtrate to dryness and dry the residue at 105°; the residue weighs not more than 10 mg (1.0 per cent).

**Substances insoluble in acetic acid.** Residue R when washed, dried and ignited at 600°, weighs not more than 2.5 mg (0.05 per cent).

**Sulphates** (2.3.17). 1 ml of solution A diluted to 15 ml with distilled water complies with the limit test for sulphates (0.3 per cent).

**Assay.** Weigh accurately about 0.15 g, dissolve in a mixture of 20 ml of water and 2 ml of 2 M hydrochloric acid and add 50 ml of water, 10 ml of strong ammonia-ammonium chloride solution and titrate with 0.05 M disodium edetate, using 0.1 g of mordant black II mixture as indicator, until a blue colour is obtained.

1 ml of 0.05 M disodium edetate is equivalent to 0.002015 g of MgO.

**Storage.** Store protected from moisture

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**Magnesium Chloride**

MgCl₂·6H₂O Mol. Wt. 203.3

Magnesium Chloride contains not less than 98.0 per cent and not more than 101.0 per cent of MgCl₂·6H₂O.

**Description.** Colourless crystals; hygroscopic.

**Identification.** Colourless crystals; hygroscopic.

**Tests**

**Appearance of solution.** Solution A is clear (2.4.1), and colourless (2.4.1).
Magnesium Hydroxide

Mg (OH)₂

Mg (OH)₂ contains not less than 95.0 per cent and not more than 100.5 per cent of Mg(OH)₂.

Description. A bulky white powder.

Identification

Dissolve about 15 mg in 2 ml of 2 M nitric acid and neutralise with 2 M sodium hydroxide. The resulting solution gives reaction A of magnesium salts (2.3.1).

Tests

Appearance of solution. Dissolve 5.0 g in a mixture 50 ml of 5 M acetic acid and 50 ml of distilled water; not more than a slight effervescence is produced. Boil for 2 minutes, cool and dilute to 100 ml with 2 M acetic acid. Filter, if necessary, through a previously ignited and weighed porcelain or silica crucible of a suitable porosity to give a clear filtrate (solution A). Reserve any residue (residue R) for the test for Substances insoluble in acetic acid. Solution A is not more intensely coloured than reference solution BS3 (2.4.1).

Arsenic (2.3.10). Dissolve 2.5 g in 18 ml of brominated hydrochloric acid and 42 ml of water and remove the excess of bromine with a few drops of stannous chloride solution AsT. The resulting solution complies with the limit test for arsenic (4 ppm).

Heavy metals (2.3.13). To 20 ml of solution A add 15 ml of 7 M hydrochloric acid and shake with 25 ml of 4-methylpentan-2-one for 2 minutes. Separate the layers, evaporate the aqueous layer to dryness, dissolve the residue in 1 ml of 5 M acetic acid and dilute to 20 ml with water. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (30 ppm). Use lead standard solution (2 ppm Pb) to prepare the standard.

Iron (2.3.14). Dissolve 0.2 g in 7 ml of 2 M hydrochloric acid and dilute to 20 ml with water. 5 ml of the resulting solution complies with the limit test for iron (0.08 per cent).

Chlorides (2.3.12). 5.0 ml of solution A diluted to 15 ml with distilled water complies with the limit test for chlorides (0.1 per cent).

 Sulphates (2.3.17). 0.6 ml of solution A diluted to 15 ml with distilled water complies with the limit test for sulphates (0.5 per cent).

Calcium. To 0.2 ml of ethanolic calcium standard solution (100 ppm Ca) add 1 ml of ammonium oxalate solution. After 1 minute add a mixture of 1 ml of 2 M acetic acid and 15 ml of a solution prepared by diluting 1.3 ml of solution A to 150 ml with distilled water and shake. After 15 minutes any opalescence produced is not more intense than that of a standard prepared in the same manner using a mixture of 10 ml of calcium standard solution (10 ppm Ca) and 5 ml of distilled water in place of solution A (1.5 per cent).

Soluble substances. Mix 2.0 g with 100 ml of water, boil for 5 minutes, filter whilst hot through a sintered-glass filter (porosity No. 3), allow to cool and dilute to 100.0 ml with water. Evaporate 50.0 ml of the filtrate to dryness and dry the residue at 105°C; the residue weighs not more than 10 mg (1.0 per cent).

Substances insoluble in acetic acid. Residue R when washed, dried and ignited at 600°C, weighs not more than 5.0 mg (0.1 per cent).

Loss on ignition (2.4.20). 30.0 to 32.5 per cent, determined on 0.5 g by igniting at 900°C increasing the heat gradually.

Assay. Weigh accurately about 0.1 g, dissolve in 20 ml of 2 M hydrochloric acid and dilute to 100.0 ml with water. To 50.0 ml of this solution add 5 ml of strong ammonia-ammonium chloride solution and titrate with 0.05 M disodium edetate, using about 50 mg of mordant black II mixture as indicator. 1 ml of 0.05 M disodium edetate is equivalent to 0.002916 g of Mg(OH)₂.

Storage. Store protected from moisture.

Magnesium Hydroxide Oral Suspension

Magnesium Hydroxide Mixture; Milk of Magnesia; Cream of Magnesia

Magnesium Hydroxide Oral Suspension is an aqueous suspension of hydrated magnesium oxide. It may be prepared from a suitable grade of Light Magnesium Oxide.

Magnesium Hydroxide Oral Suspension contains not less than 7.0 per cent and not more than 8.5 per cent w/w of hydrated magnesium oxide, calculated as Mg(OH)₂.

Description. A white, uniform suspension, which does not separate readily on standing.

Identification

A solution of 1 ml in 2 ml of dilute hydrochloric acid gives the reactions of magnesium salts (2.3.1).

Tests

Soluble alkalis. Filter about 25 ml and discard the first 10 ml of the filtrate. Dilute 5 ml of the filtrate with 40 ml of water, add 0.05 ml of methyl red solution and titrate with 0.05 M sulphuric
acid to a persistent pink colour. Not more than 1.0 ml of 0.05 M sulphuric acid is required.

**Soluble salts.** To 5 ml of the clear filtrate obtained in the test for Soluble alkalis add 0.15 ml of sulphuric acid, evaporate to dryness on a water-bath and then ignite gently to constant weight; the residue does not weigh more than 12 mg.

**Heavy metals** (2.3.13). Dissolve 12.5 g in 10 ml of hydrochloric acid and 20 ml of water, add 0.5 ml of nitric acid, boil to remove any carbon dioxide and filter. To the cooled filtrate add 2 g of ammonium chloride and 2 g of ammonium thiocyanate and extract with two successive quantities, each of 10 ml, of ether. To the aqueous layer add 2 g of citric acid and sufficient water to produce 50 ml. 12 ml of the solution complies with the limit test for heavy metals, Method D (4 ppm). Use lead standard solution (1 ppm Pb) to prepare the standard.

**Sulphates** (2.3.17). Dissolve 2.5 ml in 20 ml of hydrochloric acid and dilute to 500 ml with water. 15 ml of the resulting solution, filtered if necessary, complies with the limit test for sulphates (0.2 per cent).

**Microbial contamination** (2.2.9). Total microbial count, not more than 100 per ml; 1 ml is free from *Escherichia coli*.

**Other tests.** Complies with the tests stated under Oral Liquids.

**Assay.** Weigh accurately about 10.0 g, mix with 50 ml of water, add 50.0 ml of 0.5 M sulphuric acid and titrate the excess of acid with 1 M sodium hydroxide using methyl orange solution as indicator.

1 ml of 0.5 M sulphuric acid is equivalent to 0.02916 g of hydrated magnesium oxide calculated as Mg(OH)$_2$.

**Storage.** Store protected from moisture. Do not keep in a refrigerator.

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**Heavy Magnesium Oxide**

Heavy Magnesia

MgO \[\text{Mol. Wt. 40.3}\]

Heavy Magnesium Oxide contains not less than 98.0 per cent and not more than 100.5 per cent of MgO, calculated on the ignited basis.

**Description.** A fine, white powder. 15 g occupies a volume of about 30 ml.

**Identification.** Dissolve about 15 mg in 2 ml of 2 M nitric acid and neutralise with 2 M sodium hydroxide. The resulting solution gives reaction A of magnesium salts (2.3.1).

**Tests**

**Appearance of solution.** Dissolve 5.0 g in a mixture of 70 ml of 5 M acetic acid and 30 ml of water. Filter, if necessary, through a previously ignited and weighed porcelain or silica crucible of a suitable porosity to give a clear filtrate (solution A). Reserve any residue (residue R). Solution A is not more intensely coloured than reference solution BS3 (2.4.1).

**Arsenic** (2.3.10). Dissolve 2.5 g in 15 ml of brominated hydrochloric acid, add 45 ml of water and remove the excess of bromine with a few drops of stannous chloride solution AsT. The resulting solution complies with the limit test for arsenic (4 ppm).

**Heavy metals** (2.3.13). 20 ml of solution A complies with the limit test for heavy metals, Method D (30 ppm).

**Iron** (2.3.14). Dissolve 0.2 g in 7 ml of 2 M hydrochloric acid and dilute to 20 ml with water. 5 ml of the resulting solution complies with the limit test for iron (800 ppm).

**Chlorides** (2.3.12). 5.0 ml of solution A complies with the limit test for chlorides (0.1 per cent).

**Sulphates** (2.3.17). 0.3 ml of solution A complies with the limit test for sulphates (1.0 per cent).

**Calcium.** To 0.2 ml of ethanolic calcium standard solution (100 ppm Ca) add 1 ml of ammonium oxalate solution. After 1 minute add a mixture of 1 ml of 2 M acetic acid and 15 ml of a solution prepared by diluting 1.3 ml of solution A to 150 ml with distilled water and shake. After 1 minutes any opalescence produced is not more intense than that of a standard prepared in the same manner using a mixture of 10 ml of calcium standard solution (10 ppm Ca) and 5 ml of distilled water in place of solution A (1.5 per cent).

**Soluble substances.** Mix 2.0 g with 100 ml of water, boil for 5 minutes, filter whilst hot through a sintered-glass filter (porosity No. 3), allow to cool and dilute to 100.0 ml with water. Evaporate 50.0 ml of the filtrate to dryness and dry the residue at 105°. The residue weighs not more than 20 mg (2.0 per cent).

**Substances insoluble in acetic acid.** Residue R when washed with water, dried and ignited at 600°, weighs not more than 5 mg (0.1 per cent).

**Loss on ignition** (2.4.20). Not more than 8.0 per cent, determined on 0.5 g when ignited at 900°.

**Assay.** Weigh accurately about 0.35 g, dissolve in 10 ml of 2 M hydrochloric acid and dilute with water to 100.0 ml. To 10.0 ml add 5 ml of strong ammonia-ammonium chloride solution and titrate with 0.05 M disodium edetate using about 50 mg of mordant black II mixture as indicator.

1 ml of 0.05 M disodium edetate is equivalent to 0.002015 g of MgO.

**Storage.** Store protected from moisture.
Light Magnesium Oxide

Light Magnesia

MgO Mol. Wt. 40.3

Light Magnesium Oxide contains not less than 98.0 per cent and not more than 100.5 per cent of MgO, calculated on the ignited basis.

**Description.** A very fine, light, white powder. 15 g occupies a volume of about 150 ml.

**Tests**

**Appearance of solution.** Dissolve 5.0 g in a mixture of 70 ml of 5 M acetic acid and 30 ml of water. Filter, if necessary, through a previously ignited and weighed porcelain or silica crucible of a suitable porosity to give a clear filtrate (solution A). Reserve any residue (residue R) for the test for Substances insoluble in acetic acid. Solution A is not more intensely coloured than reference solution BS2 (2.3.1).

**Arsenic (2.3.10).** Dissolve 2.5 g in 15 ml of brominated hydrochloric acid, add 45 ml of water and remove the excess of bromine with a few drops of stannous chloride solution asT. The resulting solution complies with the limit test for arsenic (4 ppm).

**Heavy metals (2.3.13).** To 20 ml of solution A add 15 ml of 7 M hydrochloric acid and shake with 25 ml of 4-methylpentan-2-one for 2 minutes. Separate the layers, evaporate the aqueous layer to dryness, dissolve the residue in 1 ml of 5 M acetic acid and dilute to 20 ml with water. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (30 ppm). Use 7.5 ml of lead standard solution (2 ppm Pb) for preparing the standard.

**Iron (2.3.14).** Dissolve 40 mg in 5 ml of 2 M hydrochloric acid and dilute to 10 ml with water. The resulting solution complies with the limit test for iron (0.1 per cent).

**Chlorides (2.3.12).** 4.0 ml of solution A complies with the limit test for chlorides (0.125 per cent).

**Sulphates (2.3.17).** 0.3 ml of solution A complies with the limit test for sulphates (1.0 per cent).

**Calcium.** To 0.2 ml of ethanolic calcium standard solution (100 ppm Ca) add 1 ml of ammonium oxalate solution. After 1 minute add a mixture of 1 ml of 2 M acetic acid and 15 ml of a solution prepared by diluting 1.3 ml of solution A to 150 ml with distilled water and shake. After 15 minutes any opalescence produced is not more intense than that of a standard prepared in the same manner using a mixture of 10 ml of calcium standard solution (10 ppm Ca) and 5 ml of distilled water in place of solution A (1.5 per cent).

**Soluble substances.** Mix 2.0 g with 100 ml of water, boil for 5 minutes, filter whilst hot through a sintered-glass filter (porosity No. 3), allow to cool and dilute to 100.0 ml with water. Evaporate 50.0 ml of the filtrate to dryness and dry the residue at 105°. The residue weighs not more than 20 mg (2.0 per cent).

**Substances insoluble in acetic acid.** Residue R when washed with water, dried and ignited at 600°, weighs not more than 5 mg (0.1 per cent).

**Loss on ignition (2.4.20).** Not more than 8.0 per cent, determined on 0.5 g when ignited at 900°.

**Assay.** Weigh accurately about 0.35 g, dissolve in 10 ml of 2 M hydrochloric acid and dilute with water to 100.0 ml. To 10.0 ml add 5 ml of strong ammonia-ammonium chloride solution and titrate with 0.05 M disodium edetate using about 50 mg of mordant black II mixture as indicator.

1 ml of 0.05 M disodium edetate is equivalent to 0.002015 g of MgO.

**Storage.** Store protected from moisture.

Magnesium Stearate

Magnesium Stearate consists mainly of magnesium stearate \((C_{17}H_{33}CO_2)_2Mg\) with variable proportions of magnesium palmitate, \((C_{15}H_{31}CO_2)_2Mg\) and magnesium oleate, \((C_{17}H_{35}CO_2)_2Mg\) with variable proportions of magnesium stearate, \((C_{17}H_{33}CO_2)_2Mg\) with variable proportions of magnesium palmitate, \((C_{15}H_{31}CO_2)_2Mg\) and magnesium oleate, \((C_{17}H_{35}CO_2)_2Mg\) with variable proportions of magnesium stearate.

Magnesium Stearate contains not less than 3.8 per cent and not more than 5.0 per cent of Mg, calculated on the dried basis.

**Description.** A very fine, light, white powder; odourless or with a very faint odour of stearic acid; unctuous and free from grittiness.

**Identification**

A. To 5.0 g add 50 ml of ether, 20 ml of 2 M nitric acid and 20 ml of distilled water and heat under a reflux condenser until dissolution is complete. Allow to cool, separate the aqueous layer and shake the ether layer with two quantities, each of 4 ml, of distilled water. Combine the aqueous layers, wash with 15 ml of ether and dilute to 50 ml with distilled water (solution A). Evaporate the ether layer to dryness and dry the residue at 105°. The freezing point of the residue is not lower than 53° (2.4.11).
B. 1 ml of solution A obtained in test A gives reaction A of magnesium salts (2.3.1).

Tests

Appearance of solution. Solution A is not more intensely coloured than reference solution YS6 (2.4.1).

Appearance of solution of the fatty acids. Dissolve 0.5 g of the residue obtained in the preparation of solution A in 10 ml of chloroform. The solution is clear (2.4.1), and not more intensely coloured than reference solution YS5 (2.4.1).

Acidity or alkalinity. Mix 1.0 g with 20 ml of carbon dioxide-free water, boil for 1 minute, shaking continuously, cool and filter. To 10 ml of filtrate add 0.05 ml of bromothymol blue solution. Not more than 0.05 ml of 0.1 M hydrochloric acid or 0.1 M sodium hydroxide is required to change the colour of the solution.

Acid value of the fatty acids. 195 to 210, determined on 0.2 g of the residue obtained in the preparation of solution A, dissolved in 25 ml of the prescribed mixture of solvents (2.3.23).

Free stearic acid. Not more than 3 per cent, determined by the following method. Weigh accurately about 1.0 g into a stoppered flask, add 50 ml of chloroform, stopper the flask and shake well. Filter into a beaker through two thicknesses of filter paper taking care to avoid evaporation of the solvent. Wash the filter with 10 ml of chloroform and collect the washings in the beaker. Evaporate the chloroform on a water-bath in a current of air. Dissolve the residue in about 10 ml of ethanol (95 per cent) previously neutralised to phenolphthalein solution and titrate with 0.1 M sodium hydroxide using phenolphthalein solution as indicator.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.0284 g of stearic acid.

Zinc stearate. Heat 5.0 g with shaking with a mixture of 50 ml of water and 50 ml of dilute sulphuric acid until the fatty acids separate as an oily layer. Cool, filter the aqueous layer and wash the residue with two successive quantities, each of 5 ml, of hot water, combine the filtrate and the washings and dilute to 100 ml with water. To 5 ml of the resulting solution add 0.5 ml of ammonium mercurithiocyanate solution and 0.05 ml of copper sulphate solution. Scratch the walls of the container with a glass rod and allow to stand for 15 minutes; no violet precipitate is formed.

Heavy metals (2.3.13). Heat 5.0 g with 40 ml of 2 M acetic acid and allow to cool. Filter, wash the residue with two quantities, each of 5 ml, of warm water and dilute to 100 ml with water. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (20 ppm). Use 1.0 ml of lead standard solution (10 ppm Pb) to prepare the standard.

Chlorides (2.3.12). 10.0 ml of solution A diluted to 15 ml complies with the limit test for chlorides (250 ppm).

Sulphates (2.3.17). Dilute 5.0 ml of solution A to 50.0 ml with water. 2.5 ml of this solution diluted to 15 ml with water complies with the limit test for sulphates (0.6 per cent)

Loss on drying (2.4.19). Not more than 6.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.75 g, add 50 ml of a mixture of equal volumes of 1-butanol and ethanol, 5 ml of strong ammonia solution, 3 ml of ammonia buffer pH 10.0, 30.0 ml of 0.1 M disodium edetate and 15 mg of mordant black II mixture, heat to 45° to 50° and titrate with 0.1 M zinc sulphate until the colour changes from blue to violet. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of disodium edetate required.

1 ml of 0.1 M disodium edetate is equivalent to 0.002431 g of Mg.

Magnesium Sulphate

Epsom Salts

MgSO₄·7H₂O Mol. Wt. 246.5

Magnesium Sulphate contains not less than 99.0 per cent and not more than 100.5 per cent of MgSO₄, calculated on the dried basis.

Description. Colourless crystals or a white, crystalline powder.

Identification

A. Gives the reactions of sulphates (2.3.1).
B. Gives reaction A of magnesium salts (2.3.1).

Tests

Appearance of solution. Dissolve 5.0 g in sufficient carbon dioxide-free water to produce 50 ml (solution A). Solution A is clear (2.4.1), and colourless (2.4.1).

Acidity or alkalinity. To 10 ml of solution A add 0.05 ml of phenol red solution. Not more than 0.2 ml of either 0.01 M hydrochloric acid or 0.01 M sodium hydroxide is required to change the colour of the solution.

Arsenic (2.3.10). Dissolve 5.0 g in 50 ml of water and add 10 ml of stannated hydrochloric acid. The resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals (2.3.13). Dissolve 2.0 g in 10 ml of water, 2 ml of dilute acetic acid and sufficient water to produce 25 ml. The solution complies with the limit test for heavy metals, Method A (10 ppm).
Iron (2.3.14). 2.0 ml of solution A diluted to 20 ml with water complies with the limit test for iron (200 ppm).

Chlorides (2.3.12). 8.0 ml of solution A complies with the limit test for chlorides (300 ppm).

Loss on drying (2.4.19). 48.0 to 52.0 per cent, determined on 0.5 g by drying in an oven at 110° to 120° for 1 hour and then at 400°.

**Assay.** Weigh accurately about 0.3 g, dissolve in 50 ml of water, add 10 ml of strong ammonia-ammonium chloride solution and titrate with 0.05 M disodium edetate, using 0.1 g of mordant black II mixture as indicator, until a blue colour is obtained.

1 ml of 0.05 M disodium edetate is equivalent to 0.00602 g of MgSO4.

**Storage.** Store protected from moisture.

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**Magnesium Trisilicate**

Magnesium Trisilicate is a hydrated magnesium silicate of the approximate composition 2MgO,3SiO2·xH2O.

Magnesium Trisilicate contains not less than the equivalent of 29.0 per cent of MgO and not less than the equivalent of 65.0 per cent of SiO2, both calculated on the ignited basis.

**Description.** A fine, white or nearly white powder, free from grittiness; slightly hygroscopic.

**Identification**

A. To 2.0 g add a mixture of 4 ml of nitric acid and 4 ml of distilled water and heat to boiling, shaking frequently. Add 12 ml of distilled water, allow to cool, filter or centrifuge to obtain a clear solution and dilute the filtrate to 20 ml with distilled water (solution A). 1 ml of solution A, after neutralisation with 2 M sodium hydroxide, gives reaction A of magnesium salts (2.3.1).

B. 0.25 g gives the reaction of silicates (2.3.1).

**Tests**

**Alkalinity.** In a tared 200-ml conical flask on a water-bath heat 10 g with 100 g of water for 30 minutes, allow to cool and restore the initial weight with water. Allow to stand and filter or centrifuge until a clear liquid is obtained. To 10 ml of the clear liquid add 0.1 ml of phenolphthalein solution. Not more than 1.0 ml of 0.1 M hydrochloric acid is required to change the colour of the solution.

**Arsenic** (2.3.10). Disperse 2.5 g in 50 ml of water and add 10 ml of stannated hydrochloric acid AsT. The resulting solution complies with the limit test for arsenic (4 ppm).

**Heavy metals** (2.3.13). Neutralise 7.5 ml of solution A with dilute ammonia solution using metanil yellow solution as external indicator, dilute to 15 ml with water and filter if necessary. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (40 ppm).

**Chlorides** (2.3.12). 0.5 ml of solution A diluted to 15 ml with distilled water complies with the limit test for chlorides (500 ppm).

**Sulphates** (2.3.17). 0.3 ml of solution A diluted to 15 ml with distilled water complies with the limit test for sulphates (0.5 per cent).

**Acid absorption.** Not less than 100 ml of 0.1 M hydrochloric acid per g, determined by the following method. Suspend 0.25 g in 100.0 ml of 0.1 M hydrochloric acid, allow to stand in a water-bath at 37° ± 0.5° for 2 hours, shaking frequently and allow to cool. Add 0.1 ml of bromphenol blue solution to 20.0 ml of the supernatant liquid and titrate with 0.1 M sodium hydroxide until a blue colour is produced.

**Water-soluble salts.** In a tared platinum dish evaporate to dryness on a water-bath 20 ml of the clear liquid obtained in the test for Alkalinity and ignite the residue to constant weight at 900°. The residue weighs not more than 30 mg.

**Loss on ignition** (2.4.20). 17.0 to 34.0 per cent, determined on 0.5 g when ignited in a platinum crucible at 900°.

**Assay.** For MgO — Weigh accurately about 1.0 g, add 35 ml of hydrochloric acid and 50 ml of water and allow to stand for 15 minutes on a water-bath. Allow to cool, filter, wash the residue with water and dilute the combined filtrate and washings to 250.0 ml with water. Neutralise 50.0 ml with about 8 ml of 10 M sodium hydroxide, add 10 ml of ammonia buffer pH 10.0, 50 mg of mordant black II mixture, heat to 40° and titrate with 0.05 M disodium edetate until the colour changes to a deep blue.

1 ml of 0.05 M disodium edetate is equivalent to 0.002015 g of MgO.

For SiO2 — Weigh accurately about 0.7 g, add 10 ml of 1 M sulphuric acid and 10 ml of water and heat on a water-bath for 1.5 hours, shaking frequently and replacing the evaporated water. Allow to cool, decant onto an ashless filter paper (7 cm in diameter), wash the precipitate by decantation with three quantities, each of 5 ml, of hot water, transfer it to the filter paper and wash it with hot water until 1 ml of the filtrate remains clear on the addition of 2 ml of barium chloride solution and 0.05 ml of 2 M hydrochloric acid. Ignite the filter paper and its contents in a tared platinum crucible at 900° to constant weight; the residue is SiO2.

**Storage.** Store protected from moisture.
Mannitol

\[
\begin{align*}
\text{C}_6\text{H}_{14}\text{O}_6 & \quad \text{Mol. Wt. 182.2} \\
\text{Mannitol is } \text{D-mannitol, a hexahydric alcohol related to mannose.} \\
\text{Mannitol contains not less than 98.0 per cent and not more than 102.0 per cent of } \text{C}_6\text{H}_{14}\text{O}_6, \text{calculated on the dried basis.} \\
\text{Description. A white, crystalline powder or free-flowing granules.} \\
\text{Identification} \\
\text{A. To 1 ml of a saturated solution add 0.5 ml of ferric chloride test solution followed by 0.25 ml of sodium hydroxide solution and shake well; a clear solution is obtained which remains clear on the further addition of sodium hydroxide solution.} \\
\text{B. Dissolve 5 g in sufficient carbon dioxide-free water prepared from distilled water to produce 50 ml (solution A). Add 0.3 ml of solution A to 3 ml of a cooled mixture prepared by adding 6 ml of sulphuric acid to 3 ml of a freshly prepared 10 per cent w/v solution of catechol while cooling in ice. Heat gently over a naked flame for about 30 seconds; a pink colour is produced.} \\
\text{C. Melting range 165° to 170° (2.4.21).} \\
\text{Tests} \\
\text{Appearance of solution. Solution A is clear (2.4.1), and colourless (2.4.1).} \\
\text{Acidity or alkalinity. To 5 ml of solution A add 5 ml of carbon dioxide-free water and 0.05 ml of dilute phenolphthalein solution; not more than 0.2 ml of 0.01 M sodium hydroxide is required to change the colour of the solution to pink. To a further 5 ml of solution A add 5 ml of carbon dioxide-free water and 0.05 ml of methyl red solution. Not more than 0.3 ml of 0.01 M hydrochloric acid is required to change the colour of the solution to red.} \\
\text{Specific optical rotation} (2.4.22). +23.0° to +25.0°, determined in a solution prepared by dissolving 2.0 g of the substance under examination and 2.6 g of sodium tetraborate in 20 ml of water previously heated at 30° and shaking continuously for 15 to 30 minutes without further heating. Dilute the resulting clear solution to 25.0 ml with water.} \\
\text{Arsenic (2.3.10). Dissolve 5.0 g in 50 ml of water and add 10 ml of stannated hydrochloric acid. The resulting solution complies with the limit test for arsenic (2 ppm).} \\
\text{Chlorides (2.3.12). A solution of 5.0 g in 10 ml of water complies with the limit test for chlorides (50 ppm).} \\
\text{Sulphates (2.3.17). A solution of 1.5 g in 10 ml of water complies with the limit test for sulphates (100 ppm).} \\
\text{Reducing sugars. Dissolve 5.0 g in 25 ml of water with the aid of gentle heat. Cool and add 20 ml of capri-citric solution and a few glass beads. Heat so that boiling begins 4 minutes later and continue to boil for 3 minutes. Cool rapidly and add 100 ml of a 2.4 per cent v/v solution of glacial acetic acid and 20.0 ml of 0.025 M iodine. With continuous shaking add 25 ml of a mixture of 6 volumes of hydrochloric acid and 94 volumes of water and, when the precipitate has dissolved, titrate the excess of iodine with 0.05 M sodium thiosulphate using 1 ml of starch solution, added towards the end of titration, as indicator. Not less than 12.8 ml of 0.05 M sodium thiosulphate is required.} \\
\text{Sorbitol. Determine by thin-layer chromatography (2.4.17), coating the plate with a uniform 0.75-mm layer of the following mixture. Mix 0.1 g of carbomer with 110 ml of water and allow to stand, with gentle stirring, for 1 hour. Adjust to pH 7 by the gradual addition, with continuous shaking, of 2 M sodium hydroxide and add 30 g of silica gel H. Heat the plate at 110° for 1 hour, allow to cool and use immediately.} \\
\text{Mobile phase. A mixture of 85 volumes of 2-propanol and 15 volumes of a 0.2 per cent w/v solution of boric acid.} \\
\text{Test solution. Shake 0.5 g of the substance under examination, in fine powder, with 10 ml of ethanol (95 per cent) for 30 minutes and filter.} \\
\text{Reference powder. A 0.1 per cent w/v solution of sorbitol RS in ethanol (95 per cent).} \\
\text{Apply to the plate 2 µl of each solution. After development, dry the plate at 100° to 105° for 15 minutes, allow to cool, spray with a 0.5 per cent w/v solution of potassium permanganate in 1 M sodium hydroxide and heat at 100° for 2 minutes. Any spot corresponding to sorbitol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.} \\
\text{Sulphated ash (2.3.18). Not more than 0.1 per cent, determined on 2.0 g.} \\
\text{Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.}
Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 5.0 g of the substance under examination in 25 ml of water and dilute to 100.0 ml with water.

Reference solution. Dissolve 0.5 g of mannitol RS in 2.5 ml of water and dilute to 10.0 ml with water.

Chromatographic system
− a stainless steel column 30 cm x 7.8 mm packed with strong cation-exchange resin (calcium form) (9 µm),
− column temperature. 85 ± 1°,
− mobile phase: degassed water,
− flow rate. 0.5 ml per minute,
− refractometer at constant temperature,
− a 20 µl loop injector.

Inject the test solution and the reference solution. Continue the chromatography for twice the retention time of mannitol.

Calculate the content of C₆H₁₄O₆.

Mannitol intended for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 4 Endotoxin Unit per g for parenteral preparations having a concentration of 100 g per litre or less of mannitol, and less than 2.5 Endotoxin per g for parenteral preparations having a concentration of more than 100 g per litre of mannitol.

Labelling. The label states where applicable, the maximum concentration of bacterial endotoxins; whether or not the substance is suitable for use in the manufacture of parenteral preparations.

Storage. Store protected from moisture.

Mannitol Injection

Mannitol Injection is a sterile solution of Mannitol in Water for Injections.

Mannitol Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of mannitol, C₆H₁₄O₆.

Description. A colourless or almost colourless clear solution.

Identification

A. Evaporate to dryness on a water-bath a volume containing 2 g of Mannitol. The residue melts at 165° to 170° (2.4.21).

B. To the residue obtained in test A add 0.5 ml of ferric chloride test solution followed by 0.25 ml of sodium hydroxide solution and shake well; a clear solution is obtained which remains clear on the further addition of sodium hydroxide solution.

C. Dissolve 0.5 g of the residue obtained in test A in sufficient carbon dioxide-free water prepared from distilled water to produce 5 ml (solution A). Add 0.3 ml of solution A to 3 ml of a cooled mixture prepared by adding 6 ml of sulphuric acid to 3 ml of a freshly prepared 10 per cent w/v solution of catechol while cooling in ice. Heat gently over a naked flame for about 30 seconds; a pink colour is produced.

Tests

pH (2.4.24). 4.5 to 7.0, determined in a solution containing not more than 10.0 per cent w/v solution of Mannitol, diluted if necessary with water and to which 0.3 ml of a saturated solution of potassium chloride has been added for each 100 ml of solution.

Particulate contamination (2.5.9). When supplied in a container with a nominal content of 100 ml or more, complies with the limit test for particulate contamination.

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml of a solution containing not more than 10 per cent w/v of Mannitol. For solutions of higher strength, dilute the injection under examination with water BET so that the final solution contains 10 per cent w/v of Mannitol.

Other tests. Complies with the tests stated under Parenteral Preparations (Intravenous Infusions).

Assay. Dilute an accurately measured volume containing about 0.4 g of Mannitol to 100.0 ml with water, transfer 10.0 ml to a stoppered flask, add 20.0 ml of 0.1 M sodium periodate and 2 ml of 1 M sulphuric acid and heat on a water-bath for 15 minutes. Cool, add 3 g of sodium bicarbonate, in small quantities, and 25.0 ml of 0.1 M sodium arsenite, mix, add 5 ml of a 20 per cent w/v solution of potassium iodide and allow to stand for 15 minutes. Titrate with 0.05 M iodine until the first trace of yellow colour appears. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of iodine required.

1 ml of 0.05 M iodine is equivalent to 0.001822 g of C₆H₁₄O₆.

Storage. Store at temperatures between 20° and 30°. Exposure to lower temperatures may cause the deposition of crystals, which should be dissolved by warming before use.

Labelling. The label states (1) the strength as a percentage w/v of Mannitol; (2) that the injection should not be used if it contains visible solid particles that do not dissolve on warming.
Mebendazole

![Chemical structure of Mebendazole](image)

\[ C_{16}H_{13}N_3O_3 \quad \text{Mol. Wt. 295.3} \]

Mebendazole is methyl N-(5-benzoyl-1H-benimidazol-2-yl)carbamate.

Mebendazole contains not less than 98.0 per cent and not more than 102.0 per cent of \( C_{16}H_{13}N_3O_3 \), calculated on the dried basis.

**Description.** A white to slightly yellow, amorphous powder; almost odourless.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with mebendazole RS or with the reference spectrum of mebendazole.

B. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

C. To about 10 mg add 5 ml of ethanol (95 per cent), 1 ml of dinitrobenzene solution and 1 ml of sodium hydroxide solution; an intense yellow colour is produced.

D. Melts at about 290° (2.4.21).

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 90 volumes of chloroform, 5 volumes of methanol and 5 volumes of anhydrous formic acid.

**Test solution.** Dissolve 50 mg of the substance under examination in 1 ml of anhydrous formic acid and add sufficient chloroform to produce 10 ml.

**Reference solution (a).** A 0.5 per cent w/v solution of mebendazole RS in a mixture of 10 volumes of anhydrous formic acid and 90 volumes of chloroform.

**Reference solution (b).** A 0.0025 per cent w/v solution of mebendazole RS in a mixture of 10 volumes of anhydrous formic acid and 90 volumes of chloroform.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

**Assay.** Weigh accurately about 0.25 g, dissolve in 3 ml of anhydrous formic acid and 30 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, determining the endpoint potentiometrically (2.4.25). Carry out a blank titration. 1 ml of 0.1 M perchloric acid is equivalent to 0.02953 g of \( C_{16}H_{13}N_3O_3 \).

**Storage.** Store protected from light and moisture.

Mebendazole Tablets

Mebendazole Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of mebendazole, \( C_{16}H_{13}N_3O_3 \).

**Identification**

A. Shake a quantity of the powdered tablets containing 50 mg of Mebendazole with 10 ml of a mixture of 10 volumes of anhydrous formic acid and 90 volumes of chloroform for 30 minutes, filter, evaporate the filtrate to dryness and dry the residue at a pressure not exceeding 0.7 kPa. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with mebendazole RS or with the reference spectrum of mebendazole.

B. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 90 volumes of chloroform, 5 volumes of methanol and 5 volumes of anhydrous formic acid.

**Test solution.** Shake a quantity of the powdered tablets containing 50 mg of Mebendazole with 10 ml of a mixture of 10 volumes of anhydrous formic acid.

Shake the mixture for 30 minutes, filter, evaporate the filtrate to dryness and dry the residue at a pressure not exceeding 0.7 kPa. Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

**Assay.** Weigh accurately about 0.25 g, dissolve in 3 ml of anhydrous formic acid and 30 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, determining the endpoint potentiometrically (2.4.25). Carry out a blank titration. 1 ml of 0.1 M perchloric acid is equivalent to 0.02953 g of \( C_{16}H_{13}N_3O_3 \).

**Storage.** Store protected from light and moisture.
Reference solution (a). A 0.5 per cent w/v solution of mebendazole RS in a mixture of 10 volumes of anhydrous formic acid and 90 volumes of chloroform.

Reference solution (b). A 0.0025 per cent w/v solution of mebendazole RS in a mixture of 10 volumes of anhydrous formic acid and 90 volumes of chloroform.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Mebendazole, add 50 ml of 0.5 M methanolic hydrochloric acid shake for 30 minutes and dilute to 100.0 ml with 0.5 M methanolic hydrochloric acid. Filter and discard the first 10 ml of the filtrate. Dilute 10.0 ml of the filtrate to 100.0 ml with 0.5 M methanolic hydrochloric acid and mix. Further dilute 5.0 ml to 50.0 ml with the same solvent and mix. Measure the absorbance of the resulting solution at the maximum at about 234 nm (2.4.7). Calculate the content of C16H13N3O3 from the absorbance of the resulting solution at about 405 nm; absorbance at about 263 nm, about 0.79 and at about 292 nm, about 0.41.

C. Dissolve 25 mg in 2 ml of water, acidify with 2 M nitric acid and centrifuge. The supernatant liquid gives the reactions of chlorides (2.3.1).

Tests

pH (2.4.24). 4.5 to 6.5, determined in a 2.0 per cent w/v solution.

Ether-soluble extractive. Dissolve 40 mg in 25 ml of 2 M hydrochloric acid and shake with 50 ml of ether for 1 minute. Wash the ether layer with three quantities, each of 25 ml, of water, evaporate the ether to dryness using a rotary evaporator and dissolve the residue in sufficient methanol to produce 20 ml; absorbance of the resulting solution at about 260 nm, not more than 0.23 (2.4.7).

Non-tertiary amine. Dissolve 0.5 g in 5 ml of pyridine, add 5 ml of copper chloride-pyridine reagent and heat at 50° for 30 minutes. Cool, add sufficient acetone to produce 50 ml and measure the absorbance of the resulting solution at about 405 nm (2.4.7), using as the blank a solution obtained by treating 5 ml of pyridine in the same manner. The absorbance is not more than that obtained by repeating the test using 5 ml of a 0.006 per cent w/v solution of di-n-butylamine in pyridine and beginning at the words “add 5 ml of copper chloride-pyridine reagent....”.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel F254.

Mobile phase. A mixture of 50 volumes of ethanol, 50 volumes of chloroform and 1 volume of 18 M ammonia.

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of acetone.

Reference solution (a). Dissolve 10 mg of the substance under examination in 100 ml of acetone.

Reference solution (b). A 0.002 per cent w/v solution of veratric acid in acetone.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Expose the plate to iodine vapour for 1 hour. When viewed in ultraviolet light, any spot corresponding to veratric acid in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b). Using both methods of visualisation any other secondary spot in the chromatogram obtained with

Mebeverine Hydrochloride

C_{25}H_{35}NO_{5},HCl  Mol. Wt. 466.0

Mebeverine Hydrochloride is (RS)-4-[ethyl(4-methoxy-α-methylphenethyl)amino]butyl veratrate hydrochloride.

Mebeverine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of C_{25}H_{35}NO_{5},HCl, calculated on the dried basis.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with mebeverine hydrochloride RS or with the reference spectrum of mebeverine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.003 per cent w/v solution in 0.1 M hydrochloric acid shows an absorption maximum at about 263 nm and a less well-defined maximum at about 292 nm; absorbance at about 263 nm, about 0.79 and at about 292 nm, about 0.41.

C. Dissolve 25 mg in 2 ml of water, acidify with 2 M nitric acid and centrifuge. The supernatant liquid gives the reactions of chlorides (2.3.1).
the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 1 hour.

**Assay.** Weigh accurately about 0.4 g, dissolve in 75 ml of anhydrous glacial acetic acid and add 7 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.04660 g of C_{25}H_{35}NO_{5},HCl.

**Storage.** Store protected from light and moisture at a temperature not exceeding 30°.

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**Mebeverine Tablets**

Mebeverine Hydrochloride Tablets

Mebeverine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of mebeverine hydrochloride, C_{25}H_{35}NO_{5},HCl. The tablets are coated.

**Identification**

A. Suspend a quantity of the powdered tablets containing 0.2 g of Mebeverine Hydrochloride in 20 ml of water, add 5 ml of 5 M sodium hydroxide and extract with two quantities, each of 25 ml, of chloroform. Dry the combined extracts over anhydrous sodium sulphate and evaporate to dryness using a rotary evaporator.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with mebeverine hydrochloride RS or with the reference spectrum of mebeverine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows an absorption maximum at about 263 nm and a less well-defined maximum at about 292 nm.

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel F254.

*Mobile phase.* A mixture of 50 volumes of ethanol, 50 volumes of chloroform and 1 volume of 18 M ammonia.

*Test solution.* Shake a quantity of the powdered tablets containing 0.2 g of Mebeverine Hydrochloride with 10 ml of acetone and filter.

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**Meclizine Hydrochloride**

Meclizine Hydrochloride

C_{25}H_{27}ClN_{2}, 2HCl  Mol. Wt. 463.9

Meclizine Hydrochloride is (RS)-1-(4-chlorobenzhydryl)-4-(3-methylbenzyl)piperazine dihydrochloride.

Meclizine Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of C_{25}H_{27}ClN_{2}, 2HCl, calculated on the anhydrous basis.

**Description.** A white or yellowish white, crystalline powder; odour, slight.
**Identification**

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with meclizine hydrochloride RS or with the reference spectrum of meclizine hydrochloride.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.0015 per cent w/v solution in 0.1 M hydrochloric acid shows an absorption maximum at about 232 nm and weak absorption without a defined maximum in the range 260 nm to 300 nm; absorbance at the maximum at about 232 nm, 0.51 to 0.57.

C. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

D. Dissolve about 15 mg in 2 ml of ethanol (95 per cent); the solution gives reaction A of chlorides (2.3.1).

**Tests**

**Appearance of solution.** A 2.0 per cent w/v solution in ethanol (95 per cent) is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 60 volumes of dichloromethane, 30 volumes of toluene, 5 volumes of methanol and 0.5 volume of strong ammonia solution.

**Test solution (a).** Dissolve 0.5 g of the substance under examination in 10 ml of a mixture of equal volumes of dichloromethane and methanol.

**Test solution (b).** Dissolve 0.5 g of the substance under examination in 100 ml of a mixture of equal volumes of dichloromethane and methanol.

**Reference solution (a).** Dissolve 25.0 mg of the substance under examination in 100 ml of a mixture of equal volumes of dichloromethane and methanol.

**Reference solution (b).** A 0.5 per cent w/v solution of meclizine hydrochloride RS in a mixture of equal volumes of dichloromethane and methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with dilute potassium iodobismuthate solution. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). Ignore any yellowish white spot on the line of application.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 5.0 per cent, determined on 0.2 g.

**Assay.** Weigh accurately about 0.35 g, dissolve in 50 ml of chloroform, add 50 ml of anhydrous glacial acetic acid, 5 ml of acetic anhydride and 12 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid, using a 0.1 per cent w/v solution of quinaldine red in anhydrous glacial acetic acid as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02319 g of C₂₅H₂₇ClN₂, 2HCl.

**Storage.** Store protected from light and moisture.

**Meclizine Tablets**

Meclizine Hydrochloride Tablets; Meclozine Hydrochloride Tablets; Meclozine Tablets

Meclizine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of meclizine hydrochloride, C₂₅H₂₇ClN₂, 2HCl.

**Identification**

Triturate a quantity of the powdered tablets containing 0.5 g of Meclizine Hydrochloride with three quantities, each of 15 ml, of chloroform. Filter the extracts and evaporate the clear filtrate to dryness on a water-bath. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with meclizine hydrochloride RS or with the reference spectrum of meclizine hydrochloride.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.0015 per cent w/v solution in 0.1 M hydrochloric acid shows an absorption maximum at about 232 nm and weak absorption without a defined maximum in the range 260 nm to 300 nm; absorbance at the maximum at about 232 nm, 0.51 to 0.57.

C. Dissolve about 15 mg in 2 ml of ethanol (95 per cent); the solution gives reaction A of chlorides (2.3.1).

**Tests**

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and reduce to a fine powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.35 g of Meclizine Hydrochloride and extract with three quantities, each of 50 ml, of chloroform, stirring the mixture each time for 30 minutes, then allowing the undissolved matter to settle and decanting the supernatant liquid on to a sintered-glass filter...
(porosity No. 4). Transfer the residue to the filter with the aid of chloroform and wash the vessel and filter with 20 ml of chloroform. Combine the extracts and washing and evaporate on a water-bath to 50 ml. Cool and add 50 ml of anhydrous glacial acetic acid, 5 ml of acetic anhydride and 12 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid using a 0.1 per cent w/v solution of quinaldine red in anhydrous glacial acetic acid as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02319 g of C_{25}H_{27}ClN_{2}, 2HCl.

Storage. Store protected from light and moisture.

Medroxyprogesterone Acetate

Medroxyprogesterone Acetate is 6α-methyl-3,20-dioxopregn-4-en-17α-yl acetate.

Medroxyprogesterone Acetate contains not less than 97.0 per cent and not more than 103.0 per cent of C_{24}H_{34}O_{4}, calculated on the dried basis.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with medroxyprogesterone acetate RS.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 70 volumes of toluene, 40 volumes of ethyl acetate and 10 volumes of light petroleum (50° to 70°).

Test solution. Dissolve 0.1 g of the substance under examination in 100 ml of a mixture of 90 volumes of chloroform and 10 volumes of methanol.

Reference solution (a). A 0.1 per cent w/v solution of medroxyprogesterone acetate RS in a mixture of 90 volumes of chloroform and 10 volumes of methanol.

Reference solution (b). A solution containing 0.05 per cent w/v each of progesterone RS and medroxyprogesterone acetate RS in a mixture of 90 volumes of chloroform and 10 volumes of methanol.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

Spray with ethanolic sulphuric acid (20 per cent), heat at 120° for 10 minutes or until spots appear and allow to cool. Examine the plate in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. Melting range 205° to 209° (2.4.21).

Tests

Specific optical rotation (2.4.22). +45.0° to +51.0°, determined in a 1.0 per cent w/v solution in dioxan.

Related substances. Determine by liquid chromatography (2.4.17).

Test solution (a). Dissolve 5 mg of the substance under examination in 100 ml of the mobile phase.

Test solution (b). Dissolve 0.25 g of the substance under examination in 100 ml of the mobile phase.

Reference solution. Evaporate 1 ml of a solution containing 0.1 per cent w/v each of medroxyprogesterone acetate RS and megestrol acetate RS in ethanol, to dryness in a water bath at 45° and dissolve the residue in sufficient mobile phase to produce 25 ml.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsiline bonded to porous silica (5 µm),
- mobile phase: a mixture of 600 volumes of acetonitrile and 350 volumes of water and diluted to 1000 volumes with water,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.
Adjust the sensitivity so that the height of the principal peak in the chromatogram obtained with test solution (a) is 70 to 90 per cent of the full-scale deflection.

Equilibrate the column with the mobile phase at a flow rate of 1 ml per minute for about 45 minutes. When the chromatograms are recorded under the conditions described above, the retention times are 12.5 minutes for megestrol acetate and 13.5 minutes for medroxyprogesterone acetate.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to megestrol acetate and medroxyprogesterone acetate is at least 2.0. If this resolution is not achieved, adjust the concentration of acetonitrile in the mobile phase. Verify the repeatability by making five separate injections of test solution (a). The system is not suitable unless the relative standard deviation for the area of the principal peak in the chromatogram obtained with test solution (a). The system is not suitable unless the relative standard deviation for the area of the principal peak in the chromatogram obtained with test solution (a) is less than 2.0 per cent.

Inject separately test solutions (a) and (b) and record the chromatograms for 1.5 times the retention time of the principal peak. In the chromatogram obtained with test solution (b) the area of any secondary peak is not greater than half the area of the principal peak in the chromatogram obtained with test solution (a) and the sum of the areas of the secondary peaks is not greater than the area of the principal peak in the chromatogram obtained with test solution (a). Ignore any peak the area of which is less than 0.025 times the area of the principal peak in the chromatogram obtained with test solution (a) is less than 2.0 per cent.

Loss on drying. Weigh accurately about 25 mg, dissolve in sufficient ethanol (95 per cent) and measure the absorbance of the resulting solution at the maximum at about 241 nm (2.4.7). Calculate the content of C_{15}H_{15}NO_{2} taking 426 as the specific absorbance at 241 nm.

Storage. Store protected from light and moisture.

Mefenamic Acid

![Mefenamic Acid Structure](image)

C_{15}H_{15}NO_{2} Mol. Wt. 241.3

Mefenamic Acid is N-(2,3-xylyl)anthranilic acid.
Apply to the plate 20 µl of each solution. After development, dry the plate in air, expose to iodine vapour for 5 minutes and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

2,3-Dimethylaniline. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 90 volumes of toluene, 25 volumes of dioxan and 1 volume of 18 M ammonia.

Test solution. Dissolve 0.25 g of the substance under examination in 10 ml of a mixture of 3 volumes of chloroform and 1 volume of methanol.

Reference solution. A 0.00025 per cent w/v solution of 2,3-dimethylaniline in a mixture of 3 volumes of chloroform and 1 volume of methanol.

Apply to the plate 40 µl of each solution. After development, dry the plate in a current of warm air. Spray the plate with 1 volume of dimethylaniline generated by adding dilute sulphuric acid dropwise to a solution containing 10 per cent w/v of sodium nitrate and 3 per cent w/v of potassium iodide). Place the plate in a current of warm air for 15 minutes and spray with a 0.5 per cent w/v solution of N-(1-naphthyl)ethylenediamine dihydrochloride in ethanol (95 per cent). If necessary, allow to dry and repeat the spraying. Any spot corresponding to 2,3-dimethylaniline in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.5 g and dissolve in 100 ml of warm ethanol previously neutralised to phenol red solution and titrate with 0.1 M sodium hydroxide using phenol red solution as indicator.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.02413 g of C₁₅H₁₅NO₂.

Storage. Store protected from light and moisture.

Mefenamic Acid Capsules

Mefenamic Acid Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of mefenamic acid, C₁₅H₁₅NO₂.

Identification

Extract a quantity of the contents of the capsules containing 0.25 g of Mefenamic Acid with two quantities, each of 30 ml, of ether. Wash the combined extracts with water and evaporate to dryness on a water-bath. The residue, after drying at 105°, complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with mefenamic acid RS or with the reference spectrum of mefenamic acid.

B. Dissolve 25 mg in 15 ml of chloroform and examine in ultraviolet light at 365 nm; the solution exhibits a strong greenish-yellow fluorescence. Carefully add 0.5 ml of a saturated solution of trichloroacetic acid drop wise and examine again in ultraviolet light at 365 nm; the solution does not exhibit fluorescence.

C. Dissolve 5 mg in 2 ml of sulphuric acid and add 0.05 ml of 0.0167 M potassium dichromate; an intense blue colour is produced immediately which fades rapidly to brownish-green.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 90 volumes of toluene, 25 volumes of dioxan and 1 volume of glacial acetic acid.

Test solution. The supernatant liquid obtained by shaking a quantity of the contents of the capsules containing 0.25 g of Mefenamic Acid with a mixture of 7.5 ml of chloroform and 2.5 ml of methanol.

Reference solution. Dissolve 5.0 mg of the substance under examination in 100 ml of a mixture of 3 volumes of chloroform and 1 volume of methanol.

Apply to the plate 20 µl of each solution. After development, dry the plate in air, expose to iodine vapour for 5 minutes and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

2,3-Dimethylaniline. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 90 volumes of toluene, 25 volumes of dioxan and 1 volume of 18 M ammonia.

Test solution. The supernatant liquid obtained in the test for Related substances.

Reference solution. A 0.00025 per cent w/v solution of 2,3-dimethylaniline in a mixture of 3 volumes of chloroform and 1 volume of methanol.
Apply to the plate 40 µl of each solution. After development, dry the plate in a current of warm air. Spray the plate with ethanolic sulphuric acid (20 per cent), heat at 105° for 30 minutes and immediately expose to nitrous fumes in a closed glass chamber for 15 minutes (the nitrous fumes may be generated by adding dilute sulphuric acid dropwise to a solution containing 10 per cent w/v of sodium nitrite and 3 per cent w/v of potassium iodide). Place the plate in a current of warm air for 15 minutes and spray with a 0.5 per cent w/v solution of N-(1-naphthyl)ethylenediamine dihydrochloride in ethanol (95 per cent). If necessary, allow to dry and repeat the spraying. Any spot corresponding to 2,3-dimethylaniline in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Dissolution (2.5.2).

Apparatus. No 2

Medium. 900 ml of 0.05 M tris buffer prepared by dissolving 60.5 g tris(hydroxymethyl)aminomethane in 6000 ml water, diluting to 10,000 ml with water and adjusting with phosphoric acid to a pH 9.0 ± 0.05. 100 g of sodium lauryl sulphate is dissolved in 6000 ml of the above solution and further mixed with the remaining quantity of the solution.

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate as the test solution.

Reference solution. A 0.02 per cent w/v solution of mefenamic acid RS in the dissolution medium.

Use the chromatographic system described under Assay.

Calculate the content of C₁₅H₁₅NO₂ in the medium

D. Not less than 75 per cent of the stated amount of C₁₅H₁₅NO₂.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 50 mg of Mefenamic acid in a 250.0 ml volumetric flask, add 5 ml of tetrahydrofuran, shake for 10 minutes with the aid of ultrasound, dilute to volume with the mobile phase and filter.

Reference solution. A solution containing 0.02 per cent w/v of mefenamic acid RS in the mobile phase.

Protect the solutions from light.

Chromatographic system

- mobile phase: a mixture of 23 volumes acetonitrile, 20 volumes of 0.05 M of monobasic ammonium phosphate adjusted to a pH of 5.0 with 3 M ammonia and 7 volumes of tetrahydrofuran,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the column efficiency determined from the mefenamic acid peak is not less than 8200 theoretical plates, the tailing factor is not more than 1.6 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of C₁₅H₁₅NO₂ in the capsules.

Megestrol Acetate

Megestrol Acetate is 6-methyl-3,20-dioxopregna-4,6-dien-17-yl acetate.

Megestrol Acetate contains not less than 97.0 per cent and not more than 103.0 per cent of C₂₄H₃₂O₄, calculated on the dried basis.

Description. A white to creamy-white, crystalline powder; odourless or almost odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with megestrol acetate RS or with the reference spectrum of megestrol acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Solvent mixture. A mixture of 90 volumes of acetone and 1,2-propanediol.

Mobile phase. A mixture of 40 volumes of cyclohexane and 10 volumes of toluene.
**Test solution.** Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

**Reference solution (a).** Dissolve 25 mg of megestrol acetate RS in 10 ml of the solvent mixture.

**Reference solution (b).** Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 1 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with ethanolic sulphuric acid (20 per cent v/v). Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. Gives the reaction of acetyl groups (2.3.1).

**Tests**

**Specific optical rotation** (2.4.22). +9.0° to +12.0°, determined at 20° in a 5.0 per cent w/v solution in chloroform.

**Light absorption.** When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows an absorption maximum only at about 287 nm; ratio of the absorbance at about 240 nm to that at the maximum at about 287 nm, not more than 0.17.

**Related foreign steroids.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 92 volumes of 1,2-dichloroethane, 8 volumes of methanol and 0.5 volume of water.

Test solution. Dissolve 0.5 g of the substance under examination in 10 ml of a mixture of 9 volumes of chloroform and 1 volume of methanol.

Reference solution. A 0.025 per cent w/v solution of megestrol RS in a mixture of 9 volumes of chloroform and 1 volume of methanol.

Apply to the plate 1 µl of each solution. After development, dry the plate in air, spray with a 10 per cent v/v solution of sulphuric acid in ethanol, heat at 110° for 10 minutes and examine in ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Heavy metals** (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 20 mg, dissolve in sufficient ethanol to produce 100.0 ml, dilute 5.0 ml to 100.0 ml with ethanol and measure the absorbance of the resulting solution at the maximum at about 287 nm (2.4.7). Calculate the content of C₂₄H₃₂O₄ taking 630 as the specific absorbance at 287 nm.

**Storage.** Store protected from light and moisture.

**Megestrol Tablets**

Megestrol Acetate Tablets

Megestrol Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of megestrol acetate, C₂₄H₃₂O₄.

**Identification**

Extract a quantity of the powdered tablets containing 40 mg of Megestrol Acetate with 10 ml of chloroform, filter and evaporate the filtrate to dryness in a current of air. Dry the residue at 60° at a pressure not exceeding 0.7 kPa for 1 hour. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with megestrol acetate RS or with the reference spectrum of megestrol acetate.

**Tests**

**Disintegration** (2.5.1). 30 minutes.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Megestrol Acetate and dissolve as completely as possible in sufficient methanol to produce 100.0 ml. Mix well and filter. Dilute 2.0 ml of the filtrate with methanol to 100.0 ml and mix. Measure the absorbance of the resulting solution at the maximum at about 287 nm (2.4.7). Calculate the content of C₂₄H₃₂O₄ taking 630 as the specific absorbance at 287 nm.

**Storage.** Store protected from light and moisture.
Melphalan

\[
\text{C}_{13}\text{H}_{18}\text{Cl}_{2}\text{N}_{2}\text{O}_{2} \quad \text{Mol. Wt. 305.2}
\]

Melphalan is 4-bis(2-chloroethyl)amino-L-phenylalanine.

Melphalan contains not less than 93.0 per cent and not more than 100.5 per cent of \( \text{C}_{13}\text{H}_{18}\text{Cl}_{2}\text{N}_{2}\text{O}_{2} \), calculated on the dried basis.

**Description.** A white or almost white powder; odourless or almost odourless.

**Identification**

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in methanol shows an absorption maximum at about 260 nm and a less well-defined maximum at about 301 nm.

B. Dissolve 20 mg in 50 ml of methanol with the aid of gentle heat, add 1 ml of a 5 per cent w/v solution of 4-(4-nitrobenzyl)pyridine in acetone and evaporate to dryness. Dissolve the residue in 1 ml of hot methanol and add 0.1 ml of strong ammonia solution; a red colour is produced.

C. Heat 0.1 g with 10 ml of 0.1 M sodium hydroxide for 10 minutes on a water-bath. The resulting solution, after acidification with 2 M nitric acid, gives reaction A of chlorides (2.3.1).

**Tests**

**Specific optical rotation** (2.4.22). –30.0° to –36.0°, determined in a 0.7 per cent w/v solution in methanol prepared with the aid of gentle heat.

**Ionisable chlorine.** Dissolve 0.4 g in a mixture of 75 ml of water and 2 ml of nitric acid. Allow to stand for 2 minutes and titrate with 0.1 M silver nitrate, determining the end-point potentiometrically (2.4.25). Subtract the equivalent volume of 0.1 M silver nitrate used in the test for Ionisable chlorine. The difference between the volumes represents the amount of 0.1 M silver nitrate required by melphalan.

1 ml of 0.1 M silver nitrate is equivalent to 0.01526 g of \( \text{C}_{13}\text{H}_{18}\text{Cl}_{2}\text{N}_{2}\text{O}_{2} \).

**Storage.** Store protected from light and moisture.

**Melphalan Injection**

Melphalan Injection is a sterile material consisting of Melphalan Hydrochloride with or without auxiliary substances. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirement for Particulate matter stated under Parenteral Preparations (Injections) and with the following tests.*

**Appearance of solution.** Not more opalescent than opalescence standard OS2 (2.4.1).

**pH** (2.4.24). 6.0 to 7.0.

**Storage.** The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Melphalan Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous melphalan, \( \text{C}_{13}\text{H}_{18}\text{Cl}_{2}\text{N}_{2}\text{O}_{2} \).

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

**Identification**

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in methanol shows an absorption maximum at about 260 nm and a less well-defined maximum at about 301 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. Dissolve a quantity containing 20 mg of anhydrous melphalan in 50 ml of methanol with the aid of gentle heat, add 1 ml of a 5 per cent w/v solution of 4-(4-nitrobenzyl)pyridine in acetone and evaporate to dryness. Dissolve the residue in 1 ml of hot methanol and add 0.1 ml of strong ammonia solution; a red colour is produced.
D. Heat a quantity of the powder containing 0.1 g of anhydrous melphalan with 10 ml of 0.1 M sodium hydroxide for 10 minutes on a water-bath. The resulting solution, after acidification with 2 M nitric acid, gives reaction A of chlorides (2.3.1).

**Tests**

**Ionisable chlorine.** Dissolve a quantity containing 0.1 g of anhydrous melphalan in a mixture of 15 ml of water and 0.5 ml of nitric acid, allow to stand for 2 minutes and titrate with 0.02 M silver nitrate determining the end-point potentiometrically (2.4.25); not more than 1.7 ml is required.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh accurately a quantity of the mixed contents of 10 containers containing about 50 mg of Melphalan, dissolve in a mixture of 4 volumes of acetonitrile and 1 volume of 0.1 M hydrochloric acid and dilute with sufficient of the same solvent mixture to produce a final solution containing the equivalent of 0.01 per cent w/v of anhydrous melphalan.

**Reference solution.** A 0.01 per cent w/v solution of melphalan RS in the same solvent.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadeclsilane chemically bonded to porous silica (10 µm),
- mobile phase: 200 volumes of a 0.375 per cent w/v solution of ammonium carbonate, 180 volumes of methanol and 2.7 volumes of glacial acetic acid,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject alternately the test solution and the reference solution.

Calculate the amount of C$_{13}$H$_{18}$Cl$_2$N$_2$O$_2$ in the injection.

**Storage.** Store protected from light and moisture at a temperature not exceeding 30°C.

**Labelling.** The label on the sealed container states (1) the equivalent amount of anhydrous melphalan contained in it; (2) that it should be used immediately after preparation.

**Melphalan Tablets**

Melphalan Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of melphalan, C$_{13}$H$_{18}$Cl$_2$N$_2$O$_2$. The tablets are coated.

**Identification**

A. Shake a quantity of the powdered tablets containing about 5 mg of Melphalan with 100 ml of hot methanol. Filter and dilute 10 ml of the filtrate to 50 ml with methanol.

When examined in the range 230 nm to 360 nm (2.4.7), the filtrate shows an absorption maximum at about 260 nm and a less well-defined maximum at about 301 nm.

B. To the remainder of the filtrate obtained in test A add 1 ml of a 5 per cent w/v solution of 4-(4-nitrobenzyl)pyridine in acetone and evaporate to dryness. Dissolve the residue in 1 ml of hot methanol and 0.1 ml of strong ammonia solution; a red colour is produced.

**Tests**

**Uniformity of content.** Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14)

**Test solution.** Add 20 ml of a mixture of 4 volumes of acetonitrile and 1 volume of 0.1 M hydrochloric acid to one tablet, mix with the aid of ultrasound for 10 minutes or until the tablet disintegrates, filter, discarding the first 5 ml of filtrate, and use the filtrate.

**Reference solution.** A 0.01 per cent w/v solution of melphalan RS in the same solvent.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadeclsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 200 volumes of a 0.375 per cent w/v solution of ammonium carbonate, 180 volumes of methanol and 2.7 volumes of glacial acetic acid,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

Inject the test solution and the reference solution.

Calculate the content of C$_{13}$H$_{18}$Cl$_2$N$_2$O$_2$ in the tablet.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh and powder 20 tablets and add about 150 ml of a mixture of 4 volumes of acetonitrile and 1 volume of 0.1 M hydrochloric acid to an accurately weighed quantity of the powdered tablets containing about 25 mg of Melphalan, shake and mix with the aid of ultrasound for 5 minutes, dilute to 250 ml with the same solvent. Filter, discarding the first 20 ml of filtrate, and use the filtrate.

**Reference solution.** A 0.01 per cent w/v solution of melphalan RS in the same solvent.

Use the chromatographic system described under Uniformity of content.

Calculate the content of C$_{13}$H$_{18}$Cl$_2$N$_2$O$_2$ in the tablets (for tablets containing more than 2 mg of Melphalan).
For tablets containing 2 mg or less of Melphalan, use the average of 10 individual results obtained in the test for Uniformity of content.

**Storage.** Store protected from light and moisture in a cool place.

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**Menadione**

Menaphthone; Vitamin K₃

![Menadione structure](image)

C₁₁H₈O₂ \[\text{Mol. Wt. 172.2}\]

Menadione is 2-methyl-1,4-naphthaquinone.

Menadione contains not less than 98.5 per cent and not more than 101.0 per cent of C₁₁H₈O₂, calculated on the dried basis.

**Description.** A pale yellow, crystalline powder; odour, faint and characteristic. It decomposes on exposure to light, darkening in colour to light brown.

**Identification**

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with menadione RS or with the reference spectrum of menadione.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0005 per cent w/v solution in ethanol (95 per cent) shows an absorption maximum only at about 250 nm.

C. Dissolve about 1 mg in 5 ml of ethanol (95 per cent) and add 2 ml of dilute ammonia solution and 0.2 ml of ethyl cyanoacetate; an intense bluish violet colour develops which disappears on the addition of 2 ml of hydrochloric acid.

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

*Mobile phase.** A mixture of 90 volumes of cyclohexane, 5 volumes of 1,2-dichloroethane, 2 volumes of acetone and 1 volume of nitromethane.

*Test solution.* Dissolve 0.2 g of the substance under examination in 10 ml of acetone.

**Reference solution.** Dissolve 10 mg of the substance under examination in 100 ml of acetone.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of hot air and repeat the development and drying twice. Examine the plate in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Sulphated ash (2.3.18).** Not more than 0.1 per cent.

**Loss on drying (2.4.19).** Not more than 0.5 per cent, determined on 1.0 g by drying over phosphorus pentoxide at a pressure of 2 to 3 kPa for 4 hours.

**Assay.** Weigh accurately about 0.15 g, dissolve in 15 ml of glacial acetic acid in a flask with a stopper fitted with a valve, add 15 ml of 2 M hydrochloric acid and 1 g of zinc powder, close the flask and allow to stand in the dark for 60 minutes, shaking occasionally. Filter the solution through absorbent cotton and wash the filter with three quantities, each of 10 ml, of carbon dioxide-free water, adding the washings to the filtrate. Add 0.1 ml of ferroin solution and immediately titrate the combined filtrate and washings with 0.1 M ceric ammonium sulphate.

1 ml of 0.1 M ceric ammonium sulphate is equivalent to 0.00861 g of C₁₁H₈O₂.

**Storage.** Store protected from light and moisture.

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**Menthol**

![Menthol structure](image)

C₁₀H₁₉O \[\text{Mol. Wt. 156.3}\]

Menthol is 2-isopropyl-5-methylcyclohexanol. It is obtained from the volatile oils of various species of *Mentha* or prepared synthetically. It may be laevo-rotatory [(−)-menthol] or racemic [(±)-menthol].

**Description.** Colourless, hexagonal or needle-like crystals, or infused masses or a crystalline powder; odour, pleasant and characteristic.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with menthol RS or with the reference spectrum of menthol.
B. Dissolve 10 mg in 1 ml of sulphuric acid and add 1 ml of a 1 per cent w/v solution of vanillin in sulphuric acid; an orange-yellow colour is produced. Add 1 ml of water; the colour changes to violet (distinction from thymol).

C. When triturated with about an equal weight of camphor or chloral hydrate or phenol, the mixture liquefies.

**Tests**

**Appearance of solution.** Dissolve 1.0 g in 10 ml of ethanol (95 per cent). The solution is not more opalescent than opalescence standard OS4 (2.4.1), and not more intensely coloured than reference solution RS6 (2.4.1).

**Acidity.** To 1.0 g in a 100-ml glass-stoppered conical flask add 20 ml of water, boil until dissolution is complete, cool, stopper the flask and shake vigorously for 1 minute. Add a few crystals of the substance under examination to initiate crystallisation, shake vigorously for 1 minute and filter. To 5 ml of the filtrate add 0.05 ml of methyl red solution and 0.05 ml of 0.01M sodium hydroxide; the solution is yellow.

**Specific optical rotation** (2.4.22). (for (–)-menthol) –49.0° to –51.0°; (for (±)-menthol) –2.0° to +2.0°, determined in a 10.0 per cent w/v solution in ethanol (95 per cent).

**Congealing range** (2.4.10). (for (±)-menthol) 27.0° to 28.0°; on prolonged stirring, the temperature rises 30° to 32°.

**Related substances.** Determine by gas chromatography (2.4.13).

**Test solution.** Dissolve 0.1 g in sufficient ethanol (95 per cent) to produce 10 ml.

**Reference solution (a).** Dilute 1 ml of the test solution to 100 ml with ethanol (95 per cent).

**Reference solution (b).** Dilute 1 ml of reference solution (a) to 20 ml with ethanol (95 per cent).

**Chromatographic system**

- a glass or stainless steel column 4 m x 2 mm, packed with diatomaceous support (125 to 180 mesh) impregnated with a mixture suitable for the separation of free fatty acids,
- temperature: column 80°, injection port at 250° and the detector at 240°,
- flow rate 30 ml per minute of the carrier gas.

Inject separately 1µl of each solution, and, after 2 minutes, increase the temperature of the column to 240° at a rate of 8° per minute and maintain at this temperature for 15 minutes.

In the chromatogram obtained with the test solution the sum of the areas of any secondary peaks is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a). Ignore any peak with an area less than the principal peak in the chromatogram obtained with reference solution (b).

**Residue on evaporation.** Evaporate 2.0 g on a water-bath and heat at 105° for 1 hour. The residue weighs not more than 1.0 mg (0.05 per cent).

**Storage.** Store protected from light and moisture at a temperature not exceeding 30°.

**Labelling.** The label states whether the contents are laevo-rotatory or racemic menthol.

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**Mephentermine Sulphate**

\[
\text{(C}_{11}\text{H}_{17}\text{N})_{2}\text{H}_{2}\text{SO}_{4},2\text{H}_{2}\text{O} \quad \text{Mol. Wt.460.6}
\]

Mephentermine Sulphate is \(N,\alpha,\alpha\)-trimethylphenethylamine sulphate dihydrate.

Mephentermine Sulphate contains not less than 98.0 per cent and not more than 102.0 per cent of \((\text{C}_{11}\text{H}_{17}\text{N})_{2}\text{H}_{2}\text{SO}_{4}\), calculated on the dried basis.

**Description.** White crystals or a crystalline powder.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with mephentermine sulphate RS.

B. A 0.2 per cent w/v solution yields a precipitate with iodine solution and with potassium mercuri-iodide solution.

C. Dissolve 0.1 g in 5 ml of water, add with stirring 10 ml of picric acid solution. Allow to stand for 30 minutes, filter and wash the precipitate with small quantities of cold water until the last washing is colourless; the precipitate, after drying at 105° melts at 154° to 158° (2.4.21).

D. Gives the reactions of sulphates (2.3.1).

**Tests**

**pH** (2.4.24). 4.0 to 6.5, determined in a 2.0 per cent w/v solution in carbon dioxide-free water.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). 5.0 to 8.0 per cent, determined on 0.5 g by drying in an oven at 105° for 3 hours.
**Assay.** Weigh accurately about 0.4 g, dissolve in 250 ml of water, add 5 g of sodium chloride, shake well and add 5 ml of 5 M sodium hydroxide. Extract with 30 ml and then with further quantities, each of 20 ml, of ether until the base is completely extracted. Combine the ether extracts, wash with two quantities, each of 10 ml, of water and extract the aqueous washings with 10 ml of ether, adding this ether to the main ether extract. Add to the ether solution 30.0 ml of 0.05 M sulphuric acid, stir thoroughly and warm gently until the ether is evaporated. Cool and titrate with 0.1 M sodium hydroxide using methyl red solution as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of sulphuric acid required.

1 ml of 0.05 M sulphuric acid is equivalent to 0.02123 g of (C\textsubscript{11}H\textsubscript{17}N\textsubscript{2})\textsubscript{2}H\textsubscript{2}SO\textsubscript{4}.

**Storage.** Store protected from light and moisture.

**Mephentermine Injection**

Mephentermine Sulphate Injection

Mephentermine Injection is a sterile solution of Mephentermine Sulphate in Water for Injections.

Mephentermine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of mephentermine, C\textsubscript{11}H\textsubscript{17}N.

**Identification**

A. A 0.2 per cent w/v solution yields a precipitate with iodine solution and with potassium mercuri-iodide solution.

B. Dissolve 0.1 g in 5 ml of water, add with stirring 10 ml of picric acid solution. Allow to stand for 30 minutes, filter and wash the precipitate with small quantities of cold water until the last washing is colourless; the precipitate, after drying at 105° melts at 154° to 158° (2.4.21).

C. Gives the reactions of sulphates (2.3.1).

**Tests**

**pH.** (2.4.24). 4.0 to 6.5.

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** Measure accurately a volume containing about 0.2 g of mephentermine, add water if necessary to produce 20 ml add 5 g of sodium chloride, shake well and add 5 ml of 5 M sodium hydroxide. Extract with 30 ml and then with further quantities, each of 20 ml, of ether until the base is completely extracted. Combine the ether extracts, wash with two quantities, each of 10 ml, of water and extract the aqueous washings with 10 ml of ether, adding this ether to the main ether extract. Add to the ether solution 30.0 ml of 0.05 M sulphuric acid, stir thoroughly and warm gently until the ether is evaporated. Cool and titrate with 0.1 M sodium hydroxide using methyl red solution as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of sulphuric acid required.

1 ml of 0.05 M sulphuric acid is equivalent to 0.0163 g of C\textsubscript{11}H\textsubscript{17}N.

**Storage.** Store protected from light and moisture.

**Mepyramine Maleate**

Pyrilamine Maleate

![Chemical structure of Mepyramine Maleate](https://example.com/mepyramine_maleate_structure)

Mepyramine Maleate is 2-(N-4-anisyl-N-2-pyridylamino)ethyldimethylamine hydrogen maleate.

Mepyramine Maleate contains not less than 99.0 per cent and not more than 101.0 per cent of C\textsubscript{17}H\textsubscript{23}N\textsubscript{3}O\textsubscript{4}C\textsubscript{4}H\textsubscript{4}O\textsubscript{4}, calculated on the dried basis.

**Description.** A white or slightly yellowish, crystalline powder; odourless or almost odourless.

**Identification**

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with mepyramine maleate RS or with the reference spectrum of mepyramine maleate.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in 0.01 M hydrochloric acid shows absorption maxima, at about 239 nm and 316 nm; absorbance at about 239 nm, 0.43 to 0.477 and at about 316 nm, 0.2 to 0.22.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).
**Tests**

**Appearance of solution.** A 4.0 per cent w/v solution in carbon dioxide-free water is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

**pH** (2.4.24). 4.9 to 5.2, determined in a 2.0 per cent w/v solution.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 100 volumes of ethyl acetate and 2 volumes of diethylyamine.

*Prepare the following solutions immediately before use.*

**Test solution (a).** Dissolve 0.4 g of the substance under examination in 10 ml of chloroform.

**Test solution (b).** Dissolve 0.4 g of the substance under examination in 100 ml of chloroform.

**Reference solution (a).** A 4.0 per cent w/v solution of mepyramine maleate RS in chloroform.

**Reference solution (b).** A 0.4 per cent w/v solution of mepyramine maleate RS in chloroform.

**Reference solution (c).** A 0.008 per cent w/v solution of mepyramine maleate RS in chloroform.

**Reference solution (d).** A 0.004 per cent w/v solution of mepyramine maleate RS in chloroform.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (c). The test is not valid unless the Rf values of the principal spots in the chromatograms obtained with test solution (a) and reference solution (c) are at least 0.2 and unless the spot in the chromatogram obtained with reference solution (d) is clearly visible. Ignore the spot due to maleic acid on the line of application.

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

**Chlorides** (2.3.12). 2.5 g dissolved in 15 ml of water complies with the limit test for chlorides (100 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.25 per cent, determined on 2.0 g by drying in an oven at 80°.

**Assay.** Weigh accurately about 0.15 g, dissolve in 40 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration. 1 ml of 0.1 M perchloric acid is equivalent to 0.02007 g of C17H23N3O,C4H4O4.

**Storage.** Store protected from light and moisture.

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**Mepyramine Tablets**

Mepyramine MaleateTablets; Pyrilamine Maleate Tablets; Pyrilamine Tablets

Mepyramine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of mepyramine maleate, C17H23N3O,C4H4O4. The tablets may be coated.

**Identification**

A. Shake a quantity of the powdered tablets containing 0.1 g of Mepyramine Maleate with 10 ml of dichloromethane, filter and evaporate the filtrate to dryness. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with mepyramine maleate RS or with the reference spectrum of mepyramine maleate.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Dissolve a quantity of the powdered tablets containing 0.2 g of Mepyramine Maleate, freed as far as possible from any sugar coating, in 3 ml of water, add 2 ml of 5 M sodium hydroxide and shake with three quantities, each of 3 ml, of ether. Warm the aqueous layer in a water-bath for 10 minutes with 2 ml of bromine solution, heat to boiling, cool and add 0.2 ml to a solution of 10 mg of resorcinol in 3 ml of sulphuric acid; a bluish black colour is produced on heating for 15 minutes in a water-bath.

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 100 volumes of ethyl acetate and 2 volumes of diethylyamine.

*Prepare the following solutions immediately before use.*

**Test solution (a).** Shake a quantity of the powdered tablets containing a 4.0 per cent w/v solution of mepyramine maleate RS in chloroform.

**Test solution (b).** A 0.4 per cent w/v solution of mepyramine maleate RS in chloroform.

**Reference solution (a).** A 4.0 per cent w/v solution of mepyramine maleate RS in chloroform.

**Reference solution (b).** A 0.4 per cent w/v solution of mepyramine maleate RS in chloroform.

**Reference solution (c).** A 0.008 per cent w/v solution of mepyramine maleate RS in chloroform.

**Reference solution (d).** A 0.004 per cent w/v solution of mepyramine maleate RS in chloroform.
Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (c). The test is not valid unless the Rf values of the principal spots in the chromatograms obtained with test solutions (a) and reference solution (a) are at least 0.2 and unless the spot in the chromatogram obtained with reference solution (d) is clearly visible. Ignore the spot due to maleic acid on the line of application.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Mepyramine Maleate, add 75 ml of water and 5 ml of 2 M hydrochloric acid, shake vigorously for 15 minutes and dilute to 100.0 ml with water. Centrifuge and dilute 10.0 ml of the clear supernatant liquid to 100.0 ml with water. To 10.0 ml add 10 ml of 0.1 M hydrochloric acid and dilute to 50.0 ml with water. Measure the absorbance of the resulting solution at the maximum about 316 nm (2.4.7). Calculate the content of C17H23N3O, C4H4O4 taking 206 as the specific absorbance at 316 nm.

**Storage.** Store protected from light and moisture.

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**Mercaptopurine**

\[\text{C}_7\text{H}_8\text{N}_3\text{S}_2\text{H}_3\text{O}_4\]  Mol. Wt. 170.2

Mercaptopurine is purine-6-thiol monohydrate.

Mercaptopurine contains not less than 98.5 per cent and not more than 101.0 per cent of \(\text{C}_7\text{H}_8\text{N}_3\text{S}_2\), calculated on the anhydrous basis.

**Description.** A yellow, crystalline powder.

**Identification**

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with mercaptopurine RS.

B. Dissolve 20 mg in 5 ml of dimethyl sulphoxide and add sufficient 0.1 M hydrochloric acid to produce 100 ml. Dilute 5 ml to 200 ml with 0.1 M hydrochloric acid.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum only at about 325 nm.

C. Dissolve 20 mg in 20 ml of ethanol (95 per cent) heated to 60° and add 1 ml of a saturated solution of mercuric acetate in ethanol (95 per cent); a white precipitate is produced.

D. Dissolve 20 mg in 20 ml of ethanol (95 per cent) heated to 60° and add 1 ml of a 1 per cent w/v solution of lead acetate in ethanol (95 per cent); a yellow precipitate is produced.

**Tests**

**Hypoxanthine.** Dissolve 50 mg in 5 ml of dimethyl sulphoxide and add sufficient 0.1 M hydrochloric acid to produce 500 ml. Dilute 25 ml to 1000 ml with 0.1 M hydrochloric acid and measure the absorbance of the resulting solution at about 325 nm (2.4.7). Dilute a further 50 ml of the original solution to 100 ml with 0.1 M hydrochloric acid and measure the absorbance of the resulting solution at about 255 nm; the ratio of the absorbance at about 255 nm to that at about 325 nm, not greater than 1.05.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). 10.0 to 12.0 per cent, determined on 0.25 g.

**Assay.** Weigh accurately about 0.15 g, dissolve in 50 ml of dimethylformamide. Titrate with 0.1 M tetrabutylammonium hydroxide, determining the end-point potentiometrically (2.4.25). Carry out a blank titration. 1 ml of 0.1 M tetrabutylammonium hydroxide is equivalent to 0.01522 g of \(\text{C}_7\text{H}_8\text{N}_3\text{S}_2\).

**Storage.** Store protected from light and moisture.

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**Mercaptopurine Tablets**

Mercaptopurine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of mercaptopurine, \(\text{C}_7\text{H}_8\text{N}_3\text{S}_2\text{H}_3\text{O}_4\).

**Identification**

Shake a quantity of the powdered tablets containing 50 mg of Mercaptopurine with a mixture of 20 ml of water and 0.5 ml of 5 M sodium hydroxide for about 3 minutes, add sufficient water to produce 100 ml, mix and filter. Dilute a suitable aliquot of the filtrate with sufficient 0.1 M hydrochloric acid to give a solution containing 5 µg of Mercaptopurine per ml. The resulting solution shows an absorption maximum at about 325 nm (2.4.7).

**Tests**

**Dissolution** (2.5.2).

Apparatus. No 1
Medium. 900 ml of 0.1 M hydrochloric acid
Speed and time. 50 rpm and 60 minutes.
Withdraw a suitable volume of the medium and filter.
Determine by liquid chromatography (2.4.14).
Test solution. Use the filtrate as the test solution.
Reference solution. A solution containing 0.0055 per cent w/v of mercaptopurine RS in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: 0.1 per cent v/v solution of acetic acid in water,
- flow rate. 2.5 ml per minute,
- spectrophotometer set at 230 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the retention time for mercaptopurine is not less than 4 minutes and the relative standard deviation for replicate injections is not more than 2.0 per cent.
Inject alternately the test solution and the reference solution.
Calculate the content of C₅H₄N₄S in the medium.
D. Not less than 75 per cent of the stated amount of C₅H₄N₄S.

Other tests. Comply with the tests stated under Tablets.
Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Mercaptopurine, dissolve as completely as possible in 5 ml of dimethyl sulfoxide and add sufficient 0.1 M hydrochloric acid to produce 500.0 ml. Dilute 5.0 ml to 100.0 ml with 0.1 M hydrochloric acid, filter if necessary and measure the absorbance of the resulting solution at the maximum at about 325 nm (2.4.7). Calculate the content of C₅H₄N₄S, H₂O taking 1165 as the specific absorbance at 325 nm.

Storage. Store protected from light and moisture.

Meropenem

Meropenem is (1R,5S,6S)-2-[(3S,5S)-5-(dimethylaminocarbonyl)pyrrolidin-3-ylthio-6-[(R)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylic acid.

Meropenem contains not less than 98.0 per cent and not more than 101.0 per cent of C₁₇H₂₅N₃O₅S, calculated on the anhydrous basis.

Description. A white to off-white crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with meropenem RS or with the reference spectrum of meropenem.

Tests

pH (2.4.24). 4.0 to 6.0, determined in 1.0 per cent w/v solution in water.
Specific optical rotation (2.4.22). –17.0º to -21.0º, determined in a 0.5 per cent w/v solution.

Acetone (5.4). Not more than 0.05 per cent.
Determine by gas chromatography (2.4.13).

Test solution. Dissolve 100 mg of the substance under examination in 0.2 ml of dimethylformamide and 2.0 ml of internal standard solution.
Reference solution. Weigh accurately about 50 mg of acetone, dissolve in a small quantity of dimethylformamide, dilute to 100.0 ml with dimethylformamide and mix. To 1.0 ml of this solution, add 10.0 ml of the internal standard solution, and mix.

Internal standard solution. A 0.000005 per cent w/v solution of ethyl acetate in dimethylformamide.

Chromatographic system
- a glass column 2 m × 3 mm, packed with styrenedivinylbenzene copolymer (such as Chromosorb 101),
- temperature: column. 150º, inlet port and detector 170º,
- flow rate adjusted so that the retention time for acetone is about 3 minutes of the carrier gas.

Inject 1 µl of the test solution and the reference solution.
Calculate the percentage of acetone.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. To 900 ml of water add 1.0 ml of triethylamine, adjust the pH to 5.0 with dilute phosphoric acid, dilute to 1000 ml with water and mix.

NOTE – Prepare the solutions immediately before use.
Test solution. Dissolve 0.5 g of the substance under examination in the solvent mixture and dilute to 100 ml of the solvent mixture.

Reference solution. A 0.0025 per cent w/v solution of meropenem RS in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecysilane bonded to porous silica (5 µm),
- column temperature 40º,
- mobile phase: mix 1.0 ml of triethylamine and 900 ml of water, adjust the pH to 5.0 with dilute phosphoric acid, dilute with water to 1000 ml, add 70 volumes of acetonitrile and mix,
- flow rate. 1.6 ml per minute,
- spectrophotometer set at 220 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5, the column efficiency is not less than 2500 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Any individual impurity is not more than 0.5 per cent and the sum of all impurities found is not more than 2.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent, igniting at 500 ± 50º, instead of at 800 ± 25º. Use a desiccator containing silica gel.

Water (2.3.43). 11.4 per cent to 13.4 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).
Solvent mixture. To 900 ml of water add 1.0 ml of triethylamine, adjust the pH to 5.0 with dilute phosphoric acid, dilute to 1000 ml with water and mix.

NOTE – Prepare the solutions immediately before use.
Test solution. Dissolve 50.0 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution. A 0.05 per cent w/v solution of meropenem RS in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecysilane bonded to porous silica (5 µm),
- column temperature 30º,
- mobile phase: a mixture of 50 volumes of the solvent mixture and 10 volumes of methanol,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 300 nm,
- a 5 µl loop injector.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Injections) and with the following requirements.

Identification
In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.
Tests

**pH** (2.4.24). 7.3 to 8.3, determined in 5.0 per cent w/v solution.

**Related substances.** Determine by liquid chromatography (2.4.14).

*Solvent mixture.* Dissolve 1.0 ml of triethylamine in 900 ml of water. Adjust the pH to 5.0 with dilute phosphoric acid and dilute to 1000 ml with water.

**NOTE** – Prepare the solutions immediately before use.

**Test solution.** Determine the weight of the contents of 10 containers. Dissolve an accurately weighed quantity of the mixed contents of the 10 containers containing about 50 mg of Meropenem in 10 ml of the solvent mixture, and mix.

**Reference solution.** A 0.0025 per cent w/v solution of meropenem RS in solvent mixture.

**Chromatographic system**
- a stainless steel column 25 cm × 4.0 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature 40º,
- mobile phase: mix 1.0 ml of triethylamine and 900 ml of water, adjust the pH to 5.0 with dilute phosphoric acid, dilute to 1000 ml with water; filter and mix with 60 volumes of acetonitrile,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- a 10 µl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5 and the column efficiency is not less than 2500 theoretical plates.

Inject the test solution and the reference solution. Any individual impurity is not more than 0.8 per cent and the sum of all impurities found is not more than 2.0 per cent.

**Bacterial endotoxins** (2.2.3). Not more than 0.125 Endotoxin Unit per mg of meropenem.

**Sterility** (2.2.11). Complies with the test for sterility.

**Loss on drying** (2.4.19). 9.0 per cent to 12.0 per cent, determined on 1.0 g by drying it in vaccume oven at 65º for 6 hours.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Determine the weight of the contents of 10 containers. Dissolve an accurately weighed quantity of the mixed contents of the 10 containers containing about 10 mg of Meropenem in the mobile phase and dilute to 100.0 ml with the mobile phase.

**Reference solution.** A 0.01 per cent w/v solution of meropenem RS in the mobile phase.

**Chromatographic system**
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dilute 153 volumes of buffer solution prepared by dissolving 20 ml of 25 per cent w/v of tetrabutylammonium hydroxide to 1000 ml with water. Adjust the pH to 7.5 with dilute phosphoric acid, add 30 volumes of acetonitrile and 20 volumes of methanol and mix,
- flow rate. 1 ml per minute,
- spectrophotometer set at 300 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5, the column efficiency in not less than 2500 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the content of C₁₇H₂₅N₃O₅S in the injection.

**Storage.** Store protected from moisture.

**Labelling.** The label states the quantity in mg, of sodium (Na) in a suitable dose-volume.

**Mestranol**

C₂₃H₃₅O₂

Mestranol is 3-methoxy-19-nor-17α-pregna-1,3,5(10)-trien-20yn-17β-ol.

Mestranol contains not less than 98.0 per cent and not more than 102.0 per cent of C₂₁H₂₆O₂, calculated on the dried basis.

**Description.** A white or almost white, crystalline powder.

**Identification**

*Test B may be omitted if tests A and C are carried out. Test A may be omitted if tests B and C are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with mestranol RS.

B. In the test for Related substances the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).

C. Dissolve about 5 mg in 1 ml of sulphuric acid; a red colour is produced which appears greenish-yellow in ultraviolet light at 365 nm. On adding the solution to 10 ml of water and mixing,
the solution becomes pink and on standing a pink to violet precipitate is produced.

**Tests**

**Specific optical rotation** (2.4.22). –20.0° to –24.0°, determined in a 1.0 per cent w/v solution in anhydrous pyridine.

**Light absorption** (2.4.7). Dissolve about 25 mg in sufficient ethanol (95 per cent) to produce 25 ml and dilute 10 ml of the solution to 100 ml with ethanol (95 per cent). When examined in the range 230 nm to 360 nm, the resulting solution shows absorption maxima at about 279 nm and 288 nm and a minimum at about 286 nm. Absorbance at about 279 nm is 0.062 to 0.068 and at about 288 nm is 0.059 to 0.064, both calculated on the dried basis.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.  

*Mobile phase.* A mixture of 90 volumes of toluene and 10 volumes of ethanol (95 per cent).

*Test solution (a).* Dissolve 0.1 g of the substance under examination in 10 ml of chloroform.

*Test solution (b).* Dissolve 0.1 g of the substance under examination in 100 ml of chloroform.

*Reference solution (a).* A 0.01 per cent w/v solution of the substance under examination in chloroform.

*Reference solution (b).* A 0.005 per cent w/v solution of the substance under examination in chloroform.

*Reference solution (c).* A 0.1 per cent w/v solution of mestranol RS in chloroform.

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable, heat it at 110° for 10 minutes and spray the hot plate with ethanolic sulphuric acid (20 per cent). Heat again at 110° for 10 minutes and examine in daylight and in ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a), and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 0.5 g by drying in an oven at 105° for 3 hours.

**Assay.** Weigh accurately about 0.2 g, dissolve in 40 ml of tetrahydrofuran and add 5 ml of 10 per cent w/v solution of silver nitrate. Titrate with 0.1 M sodium hydroxide, determining the end-point potentiometrically (2.4.25).

1 ml of 0.1 M sodium hydroxide is equivalent to 0.03104 g of C₂H₅N₅SO₄.

**Storage.** Store protected from light and moisture.

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**Metformin Hydrochloride**

\[
\text{CH}_3 \quad \text{N} \quad \text{N} \quad \text{NH}_2 \quad \text{HCl}
\]

C₇H₁₃N₅.HCl  \hspace{1cm} \text{Mol. Wt. 165.6}

Metformin Hydrochloride is 1,1-dimethylbiguanide hydrochloride.

Metformin Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of C₇H₁₃N₅.HCl, calculated on the dried basis.

**Description.** A white, crystalline powder; hygroscopic.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with metformin hydrochloride RS or with the reference spectrum of metformin hydrochloride.

B. Dissolve 25 mg in 5 ml of water, add 1.5 ml of 5 M sodium hydroxide, 1 ml of 1-naphthol solution and, dropwise with shaking, 0.5 ml of dilute sodium hypochlorite solution; an orange-red colour is produced which darkens on keeping.

C. Dissolve 10 mg in 10 ml of water and add 10 ml of a solution prepared by mixing equal volumes of a 10 per cent w/v solution of sodium nitroprusside, a 10 per cent w/v solution of potassium ferricyanide and a 10 per cent w/v solution of sodium hydroxide and allowing to stand for 20 minutes; a wine red colour develops within 3 minutes.

D. Gives reaction A of chlorides (2.3.1).

**Tests**

**Related substances.** Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 50 mg of the substance under examination in 10 ml of water.

*Reference solution (a).* A 0.0005 per cent w/v solution of the substance under examination in water.

*Reference solution (b).* A 0.0001 per cent w/v solution of dicyandiamide in water.

**Chromatographic system**

- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a solution containing 0.087 per cent w/v of sodium pentanesulphonate and 0.12 per cent w/v of sodium chloride, adjusted to pH 3.5 using 1 per cent v/v solution of orthophosphoric acid,

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**Description.** A white, crystalline powder; hygroscopic.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with metformin hydrochloride RS or with the reference spectrum of metformin hydrochloride.

B. Dissolve 25 mg in 5 ml of water, add 1.5 ml of 5 M sodium hydroxide, 1 ml of 1-naphthol solution and, dropwise with shaking, 0.5 ml of dilute sodium hypochlorite solution; an orange-red colour is produced which darkens on keeping.

C. Dissolve 10 mg in 10 ml of water and add 10 ml of a solution prepared by mixing equal volumes of a 10 per cent w/v solution of sodium nitroprusside, a 10 per cent w/v solution of potassium ferricyanide and a 10 per cent w/v solution of sodium hydroxide and allowing to stand for 20 minutes; a wine red colour develops within 3 minutes.

D. Gives reaction A of chlorides (2.3.1).

**Tests**

**Related substances.** Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 50 mg of the substance under examination in 10 ml of water.

*Reference solution (a).* A 0.0005 per cent w/v solution of the substance under examination in water.

*Reference solution (b).* A 0.0001 per cent w/v solution of dicyandiamide in water.

**Chromatographic system**

- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a solution containing 0.087 per cent w/v of sodium pentanesulphonate and 0.12 per cent w/v of sodium chloride, adjusted to pH 3.5 using 1 per cent v/v solution of orthophosphoric acid,
flow rate. 1 ml per minute,
spectrophotometer set at 218 nm,
a 20 µl loop injector.

For the test solution record the chromatogram for three times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any peak corresponding to dicyandiamide is not greater than that obtained with reference solution (b) and the area of any other secondary peak is not greater than that obtained with reference solution (a).

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°C.

**Assay.** Weigh accurately about 60 mg, dissolve in 4 ml of anhydrous formic acid, add 50 ml of acetic anhydride. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration. 1 ml of 0.1 M perchloric acid is equivalent to 0.008281 g of C₄H₁₁N₅·HCl.

**Storage.** Store protected from light and moisture.

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### Metformin Tablets

Metformin Hydrochloride Tablets

Metformin Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of metformin hydrochloride, C₄H₁₁N₅·HCl. The tablets may be coated.

**Identification**

A. Shake a quantity of the powdered tablets containing 20 mg of Metformin Hydrochloride with 20 ml of ethanol, filter, evaporate the filtrate to dryness on a water-bath and dry the residue at 105°C for 1 hour.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with metformin hydrochloride RS or with the reference spectrum of metformin hydrochloride.

B. Triturate a quantity of the powdered tablets containing 50 mg of Metformin Hydrochloride with 10 ml of water and filter. To 5 ml of the filtrate, add 1.5 ml of 5 M sodium hydroxide, 1 ml of I-naphthol solution and, dropwise with shaking, 0.5 ml of dilute sodium hypochlorite solution; an orange-red colour is produced which darkens on keeping.

C. The filtrate obtained in test B gives reaction A of chlorides (2.3.1).

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### Tests

**Related substances.** Determine by liquid chromatography (2.4.14).

**Test solution.** Shake a quantity of the powdered tablets containing 0.5 g of Metformin Hydrochloride with 100 ml of water and filter.

**Reference solution (a).** Dilute 0.1 ml of the test solution to 100 ml with water.

**Reference solution (b).** A 0.0001 per cent w/v solution of dicyandiamide in water.

Chromatographic system

- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a solution containing 0.087 per cent w/v of sodium pentanesulphonate and 0.12 per cent w/v of sodium chloride, adjusted to pH 3.5 using 1 per cent v/v solution of orthophosphoric acid,
- flow rate. 1 ml per minute,
- spectrophotometer set at 218 nm,
- a 20 µl loop injector.

For the test solution record the chromatogram for three times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any peak corresponding to dicyandiamide is not greater than that obtained with reference solution (b) and the area of any other secondary peak is not greater than that obtained with reference solution (a).

**Dissolution** (2.5.2).

Apparatus. No 2

Medium. 900 ml of a 0.68 per cent w/v solution of potassium dihydrogen phosphate, adjusted to pH 6.8 by the addition of 1 M sodium hydroxide

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter, dilute suitably with water and measure the absorbance of the resulting solution at the maximum at about 233 nm (2.4.7). Calculate the content of C₄H₁₁N₅·HCl, in the medium taking 806 as the specific absorbance at 233 nm.

D. Not less than 70 per cent of the stated amount of C₄H₁₁N₅·HCl.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Metformin Hydrochloride, shake with 70 ml of water for 15 minutes, dilute to 100.0 ml with water and filter. Dilute 10.0 ml of the filtrate to 100.0 ml with water. Further dilute 10.0 ml to 100.0 ml with water and measure the absorbance of the resulting solution...
at the maximum at about 232 nm (2.4.7). Calculate the content of C_{21}H_{27}NO,HCl taking 798 as the specific absorbance at 232 nm.

**Methadone Hydrochloride**

*Amidone Hydrochloride*

![Chemical Structure](image)

\[ \text{C}_{21}\text{H}_{27}\text{NO,HCl} \quad \text{Mol. Wt. 345.9} \]

Methadone Hydrochloride is (RS)-dimethyl-(1-methyl-4-oxo-3,3-diphenylhexyl)amine hydrochloride. Methadone Hydrochloride contains not less than 98.5 per cent and not more than 100.5 per cent of C_{21}H_{27}NO,HCl, calculated on the dried basis.

**Description.** A white, crystalline powder.

**Identification**

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with methadone hydrochloride RS.

B. To 2 ml of a 5 per cent w/v solution in carbon dioxide-free water add 1 ml of 0.1 M hydrochloric acid and 6 ml of ammonium thiocyanate solution; a white precipitate is produced which becomes crystalline on stirring for a few minutes. The precipitate, after drying at 105° melts at 143° to 148° (2.4.21).

C. Dissolve 50 mg in 5 ml of carbon dioxide-free water, add 1 ml of 6 M ammonia, mix, allow to stand for 5 minutes and filter; the filtrate gives reaction A of chlorides (2.3.1).

D. Optical rotation of a 2-dm layer of a 5 per cent w/v solution in carbon dioxide-free water, is –0.05° to +0.05° (2.4.22).

**Tests**

**Appearance of solution.** A 5.0 per cent w/v solution in carbon dioxide-free water is clear (2.4.1), and colourless (2.4.1).

**Acidity or alkalinity.** To 10 ml of a 2.0 per cent w/v solution in carbon dioxide-free water add 0.2 ml of methyl red solution and 0.2 ml of 0.01 M sodium hydroxide; the solution is yellow. Add 0.4 ml of 0.01 M hydrochloric acid; the solution is red.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 60 volumes of ethanol (95 per cent), 30 volumes of glacial acetic acid and 10 volumes of water.

**Test solution.** Dissolve 0.5 g of the substance under examination in 10 ml of ethanol (95 per cent).

**Reference solution.** A 0.005 per cent w/v solution of the substance under examination in ethanol (95 per cent).

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with dilute potassium iodobismuthate solution. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.5 g, dissolve in 50 ml of anhydrous glacial acetic acid, add 5 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid, using crystal violet solution as indicator and continuing the titration until the colour changes from violet-blue to green. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.03459 g of C_{21}H_{27}NO,HCl.

**Storage.** Store protected from light and moisture.

**Methadone Injection**

Methadone Injection; Amidone Hydrochloride Injection; Amidone Injection

Methadone Injection is a sterile solution of Methadone Hydrochloride in Water for Injections.

Methadone Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of methadone hydrochloride, C_{21}H_{27}NO,HCl.

**Identification**

Make a volume containing 0.1 g of Methadone Hydrochloride alkaline with 5 M sodium hydroxide, stir with a glass rod until the precipitate solidifies, filter, wash with water and dry over phosphorus pentoxide at room temperature at a pressure of 2 kPa. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with methadone.
hydrochloride RS treated in the same manner or with the reference spectrum of methadone.

B. To 5 mg add 0.05 ml of dinitrobenzene solution and 0.05 ml of a 50 per cent w/v solution of sodium hydroxide; a purple colour is produced which changes slowly to dark brown.

Tests

pH (2.4.24). 5.0 to 6.5.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. To an accurately measured volume containing about 10 mg of Methadone Hydrochloride add 1 ml of glacial acetic acid and dilute to 100.0 ml with water. To 10.0 ml of this solution add 10 ml of a 0.4 per cent w/v solution of picric acid and 10 ml of phosphate buffer pH 4.9, extract with three quantities, each of 15 ml, of chloroform, dilute the combined chloroform extracts to 50.0 ml with chloroform. To 10.0 ml add sufficient chloroform to produce 20.0 ml and measure the absorbance of the resulting solution at the maximum at about 350 nm (2.4.7), using as the blank a solution prepared in the same manner but omitting the substance under examination. Calculate the content of C\textsubscript{21}H\textsubscript{27}NO\textsubscript{2}HCl taking 448 as the specific absorbance at 350 nm.

Storage. Store protected from light, in single dose container.

Methadone Tablets

Methadone Tablets; Amidone Hydrochloride Tablets; Amidone Tablets

Methadone Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of methadone hydrochloride, C\textsubscript{21}H\textsubscript{27}NO\textsubscript{2}HCl.

Identification

A. Shake a quantity of the powdered tablets containing 0.1 g of Methadone Hydrochloride with 20 ml of water and centrifuge. Make the supernatant liquid alkaline with 5 M sodium hydroxide, stir with a glass rod until the precipitate solidifies, filter, wash with water and dry over phosphorus pentoxide at room temperature at a pressure of 2 kPa.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with methadone hydrochloride RS or with the reference spectrum of methadone.

B. Extract a quantity of the powdered tablets containing 0.1 g of Methadone Hydrochloride with 10 ml of water, filter and wash the residue with sufficient water to bring the volume of the filtrate to 10 ml. Add to the filtrate 0.125 g of picrolonic acid dissolved in 50 ml of boiling water, stir and allow to stand for 2 hours. The residue, after recrystallisation from ethanol (20 per cent), washing with ethanol (20 per cent) and drying at 105°, melts at about 160° or 180° (2.4.21).

Tests

Uniformity of content. Comply with the test stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Methadone Hydrochloride, add 60 ml of water and 5 ml of glacial acetic acid, heat on a water-bath for 5 minutes, mix with the aid of ultrasound for 10 minutes and dilute to 100.0 ml with water. Filter, discarding the first 10 ml of the filtrate, and dilute 20.0 ml to 100.0 ml with water. To 10.0 ml of the resulting solution, add 10 ml of a 0.4 per cent w/v solution of picric acid and 10 ml of phosphate buffer pH 4.9, extract with three quantities, each of 15 ml, of chloroform, dilute the combined chloroform extracts to 50.0 ml with chloroform. To 10.0 ml add sufficient chloroform to produce 20.0 ml and measure the absorbance of the resulting solution at the maximum at about 350 nm (2.4.7). Calculate the content of C\textsubscript{21}H\textsubscript{27}NO\textsubscript{2}HCl in the tablet from the absorbance obtained by repeating the operation on an accurately weighed quantity of methadone hydrochloride RS.

Other tests. Comply with the tests stated under Tablets.

Methadilazine Hydrochloride

\[
\text{Methadilazine Hydrochloride} \quad \text{HCl}
\]

C\textsubscript{18}H\textsubscript{20}N\textsubscript{2}S\textsubscript{2}HCl, Mol. Wt. 332.9

Methadilazine Hydrochloride is 10-(1-methylpyrrolidin-3-ylmethyl)phenothiazine hydrochloride.
Methdilazine Hydrochloride contains not less than 97.0 per cent and not more than 103.0 per cent of \( \text{C}_{18}\text{H}_{20}\text{N}_2\text{S},\text{HCl} \), calculated on the dried basis.

**Description.** A light tan powder, turning pale pink to brown on exposure to light; odour, slight and characteristic.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with methdilazine hydrochloride RS or with the reference spectrum of methdilazone hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0005 per cent w/v solution shows an absorption maximum at about 252 nm and an inflection at about 275 nm; absorbance at about 252 nm, about 0.46.

C. Dissolve 50 mg in dilute hydrochloric acid, add 3 ml of buffered palladium chloride solution and 1 ml of a 1 per cent w/v solution of sodium lauryl sulphate and mix; a dark blue colour is formed.

D. Gives the reactions of chlorides (2.3.1).

**Tests**

**pH** (2.4.24). 4.8 to 6.0, determined in a 1.0 per cent w/v solution.

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.5 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g, by drying in an oven at 65°C at a pressure not exceeding 0.7 kPa for 16 hours.

**Assay.** Weigh accurately about 0.1 g, dissolve in sufficient water to produce 100.0 ml. Transfer 5.0 ml of this solution to a 100-ml volumetric flask, dilute to volume with water and mix. Dilute 5.0 ml to 50.0 ml with water, mix and measure the absorbance of the resulting solution at the maximum at about 252 nm and at about 275 nm (2.4.7). Subtract the absorbance at about 275 nm from the absorbance at about 252 nm. Calculate the content of \( \text{C}_{18}\text{H}_{20}\text{N}_2\text{S},\text{HCl} \) from the difference in the absorbances obtained by carrying out the Assay simultaneously on methdilazine hydrochloride RS.

**Storage.** Store protected from light and moisture.

**Methdilazine Tablets**

Methdilazine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of methdilazine hydrochloride, \( \text{C}_{18}\text{H}_{20}\text{N}_2\text{S},\text{HCl} \).

**Identification**

Weigh accurately a quantity of finely powdered tablets containing about 8 mg of Methdilazine Hydrochloride, transfer to a 60-ml separating funnel, add 10 ml of a 10 per cent w/v solution of sodium bicarbonate and extract with 3 ml of chloroform. Filter the extract through a plug of cotton. Evaporate the chloroform carefully removing the last traces of solvent in a small flask at a pressure of 1.5 to 2.5 kPa.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with methdilazine hydrochloride RS or with the reference spectrum of methdilazine hydrochloride.

**Tests**

**Uniformity of content.** Comply with the test stated under Tablets.

*Carry out the procedure protected from light.*

Powder one tablet and dissolve in about 20 ml of methanol in a 25-ml volumetric flask, dilute to volume and mix. Filter, discarding the first 10 ml of the filtrate. To 10.0 ml of the filtrate in a 100-ml volumetric flask, add 5 ml of methanol and 4.0 ml of buffered palladium chloride solution, dilute to volume with ethanol (95 per cent) and mix. Carry out the Assay on the resulting solution beginning at the words “Measure the absorbance...”. Calculate the content of \( \text{C}_{18}\text{H}_{20}\text{N}_2\text{S},\text{HCl} \) in the tablet.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** *Carry out the procedure protected from light.*

Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 80 mg of Methdilazine Hydrochloride and transfer to a 200-ml volumetric flask. Add 60 ml of methanol, shake for 20 minutes, dilute with methanol to volume and mix. Filter, discarding the first 15 ml of the filtrate. To 10.0 ml of the filtrate in a 100-ml volumetric flask add 20 ml of methanol and 4.0 ml of buffered palladium chloride solution, dilute to volume with ethanol (95 per cent) and mix. Measure the absorbance of the resulting solution at the maximum at about 460 nm (2.4.7), using as the blank a solution prepared by treating 10 ml of methanol in the same manner but omitting the filtrate of methdilazine hydrochloride solution. Calculate the content of \( \text{C}_{18}\text{H}_{20}\text{N}_2\text{S},\text{HCl} \) from the absorbance obtained by repeating the operation using methdilazine hydrochloride RS instead of the substance under examination.

**Storage.** Store protected from light and moisture.
Methotrexate

\[
\text{C}_{20}\text{H}_{22}\text{N}_{8}\text{O}_{5} \quad \text{Mol. Wt. 454.4}
\]

Methotrexate is 4-amino-4-deoxy-10-methylpteroyl-L-glutamic acid.

Methotrexate contains not less than 97.0 per cent and not more than 102.0 per cent of \( \text{C}_{20}\text{H}_{22}\text{N}_{8}\text{O}_{5} \), calculated on the anhydrous basis.

Description. A yellow to orange-brown, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with methotrexate RS or with the reference spectrum of methotrexate.

B. When examined in the range 230 nm to 380 nm (2.4.7), a 0.001 per cent w/v solution in \( 0.1 \text{ M sodium hydroxide} \) shows absorption maxima at about 258 nm, 303 nm and 371 nm; ratio of the absorbance at the maximum at about 303 nm to that at the maximum at about 371 nm, 2.8 to 3.3.

Tests

Specific optical rotation (2.4.22). \(+19.0^\circ\) to \(+24.0^\circ\), determined in a 1.0 per cent w/v solution in a solution containing 1.4 per cent w/v of sodium carbonate.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 12.0 per cent, determined on 0.25 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 250.0 ml of the mobile phase.

Reference solution (a). Dissolve 25 mg of methotrexate RS in 250.0 ml of the mobile phase.

Reference solution (b). Dissolve 25 mg of methotrexate RS and 25 mg of folic acid in 250.0 ml of the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 92 volumes of phosphate buffer pH 6.0 and 8 volumes of acetonitrile,
- flow rate. 1.4 ml per minute,
- spectrophotometer set at 302 nm,
- a 20 µl loop injector.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b). The resolution between the peaks due to methotrexate and folic acid is not less than 5.0.

Inject separately the test solution and reference solution (a) and measure the responses for the principal peak.

Calculate the content of \( \text{C}_{20}\text{H}_{22}\text{N}_{8}\text{O}_{5} \).

Storage. Store protected from light and moisture.

CAUTION - Great care should be taken to prevent inhaling particles of Methotrexate and exposing the skin to it.

Methotrexate Injection

Methotrexate Injection is a sterile solution of Methotrexate in Water for Injections containing Sodium Hydroxide.

Methotrexate Injection contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of methotrexate, \( \text{C}_{20}\text{H}_{22}\text{N}_{8}\text{O}_{5} \).

Description. A clear, yellowish solution.

Identification

When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows absorption maxima at about 242 nm and 306 nm.

Tests

pH (2.4.24). 7.5 to 9.0.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the injection with the mobile phase to produce a solution containing 0.0025 per cent w/v of Methotrexate.

Reference solution (a). A 0.0025 per cent w/v solution of methotrexate RS in the mobile phase.

Reference solution (b). A solution containing 0.0025 per cent w/v each of methotrexate RS and folic acid in the mobile phase.
Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 92 volumes of phosphate buffer pH 6.0 and 8 volumes of acetonitrile,
- flow rate, 1.4 ml per minute,
- spectrophotometer set at 302 nm,
- a 20 µl loop injector.

Inject reference solution (a). The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject reference solution (b). The resolution between the peaks due to methotrexate and folic acid is not less than 5.0.

Inject separately the test solution and reference solution (a) and measure the responses for the principal peak.

Calculate the content of C_{20}H_{22}N_{8}O_{5} in the injection.

Storage. Store protected from light.

Labelling. The label states that the injection is not intended for intrathecal injection when an antimicrobial preservative is present.

Methotrexate Tablets
Methotrexate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of methotrexate, C_{20}H_{22}N_{8}O_{5}.

Identification
Extract a quantity of the powdered tablets containing 10 mg of Methotrexate with sufficient 0.1 M sodium hydroxide to produce 100 ml, filter and dilute 10 ml of the filtrate to 100 ml with 0.1 M sodium hydroxide.

When examined in the range 230 nm to 380 nm (2.4.7), the resulting solution shows absorption maxima at about 258 nm, 303 nm and 371 nm.

Tests
Dissolution (2.5.2).
Apparatus. No 1
Medium. 900 ml of 0.1 M hydrochloric acid.
Speed and time. 50 rpm and 45 minutes.
Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Measure the absorbance of the filtrate at the maximum at about 306 nm (2.4.7). Calculate the content of C_{20}H_{22}N_{8}O_{5} taking 430 as the specific absorbance at 306 nm.

D. Not less than 75 per cent of the stated amount of C_{20}H_{22}N_{8}O_{5}.

Uniformity of content. Comply with the test stated under Tablets.

Carry out the test as described under Assay, using the following solutions.

Test solution. Crush one tablet and mix with 100 ml of the mobile phase with the aid of ultrasound, centrifuge and use the supernatant liquid.

Reference solution (a). A 0.0025 per cent w/v solution of methotrexate RS in the mobile phase.

Reference solution (b). A solution containing 0.0025 per cent w/v each of methotrexate RS and folic acid in the mobile phase.

Calculate the content of C_{20}H_{22}N_{8}O_{5} in the tablet.

Other tests. Comply with the tests stated under Tablets.
Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 2.5 mg of Methotrexate and mix with 100.0 ml of the mobile phase with the aid of ultrasound, centrifuge and use the supernatant liquid.

Reference solution (a). A solution containing 0.0025 per cent w/v each of methotrexate RS and folic acid in the mobile phase.

Reference solution (b). A 0.0025 per cent w/v solution of methotrexate RS in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 92 volumes of phosphate buffer pH 6.0 and 8 volumes of acetonitrile,
- flow rate, 1.4 ml per minute,
- spectrophotometer set at 302 nm,
- a 20 µl loop injector.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b). The resolution between the peaks due to methotrexate and folic acid is not less than 5.0.

Inject separately the test solution and reference solution (a) and measure the responses for the principal peak.

Calculate the content of C_{20}H_{22}N_{8}O_{5} in the tablets.

Storage. Store protected from light and moisture.
Methoxamine Hydrochloride

\[
\text{CH}_3\text{NH}_2\text{OH}\quad \text{H}_3\text{CO}\quad \text{OCH}_3\quad \text{H}_3\text{CO}
\]

C\textsubscript{11}H\textsubscript{17}N\textsubscript{0}3\cdot\text{HCl} \quad \text{Mol. Wt. 247.7}

Methoxamine Hydrochloride is all-rac-2-amino-1-(2,5-dimethoxyphenyl)propan-1-ol hydrochloride.

Methoxamine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of C\textsubscript{11}H\textsubscript{17}NO\textsubscript{3},\cdot\text{HCl}, calculated on the dried basis.

**Description.** Colourless crystals or white, plate-like crystals or a white, crystalline powder; odourless or almost odourless.

**Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with methoxamine hydrochloride RS or with the reference spectrum of methoxamine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution shows an absorption maximum only at about 290 nm; absorbance at about 290 nm, about 0.69.

C. Dissolve 20 mg in 2 ml of water, add 5 ml of diazotised nitroaniline solution and 1 ml of dilute sodium carbonate solution. Allow to stand for 2 minutes and add 1 ml of 1 M sodium hydroxide; a deep red colour is produced which is extractable with 1-butanol.

D. A 5 per cent w/v solution gives the reactions of chlorides (2.3.1).

**Tests**

**pH** (2.4.24). 4.0 to 6.0, determined in a 2.0 per cent w/v solution.

**Related substances.** Determine by thin-layer chromatography (2.4.14), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 86 volumes of chloroform, 12 volumes of methanol and 2 volumes of strong ammonia solution.

**Test solution.** Dissolve 0.2 g of the substance under examination in 10 ml of methanol.

**Reference solution (a).** A 0.02 per cent w/v solution of the substance under examination in methanol.

**Reference solution (b).** A 0.01 per cent w/v solution of 2,5-dimethoxybenzaldehyde in methanol.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm.

Any spot corresponding to 2,5-dimethoxybenzaldehyde in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b). Spray the plate with a 0.3 per cent w/v solution of ninhydrin in 1-butanol containing 3 per cent v/v of glacial acetic acid and heat at 105° for 5 minutes. Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

**Assay.** Weigh accurately about 0.5 g and dissolve in 30 ml of anhydrous glacial acetic acid, 15 ml of mercuric acetate solution and 5 ml of acetic anhydride, warming if necessary. Titrate with 0.1 M perchloric acid, using crystal violet solution as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02477 g of C\textsubscript{11}H\textsubscript{17}NO\textsubscript{3},\cdot\text{HCl}.

**Storage.** Store protected from light and moisture.

Methoxamine Injection

Methoxamine Hydrochloride Injection

Methoxamine Injection is a sterile solution containing 2 per cent w/v of Methoxamine Hydrochloride in Water for Injections.

Methoxamine Injection contains not less than 1.90 per cent and not more than 2.10 per cent w/v of methoxamine hydrochloride, C\textsubscript{11}H\textsubscript{17}NO\textsubscript{3},\cdot\text{HCl}.

**Identification**

A. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows an absorption maximum only at about 290 nm.

B. Dilute 1 ml with 1 ml of water, add 5 ml of diazotised nitroaniline solution and 1 ml of dilute sodium carbonate solution. Allow to stand for 2 minutes and add 1 ml of 1 M sodium hydroxide; a deep red colour is produced which is extractable with 1-butanol.

**Tests**

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 86 volumes of chloroform, 12 volumes of methanol and 2 volumes of strong ammonia solution.
Test solution. Dilute the injection, if necessary, with water so as to contain 2.0 per cent w/v of Methoxamine Hydrochloride.

Reference solution (a). Dilute 1 volume of the test solution to 100 ml with water.

Reference solution (b). A 0.01 per cent w/v solution of 2,5-dimethoxybenzaldehyde in methanol.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. Any spot corresponding to 2,5-dimethoxybenzaldehyde in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b). Spray the plate with a 0.3 per cent w/v solution of ninhydrin in 1-butanol containing 3 per cent v/v of glacial acetic acid and heat at 105° for 5 minutes. Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a).

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. To an accurately measured volume containing about 100 mg of Methoxamine Hydrochloride add sufficient water to produce 100.0 ml. Dilute 5.0 ml of this solution to 100.0 ml with water. Measure the absorbance of the resulting solution at the maximum at about 290 nm (2.4.7). Calculate the content of C11H17NO3.HCl taking 137 as the specific absorbance at 290 nm.

Storage. Store protected from light.

Methyl Salicylate

Wintergreen Oil

\[
\text{COOCH}_3
\]

\[
\text{OH}
\]

\[
\text{C}_8\text{H}_8\text{O}_3
\]

Mol. Wt. 152.2

Methyl Salicylate is 2-hydroxybenzoic acid methyl ester.

Methyl Salicylate contains not less than 99.0 per cent w/w and not more than 100.5 per cent w/w of C8H8O3.

Description. A colourless or slightly yellow liquid; odour, strong, persistent, characteristic and aromatic.

Identification

A. To 10 ml of a saturated aqueous solution add 0.05 ml of ferric chloride test solution; a violet colour develops.

B. Heat 0.25 ml with 2 ml of 2 M sodium hydroxide on a water-bath for 5 minutes and add 3 ml of 1 M sulphuric acid. Filter and wash the precipitate with water. The precipitate after drying at 105° for 1 hour melts at 156° to 161° (2.4.21).

Tests

Appearance of solution. To 2 ml add 10 ml of ethanol (95 per cent). The resulting solution is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

Acidity. Dissolve 5.0 g in 50 ml of ethanol (95 per cent), previously neutralised to a blue colour with bromocresol green solution by the addition of 0.1 M sodium hydroxide. Not more than 0.4 ml of 0.1 M sodium hydroxide is required to restore the blue colour.

Refractive index (2.4.27). 1.534 to 1.538.

Weight per ml (2.4.29). 1.175 g to 1.185 g.

Assay. Weigh accurately about 0.5 g, dissolve in 25 ml of ethanol (95 per cent), add 0.05 ml of phenol red solution and neutralise with 0.1 M sodium hydroxide. Add 50.0 ml of 0.1 M sodium hydroxide and heat under a reflux condenser on a water-bath for 30 minutes. Cool and titrate with 0.1 M hydrochloric acid. Carry out a blank titration. 1 ml of 0.1 M sodium hydroxide is equivalent to 0.01522 g of C8H8O3.

Storage. Store protected from light.

Industrial Methylated Spirit

IMS

Industrial Methylated Spirit is a mixture of nineteen volumes of Ethanol of an appropriate strength and one volume of approved wood naphtha.

Description. Clear, colourless, mobile, volatile liquid; odour, spirituous and of wood naphtha.

Identification

Mix 0.1 ml with 0.05 ml of an 11 per cent w/w solution of phosphoric acid and 0.25 ml of dilute potassium permanganate solution. After 1 minute add a few mg of sodium metabisulphite and shake until the mixture is decolorised. Add 1.5 ml of a 50 per cent w/v solution of sulphuric acid and a few mg of finely powdered chromotropic acid sodium salt, shake well and heat on a water-bath for 5 minutes; a deep violet colour is produced.

Tests
**Relative density** (2.4.29). Not greater than 0.815.

**Acidity or alkalinity.** 25 ml requires not more than 0.2 ml of 0.1 M sodium hydroxide to produce a pink colour with phenolphthalein solution and not more than 1.0 ml of 0.1 M hydrochloric acid is required to produce a red colour with methyl red solution.

**Appearance of solution.** Dilute 5.0 ml to 100 ml with water; the solution is clear (2.4.1).

**Aldehydes.** Not more than 50 ppm, determined by the following method. To 5.0 ml add 5 ml of water and 1 ml of decolorised fuchsine solution and allow to stand for 30 minutes. Any colour produced is not more intense than that obtained by treating in the same manner 5 ml of a 0.005 per cent w/v solution of redistilled acetaldehyde in aldehyde-free ethanol (95 per cent).

**Non-volatile matter.** When evaporated and dried at 105°, leaves not more than 0.01 per cent w/v of residue.

**Storage.** Store in tightly-closed containers at a temperature not exceeding 30°.

**Labelling.** The label states that it is inflammable.

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**Methylcellulose**

Cellulose Methyl Ether

Methylcellulose is a cellulose having some of the hydroxyl groups in the form of the methyl ether. Various grades are available and are distinguished by a number indicative of the apparent viscosity in millipascal seconds of a 2 per cent w/w solution measured at 20°.

Methylcellulose contains not less than 27.5 per cent and not more than 31.5 per cent of methoxyl (-OCH₃) groups, calculated on the dried basis.

**Description.** A white or yellowish white or greyish white powder or granules; practically odourless; hygroscopic after drying.

**Identification**

A. With constant stirring add a quantity containing 1.0 g of the dried substance into 50 ml of carbon dioxide-free water previously heated to 90°. Allow to cool, dilute to 100 ml with carbon dioxide-free water and continue stirring until solution is complete (solution A). Heat 10 ml of solution A on a water-bath with stirring. At temperatures above 40° the solution becomes cloudy or a floculent precipitate is formed. On cooling, the solution becomes clear.

B. To 10 ml of solution A add 0.3 ml of 2 M acetic acid and 2.5 ml of a 10.0 per cent w/v solution of tannic acid; a yellowish white, floculent precipitate is produced which dissolves in 6 M ammonia.

C. Without heating completely dissolve 0.2 g in 15 ml of a 70 per cent w/w solution of sulphuric acid, pour the solution with stirring into 100 ml of iced water. In a test-tube kept in ice, mix thoroughly 1 ml of the solution with 8 ml of sulphuric acid, added dropwise. Heat in a water-bath for exactly 3 minutes and cool immediately in ice. When the mixture is cool, carefully add 0.6 ml of a solution containing 3 g of ninhydrin in 100 ml of a 4.55 per cent w/v solution of sodium metabisulphite, mix well and allow to stand at 25°; a pink colour is produced immediately which becomes violet within 100 minutes.

D. Place 1 ml of solution A on a glass plate. After evaporation of the water a thin film is produced.

**Tests**

**Appearance of solution.** Whilst stirring, introduce a quantity containing 1.0 g of the dried substance into 50 g of carbon dioxide-free water heated to 90°. Allow to cool, dilute to 100 g with the same solvent and continue stirring until solution is complete. Allow to stand at 2° to 8° for 1 hour. The resulting solution is not more opalescent than opalescence standard OS3 (2.4.1), and is not more intensely coloured than reference solution YS6 (2.4.1).

**pH** (2.4.24). 5.0 to 8.5, determined in solution A.

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Chlorides** (2.3.12). Dilute 5.0 ml of solution A to 15 ml with water. The resulting solution complies with the limit test for chlorides (0.5 per cent).

**Apparent viscosity.** Not less than 75 per cent and not more than 140 per cent of the declared value, determined by the following method. To 150 g of water heated to 90° add, with stirring, a quantity containing 6.0 g of the dried substance. Stir with a propeller-type stirrer for 10 minutes, place the flask in a bath of iced water, continue the stirring and allow to remain in the bath of iced water for 40 minutes to ensure that solution is complete. Adjust the weight of the solution to 300 g and centrifuge the solution to expel any trapped air. Determine the viscosity at 20° by Method C (2.4.28), using a shear rate of 10 s⁻¹.

**Sulphated ash** (2.3.18). Not more than 1.0 per cent.

**Loss on drying** (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 50 mg in a hard gelatin capsule shell place the capsule and the contents in a 50-ml boiling flask and carry out the determination of methoxyl (2.3.29).

1 ml of 0.1 M sodium thiosulphate is equivalent to 0.0005172 g of methoxyl (-OCH₃) groups.
Storage. Store protected from light and moisture.

Labelling. The label states the apparent viscosity in millipascal seconds of a 2 per cent w/w solution.

Methyldopa

\[
\text{OH} \quad \text{COOH} \quad \text{H}_2\text{NCH}_3, \ 1\frac{1}{2}\text{H}_2\text{O}
\]

C\(_{10}\)H\(_{13}\)NO\(_4\), 1\(\frac{1}{2}\)H\(_2\)O  
Mol. Wt. 238.2

Methyldopa is 3-(3,4-dihydroxyphenyl)-2-methyl-L-alanine sesquihydrate.

Methyldopa contains not less than 98.5 per cent and not more than 101.0 per cent of C\(_{10}\)H\(_{13}\)NO\(_4\), calculated on the anhydrous basis.

Description. A white to yellowish white, fine powder which may contain friable lumps.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with \textit{methyldopa RS} or with the reference spectrum of methyldopa.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.004 per cent w/v solution in 0.1 M hydrochloric acid shows an absorption maximum only at about 280 nm; absorbance at about 280 nm, about 0.46.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with microcrystalline cellulose.

Mobile phase. A mixture of 50 volumes of 1-butanol, 25 volumes of glacial acetic acid and 25 volumes of water.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of 1 M hydrochloric acid.

Reference solution (a). A 1 per cent w/v solution of \textit{methyldopa RS} in 1 M hydrochloric acid.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 10 µl of each of the test solution and reference solution (a) and 20 µl of reference solution (b). After development, dry the plate immediately in a current of warm air and spray with a mixture of 5 volumes of a 5 per cent w/v solution of sodium nitrite and 45 volumes of a 0.3 per cent w/v solution of 4-nitroaniline in a mixture of 80 volumes of hydrochloric acid and 20 volumes of water. Dry it in a current of warm air and spray with a 20 per cent w/v solution of sodium carbonate and examine immediately. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

Heavy metals (2.3.13). Dissolve 2.0 g in 10 ml of water, add 2 ml of dilute acetic acid and dilute to 25 ml with water. The solution complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.
**Methyldopa Tablets**

Methyldopa Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of anhydrous methyldopa, C_{10}H_{13}NO_{4}. The tablets are coated.

**Identification**

Remove the coating from a suitable quantity of the tablets by washing with chloroform. To a quantity of the powdered tablet cores containing 5 g of anhydrous methyldopa add 35 ml of a mixture of equal volumes of chloroform and methanol and shake for 3 minutes. Centrifuge and discard the supernatant liquid. Repeat the operation with a further 35 ml of a mixture of equal volumes of chloroform and methanol. Dry the residue in a current of nitrogen, add 20 ml of methanol and 15 ml of 2 M hydrochloric acid, shake for 3 minutes and filter. Close the pH of the filtrate to 4.9 with 5 M ammonia, allow to stand for several hours at 2° to 8° and filter. Wash the precipitate with 15 ml of water and dry it at 50° at a pressure not exceeding 0.7 kPa for 3 hours. Reserve a portion of the residue for the test for Specific optical rotation. The remainder of the residue complies with tests A and B.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with methyldopa RS or with the reference spectrum of methyldopa.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.004 per cent w/v solution in 0.1 M hydrochloric acid shows an absorption maximum only at about 280 nm; absorbance at about 280 nm, about 0.46.

C. To a quantity of the powdered tablets containing 10 mg of anhydrous methyldopa, add 3 drops of a 0.4 per cent w/v solution of ninhydrin in sulphuric acid; a dark purple colour is produced within 5 to 10 minutes. Add 0.15 ml of water; the colour changes to pale brownish yellow.

D. To 10 mg of the powdered tablets add 2 ml of 0.1 M sulphuric acid, 2 ml of ferrous sulphate-citrate solution and 0.5 ml of dilute ammonia solution; a dark purple colour is immediately produced.

**Water** (2.3.43). 10.0 to 13.0 per cent, determined on 0.4 g.

**Assay.** Weigh accurately about 0.4 g and dissolve in 15 ml of anhydrous formic acid, 30 ml of anhydrous glacial acetic acid and 30 ml of dioxan. Titrate with 0.1 M perchloric acid, using crystal violet solution as indicator. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02112 g of C_{10}H_{13}NO_{4}.

**Storage.** Store protected from light and moisture.

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**Methylergometrine Maleate**

Methylergometrine Maleate is 9,10-didehydro-N-[(S)-1-(hydroxymethyl)propyl]-6-methylergoline-8β-carboxamide hydrogen maleate.

Methylergometrine Maleate contains not less than 95.0 per cent and not more than 105.0 per cent of C_{20}H_{25}N_{3}O_{4}C_{4}H_{4}O_{4}, calculated on the dried basis.

**Description.** A white or faintly yellow, crystalline powder; odourless.
Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with methylergometrine maleate RS.

B. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

C. A 1 per cent w/v solution shows a blue fluorescence.

D. Dissolve 0.25 mg in 1 ml of glacial acetic acid containing a trace of ferric chloride solution and add carefully 1 ml of sulphuric acid and shake well; a deep blue colour is produced.

Tests

pH (2.4.24). 4.4 to 5.2, determined in a 0.02 per cent w/v solution.

Specific optical rotation (2.4.22). +44.0° to +50°, determined at 20° in a 0.5 per cent w/v solution.

Related substances. Protect the solutions from light throughout the test.

Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 10 volumes of chloroform and 1 volume of methanol.

Test solution. Dissolve 40 mg of the substance under examination in 10 ml of methanol.

Reference solution (a). A 0.4 per cent w/v solution of methylergometrine maleate RS in methanol.

Reference solution (b). A 0.012 per cent w/v solution of methylergometrine maleate RS in methanol.

Place a beaker containing 25 ml of strong ammonia solution in the developing chamber, cover the chamber and allow to equilibrate for 30 minutes. Apply to the plate 25 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Spray the plate with a solution containing 0.8 g of 4-dimethylaminobenzaldehyde in a mixture of 90 ml of ethanol and 10 ml of sulphuric acid. Dry in a current of warm air for about 2 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 1.0 g by drying at 80° at a pressure not exceeding 2.7 kPa for 3 hours.

Assay. Weigh accurately about 20 mg and dissolve in sufficient water to produce 100.0 ml; dilute 20.0 ml of this solution to 100.0 ml with water. To 3.0 ml add 6.0 ml of dimethylamino-benzaldehyde reagent, mix, cool in running water for 5 minutes and add sufficient reagent to produce 10.0 ml. Measure the absorbance of the resulting solution at the maximum at about 550 nm (2.4.7), using as the blank a solution prepared in the same manner omitting the substance under examination. Calculate the content of C_{20}H_{25}N_{3}O_{2}, C_{4}H_{4}O_{4} from the absorbance obtained by repeating the Assay using ergometrine maleate RS in place of the substance under examination.

1 mg of ergometrine maleate RS is equivalent to 1.032 mg of C_{20}H_{25}N_{3}O_{2}, C_{4}H_{4}O_{4}.

Storage. Store protected from light, in an atmosphere of nitrogen, at a temperature between 2° to 8°.

Methylergometrine Injection

Methylergometrine Maleate Injection; Methylergonovine Maleate Injection; Methylergonovine Injection

Methylergometrine Injection is a sterile solution of Methylergometrine Maleate in Water for Injections free from dissolved air.

Methylergometrine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of methylergometrine maleate, C_{20}H_{25}N_{3}O_{2}, C_{4}H_{4}O_{4}.

Identification

A. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

B. It exhibits a blue fluorescence.

C. To a volume containing 0.1 mg of Methylergometrine Maleate add 0.5 ml of water and 2 ml of 4-dimethylaminobenzaldehyde solution; after a few minutes a deep blue colour is produced.

Tests

pH (2.4.24). 2.7 to 3.5.

Related substances. Protect the solutions from light throughout the test.

Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 10 volumes of chloroform and 1 volume of methanol.

Test solution. Transfer a volume containing 1 mg of Methylergometrine Maleate to a separating funnel, add 1 ml of sodium bicarbonate solution and extract with three quantities, each of 5 ml, of chloroform. Evaporate the combined
extracts to dryness at room temperature at a pressure not exceeding 0.7 kPa. Dissolve the residue in 0.25 ml of methanol and centrifuge, if necessary. 

Reference solution (a). A 0.4 per cent w/v solution of methylergometrine maleate RS in methanol.

Reference solution (b). A 0.012 per cent w/v solution of methylergometrine maleate RS in methanol.

Place a beaker containing 25 ml of strong ammonia solution in the developing chamber, cover the chamber and allow to equilibrate for 30 minutes. Apply to the plate 25 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Spray the plate with a solution containing 0.8 g of 4-dimethylaminobenzaldehyde in a mixture of 90 ml of ethanol and 10 ml of sulphuric acid. Dry in a current of warm air for about 2 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Protect the solutions from light throughout the Assay.

To 1.0 ml add sufficient water to produce a solution containing 0.04 mg of Methylergometrine Maleate per ml. To 3.0 ml add 6.0 ml of dimethylaminobenzaldehyde reagent, mix, cool in running water for 5 minutes and add sufficient reagent to produce 10.0 ml. Measure the absorbance of the resulting solution at the maximum at about 550 nm (2.4.7), using as the blank a solution prepared in the same manner omitting the substance under examination. Calculate the content of C_{20}H_{25}N_{3}O_{2},C_{4}H_{4}O_{4} from the absorbance obtained by repeating the Assay using ergometrine maleate RS in place of the substance under examination.

1 mg of ergometrine maleate RS is equivalent to 1.032 mg of C_{20}H_{25}N_{3}O_{2},C_{4}H_{4}O_{4}.

Storage. Store protected from light.

Methylergometrine Tablets

Methylergometrine Maleate Tablets; Methylergonovine Maleate Tablets; Methylergonovine Tablets

Methylergometrine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of methylergometrine maleate, C_{20}H_{25}N_{3}O_{2},C_{4}H_{4}O_{4}. The tablets may be coated.

Identification

A. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

B. Extract a quantity of the powdered tablets containing 1 mg of Methylergometrine Maleate with 10 ml of water, filter and wash the residue with sufficient water to produce 10 ml; the solution has a blue fluorescence.

C. To 2 ml of the solution obtained in test B add 4 ml of 4-dimethylaminobenzaldehyde solution; a deep blue colour is produced after a few minutes.

Tests

Related substances. Protect the solutions from light throughout the test.

Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 10 volumes of chloroform and 1 volume of methanol.

Test solution. To a quantity of the powdered tablets containing 1 mg of Methylergometrine Maleate add 5 ml of water, 1 ml of sodium bicarbonate solution and 2 ml of chloroform. Shake, allow to separate and filter the chloroform layer through a plug of cotton moistened with chloroform. Repeat the extraction with a further 2 ml of chloroform and filter. Evaporate the combined extracts to dryness at room temperature at a pressure not exceeding 0.7 kPa, dissolve the residue in 0.25 ml of methanol and centrifuge, if necessary.

Reference solution (a). A 0.4 per cent w/v solution of methylergometrine maleate RS in methanol.

Reference solution (b). A 0.012 per cent w/v solution of methylergometrine maleate RS in methanol.

Place a beaker containing 25 ml of strong ammonia solution in the developing chamber, cover the chamber and allow to equilibrate for 30 minutes. Apply to the plate 25 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Spray the plate with a solution containing 0.8 g of 4-dimethylaminobenzaldehyde in a mixture of 90 ml of ethanol and 10 ml of sulphuric acid. Dry in a current of warm air for about 2 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

Uniformity of content. Comply with the test stated under Tablets.

Crush one tablet and transfer to a separating funnel with the aid of not more than 5 ml of water and add 3 ml of a 5 per cent w/v solution of sodium carbonate. Extract with four quantities, each of 5 ml, of chloroform. Filter the extracts through a plug of cotton moistened with chloroform into a 100-ml separating funnel. Add 2.0 ml of water and 10.0 ml of 4-dimethylaminobenzaldehyde solution and shake vigorously for at least 90 seconds. Allow to stand for 30 minutes and discard the
chloroform layer. Transfer the aqueous layer to a stoppered-tube and allow to stand for 60 minutes. Measure the absorbance of the resulting solution at the maximum at about 550 nm (2.4.7), using as the blank a mixture of 2.0 ml of water and 10.0 ml of 4-dimethylaminobenzaldehyde solution. Calculate the content of \( C_{19}H_{23}N_3O_2, C_4H_4O_4 \) in the tablet from the absorbance obtained by carrying out the following operation simultaneously. Weigh accurately about 12 mg of ergometrine maleate RS and dissolve in sufficient water to produce 200.0 ml. To 2.0 ml add 10.0 ml of 4-dimethylaminobenzaldehyde solution, mix and cool in running water for 5 minutes. Measure the absorbance of the resulting solution at the maximum at about 545 nm, using as the blank a mixture of 2.0 ml of water and 10.0 ml of 4-dimethylaminobenzaldehyde solution.

1 mg of ergometrine maleate RS is equivalent to 1.032 mg of \( C_{20}H_{25}N_3O_2, C_4H_4O_4 \).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 2 mg of Methylergometrine Maleate, dissolve in 50 ml of a 1 per cent w/v solution of tartaric acid. To 3.0 ml add 6.0 ml of dimethylaminobenzaldehyde reagent, mix, cool in running water for 5 minutes and add sufficient reagent to produce 10.0 ml. Measure the absorbance of the resulting solution at the maximum at about 550 nm (2.4.7), using as the blank a solution of \( C_{20}H_{25}N_3O_2, C_4H_4O_4 \) from the absorbance obtained by repeating the Assay using ergometrine maleate RS in place of the substance under examination.

1 mg of ergometrine maleate RS is equivalent to .001032 g of \( C_{20}H_{25}N_3O_2, C_4H_4O_4 \).

Storage. Store protected from light and moisture.

Methyparaben

Methyl Hydroxybenzoate

\[
\text{COOCH}_3
\]

\[
\text{OH}
\]

\( C_8H_8O_3 \)  
Mol. Wt. 152.2

Methyparaben is methyl 4-hydroxybenzoate.

Methyparaben contains not less than 99.0 per cent and not more than 101.0 per cent of \( C_8H_8O_3 \).
barium chloride solution; no turbidity is produced within 10 minutes.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Assay.** Weigh accurately about 80 mg, transfer to a glass-stoppered flask, add 25 ml of 2 M sodium hydroxide and boil gently under a reflux condenser for 30 minutes. Cool and add 25.0 ml of 0.0333 M potassium bromate, 5 ml of a 12.5 per cent w/v solution of potassium bromide and 40 ml of glacial acetic acid, cool in ice, add 10 ml of hydrochloric acid, immediately stopper the flask and allow to stand for 15 minutes. Add 15 ml of potassium iodide solution, mix and titrate the liberated iodine with 0.1 M sodium thiosulphate using 2 ml of starch solution, added towards the end of the titration, as indicator.

Repeat the operation without the substance under examination. The difference between the titrations represents the amount of potassium bromate required. The volume of 0.0333 M potassium bromate is equivalent to half of the volume of 0.1 M sodium thiosulphate required for the titration.

1 ml of 0.0333 M potassium bromate is equivalent to 0.005072 g of C$_8$H$_8$O$_3$.

**Storage.** Store protected from light and moisture.

### Methylprednisolone

![Methylprednisolone](image)

C$_{22}$H$_{30}$O$_5$ Mol. Wt. 374.5

Methylprednisolone is 11β,17α,21-trihydroxy-6α-methylpregna-1,4-diene-3,20-dione.

Methylprednisolone contains not less than 96.0 per cent and not more than 104.0 per cent of C$_{22}$H$_{30}$O$_5$, calculated on the dried basis.

**Description.** A white or almost white, crystalline powder.

**Identification**

Test A may be omitted if tests B and C are carried out. Test C may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with methylprednisolone RS or with the reference spectrum of methylprednisolone.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Solvent mixture.** A mixture of 90 volumes of acetone and 10 volumes of formamide.

**Mobile phase.** Chloroform.

**Test solution.** Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

**Reference solution (a).** Dissolve 25 mg of methylprednisolone RS in 10 ml of the solvent mixture.

**Reference solution (b).** Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with ethanolic sulphuric acid (20 per cent v/v). Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. Dissolve about 2 mg in 2 ml of sulphuric acid by shaking and allow to stand for 5 minutes; an intense red colour is produced and the solution exhibits a reddish brown fluorescence when examined in ultraviolet light at 365 nm. Add the solution to 10 ml of water and mix; the colour fades and the solution exhibits a yellowish green fluorescence in ultraviolet light at 365 nm.

**Tests**

**Specific optical rotation** (2.4.22). +79.0° to +86.0°, determined in a 1.0 per cent w/v solution in dioxan.

**Light absorption** (2.4.7). Absorbance of a 0.001 per cent w/v solution in ethanol (95 per cent) at the maximum at about 243 nm, 0.38 to 0.40.

**Related substances.** Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in a mixture of equal volumes of acetonitrile and methanol and dilute to 10 ml with the same solvent mixture.
Reference solution (a). Dissolve 2.0 mg of methylprednisolone RS and 2.0 mg of betamethasone RS in mobile phase A and dilute to 200 ml with the same mobile phase.

Reference solution (b). Dilute 1 ml of the test solution to 100 ml with mobile phase A.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane chemically bonded to silica gel (5 µm),
- mobile phase A. 250 volumes of acetonitrile and 700 volumes of water mixed, allowed to equilibrate and adjusted to 1000 volumes with water and mixed,
- B. acetonitrile,
- flow rate. 2.5 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
</tr>
<tr>
<td>15</td>
<td>100</td>
<td>100</td>
<td>begin linear gradient</td>
</tr>
<tr>
<td>40</td>
<td>0</td>
<td>100</td>
<td>end chromatogram, return to 100 A</td>
</tr>
<tr>
<td>41</td>
<td>100</td>
<td>0</td>
<td>begin equilibration with A</td>
</tr>
<tr>
<td>46=0</td>
<td>100</td>
<td>0</td>
<td>end equilibration, begin next chromatogram</td>
</tr>
</tbody>
</table>

Equilibrate the column for at least 30 minutes with mobile phase B and then with mobile phase A for 5 minutes. For subsequent operations use the conditions described from 40 to 46 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is at least 50 per cent of the full scale of the recorder.

Inject reference solution (a). When the chromatograms are recorded, the retention times are: methylprednisolone about 11.5 minutes, and betamethasone about 12.5 minutes. The test is not valid unless the resolution between the peaks corresponding to methylprednisolone and betamethasone is at least 1.5; if necessary, adjust the concentration of acetonitrile in mobile phase A.

Inject separately a mixture of equal volumes of acetonitrile and methanol as a blank, the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any peak other than the principal peak, is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all the peaks other than the principal peak, is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent). Ignore any peak due to the blank and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Weigh accurately about 0.1 g and dissolve in sufficient ethanol to produce 100.0 ml and mix. Dilute 2.0 ml of this solution to 100.0 ml with ethanol and mix well. Determine the absorbance of the resulting solution (2.4.7) at the maximum at about 243 nm. Calculate the content of C_{22}H_{29}FO_{5} taking 395 as the specific absorbance at 243 nm.

Storage. Store protected from light and moisture.

Methylprednisolone Tablets

Methylprednisolone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of methylprednisolone, C_{22}H_{30}O_{5}.

Identification

Extract a quantity of the powdered tablets containing 50 mg of Methylprednisolone with 100 ml of chloroform, filter and evaporate the filtrate to dryness. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with methylprednisolone RS or with the reference spectrum of methylprednisolone.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Solvent mixture. A mixture of 90 volumes of acetone and 10 volumes of formamide.

Mobile phase. A mixture of 30 volumes of toluene and 10 volumes of chloroform.

Test solution. Dissolve 25 mg of the residue in 10 ml of the solvent mixture.

Reference solution (a). Dissolve 25 mg of methylprednisolone acetate RS in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).
Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120°C for 15 minutes and spray the hot plate with ethanolic sulphuric acid (20 per cent v/v). Heat at 120°C for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

**Tests**

**Related substances.** Determine by liquid chromatography (2.4.14).

**Diluting solution.** A filtered mixture of 72 volumes of water, 25 volumes of tetrahydrofuran and 3 volumes of glacial acetic acid.

**Test solution.** Extract a quantity of the powdered tablets containing 25 mg of Methylprednisolone with the diluting solution and dilute to 25 ml with the same solvent. Filter and centrifuge if necessary.

**Reference solution.** A 0.001 per cent w/v solution of methylprednisolone RS in the diluting solution.

**Chromatographic system**
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilil silica gel (3 to 10 µm),
- mobile phase: a mixture of 149 volumes of water, 40 volumes of tetrahydrofuran, 10 volumes of dimethysulfoxide and 1 volume of butanol,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 10 µl loop injector.

Inject the reference solution. The column efficiency is not less than 800 theoretical plates and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution, the area of any peak other than the principal peak is not greater than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). The sum of the areas of all the peaks other than the principal peak is not greater than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent).

**Dissolution (2.5.2).**

Apparatus. No 2

Medium. 900 ml of water

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of a layer of suitable thickness of the filtered solution at the maximum at about 246 nm (2.4.7). Calculate the content of C_{22}H_{30}O_{5} in the medium taking 400 as the specific absorbance at 246 nm.

D. Not less than 70 per cent of the stated amount of C_{22}H_{30}O_{5}.

**Uniformity of content.** Comply with the test stated under Tablets.

To one tablet add 0.5 ml of water (in the case of tablets containing 10 mg or less) or 1.0 ml of water (in the case of tablets containing more than 10 mg). Allow the tablet to stand for about 2 minutes, then swirl to disperse the tablet. Add 5.0 ml of the internal standard used in the assay for each mg of methylprednisolone, shake for 15 minutes, filter and centrifuge. Use the filtrate as the test solution.

Determine by liquid chromatography (2.4.14) using the chromatographic system and the reference solution described under Assay.

Calculate the content of C_{22}H_{30}O_{5} in the tablet.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14).

**Internal standard solution.** Weigh accurately a suitable quantity of prednisolone in a 3 per cent v/v solution of glacial acetic acid in chloroform to obtain a solution having a known concentration of about 0.2 mg per ml of prednisolone.

**Test solution.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 10 mg of Methylprednisolone transfer to a suitable container and add 2.5 ml of water. Swirl to form a slurry. Add 50.0 ml of the internal standard solution, and shake for 15 minutes. Filter and centrifuge a portion of the filtrate if necessary and use this as the test solution.

**Reference solution.** Weigh accurately a suitable quantity of methylprednisolone RS in the internal standard solution to obtain a solution having a known concentration of about 0.2 mg per ml of methylprednisolone.

**Chromatographic system**
- stainless steel column 25 cm x 4 mm, packed with porous silica particles (3 to 10 µm),
- mobile phase: a mixture of 475 volumes of butyl chloride, 475 volumes of water-saturated butyl chloride, 70 volumes of tetrahydrofuran, 35 volumes of methanol, and 30 volumes of glacial acetic acid,
flow rate 1 ml per minute,
spectrophotometer set at 254 nm,
a 10 µl loop injector.

Inject the reference solution. The resolution between methylprednisolone and prednisolone is not less than 4.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. The relative retention times are 0.7 for prednisolone and 1.0 for methylprednisolone.

Calculate the content of C₂₂H₃₀O₅ in the tablets.

Storage. Store protected from light and moisture.

Methylprednisolone Acetate

C₂₄H₃₂O₆ Mol. Wt. 416.5

Methylprednisolone Acetate is 11β,17α-dihydroxy-6α-methyl-3,20-dioxopregna-1,4-dien-21-yl acetate.

Methylprednisolone Acetate contains not less than 96.0 per cent and not more than 104.0 per cent of C₂₄H₃₂O₆, calculated on the dried basis.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with methylprednisolone acetate RS or with the reference spectrum of methylprednisolone acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Solvent mixture. A mixture of 90 volumes of acetone and 10 volumes of formamide.

Mobile phase. A mixture of 30 volumes of toluene and 10 volumes of chloroform.

Tests

Specific optical rotation (2.4.22). +97.0° to +105°, determined in a 1.0 per cent w/v solution in dioxan.

Light absorption (2.4.7). Absorbance of a 0.001 per cent w/v solution in ethanol at the maximum at about 240 nm, 0.34 to 0.37. The ratio of the absorbance at the maximum at about 240 nm to that at about 263 nm is 1.50 to 1.70.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in 5 ml of tetrahydrofuran and dilute to 10 ml with water.

Reference solution (a). Dissolve 4.0 mg of methylprednisolone acetate RS and 4.0 mg of dexamethasone acetate RS in the mobile phase and dilute to 20 ml with the mobile phase.

Reference solution (b). Dilute 1 ml of the test solution to 50 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 260 ml of tetrahydrofuran and 760 ml of water; allowed to equilibrate, diluted to 1000 ml with water and mixed,
t

flow rate. 1 ml per minute,
spectrophotometer set at 254 nm,
a 20 µl loop injector.

Equilibrate the column with the mobile phase for about 45 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is at least 50 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are:
methylprednisolone acetate, about 43 minutes and dexamethasone acetate about 57 minutes. The test is not valid unless the resolution between the peaks corresponding to methylprednisolone acetate and dexamethasone acetate is not less than 6.5. If necessary, adjust the concentration of water in the mobile phase.

Inject the test solution and reference solution (b). Continue the chromatography for 1.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the sum of the areas of all the peaks other than the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak due to the solvent and any peak with an area less than 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Weigh accurately about 0.1 g and dissolve in sufficient ethanol to produce 100.0 ml and mix. Dilute 1.0 ml of this solution to 100.0 ml with ethanol and mix well. Determine the absorbance of the resulting solution (2.4.7) at about 243 nm. Calculate the content of C_{24}H_{32}O_{6}, taking 355 as the specific absorbance at 243 nm.

Storage. Store protected from light and moisture.

Methylprednisolone Acetate Injection

Methylprednisolone Acetate Injection is a sterile suspension of Methylprednisolone Acetate in Water for Injections.

Methylprednisolone Acetate Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of methylprednisolone acetate, C_{24}H_{32}O_{6}.

Description. A white suspension which settles on standing but readily disperses on shaking. On examination under a microscope, the particles are seen to be crystalline and rarely exceed 20 µm in diameter.

Identification

Dilute a volume containing 0.1 g of Methylprednisolone Acetate to 5 ml with water, centrifuge and discard the supernatant liquid. Wash the residue with five quantities, each of 5 ml of water, resuspending the residue in water each time. Centrifuge and discard the washings. The residue, after drying at 105° for 3 hours, complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with methylprednisolone acetate RS or with the reference spectrum of methylprednisolone acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Solvent mixture. A mixture of 90 volumes of acetone and 10 volumes of formamide.

Mobile phase. A mixture of 30 volumes of toluene and 10 volumes of chloroform.

Test solution. Dissolve 25 mg of the residue in 10 ml of the solvent mixture.

Reference solution (a). Dissolve 25 mg of methylprednisolone acetate RS in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Prepare a dry plate for the test. Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with ethanolic sulphuric acid (20 per cent v/v). Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

Tests

pH (2.4.24). 3.5 to 7.0.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Mix 0.12 g of prednisone RS (internal standard) with 0.6 ml of glacial acetic acid, slowly add chloroform with the aid of
ultrasound, shake to dissolve and dilute with sufficient chloroform to produce 20 ml (solution A).

**Test solution.** Add 10 ml of solution A to an accurately measured quantity of the injection containing about 40 mg of Methylprednisolone Acetate, add sufficient chloroform to produce 25.0 ml and shake for 5 minutes or until the aqueous layer is clear; to 4.0 ml of the chloroform layer, add 30 ml of chloroform and 0.4 g of anhydrous sodium sulphate, shake for 5 minutes, and use the clear solution.

**Reference solution.** Dissolve 20.0 mg of methylprednisolone acetate RS in 5 ml of solution A and add sufficient chloroform to produce 100.0 ml.

**Chromatographic system**
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane chemically bonded to porous silica (5 to 10 µm),
- mobile phase: a mixture of 30 volumes of glacial acetic acid and 35 volumes of methanol, 75 volumes of tetrahydrofuran, 475 volumes of water-saturated 1-chloro-butane and 475 volumes of 1-chloro-butane,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

The assay is not valid unless the resolution between the peaks due to methylprednisolone and the internal standard is at least 2.5.

Calculate the content of C₂₂H₂₉FO₅ in the injection.

**Storage.** Store protected from light at a temperature not exceeding 30°. The injection should not be allowed to freeze.

**Labelling.** The label states (1) that the preparation is not to be given by intravenous injection; (2) that the container should be shaken gently before a dose is withdrawn.

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**Metoclopramide Hydrochloride**

![Metoclopramide Hydrochloride](image)

C₁₄H₂₂ClN₃O₂.HCl.H₂O

Metoclopramide Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of C₁₄H₂₂ClN₃O₂.HCl, calculated on the anhydrous basis.

**Description.** White or almost white crystals or crystalline powder.

**Identification**

**Test A may be omitted if tests B, C and D are carried out. Tests B and D may be omitted if tests A and C are carried out.**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with metoclopramide hydrochloride RS or with the reference spectrum of metoclopramide hydrochloride.

B. Examine the chromatograms obtained in the test for Related substances in ultraviolet light before spraying with the 4-dimethylaminobenzaldehyde reagent. The principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. A 5 per cent w/v solution in carbon dioxide-free water gives reaction A of chlorides (2.3.1).

D. Dissolve about 2 mg in 2 ml of water. The solution gives the reaction of primary aromatic amines (2.3.1).

**Tests**

**Appearance of solution.** A 10.0 per cent w/v solution in carbon dioxide-free water is clear (2.4.1), and colourless (2.4.1).

**pH (2.4.24).** 4.5 to 6.0, determined in a 10.0 per cent w/v solution.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel HF254.

**Mobile phase.** A mixture of 90 volumes of dichloromethane, 14 volumes of methanol, 10 volumes of dioxan and 2 volumes of strong ammonia solution.

**Test solution (a).** Dissolve 0.4 g of the substance under examination in 10 ml of methanol.

**Test solution (b).** Dissolve 0.4 g of the substance under examination in 100 ml of methanol.

**Reference solution (a).** A 0.02 per cent w/v solution of the substance under examination in methanol.

**Reference solution (b).** A 0.4 per cent w/v solution of metoclopramide hydrochloride RS in methanol.

**Reference solution (c).** A 0.02 per cent w/v solution of N,N-diethyl-ethylenediamine in methanol.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution.
a. Spray the plate with dimethylaminobenzaldehyde reagent and allow it to dry in air. Any secondary spot in the chromatogram obtained with test solution (a) that has not been visualised in ultraviolet light at 254 nm is not more intense than the spot in the chromatogram obtained with reference solution (c).

**Heavy metals** (2.3.13). 12 ml of a 10 per cent w/v solution in carbon dioxide-free water complies with the limit test for heavy metals, Method D (20 ppm). Use lead standard solution (2 ppm Pb) to prepare the standard.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). 4.5 to 5.5 per cent, determined on 0.5 g.

**Assay.** Weigh accurately about 0.25 g, dissolve in a mixture of 50 ml of ethanol (95 per cent) and 5.0 ml of 0.01 M hydrochloric acid. Titrate with 0.1 M sodium hydroxide, determining the end-point potentiometrically (2.4.25). Note the volume added between the two inflections.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.03363 g of C14H22ClN3O2.HCl.

**Storage.** Store protected from light and moisture.

### Metoclopramide Injection

Metoclopramide Hydrochloride Injection

Metoclopramide Injection is a sterile solution of Metoclopramide Hydrochloride in Water for Injections free from dissolved air. It contains suitable buffering and stabilising agents.

Metoclopramide Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous metoclopramide hydrochloride, C14H22ClN3O2.HCl.

**Description.** A clear, colourless solution.

**Identification**

A. Dilute a volume containing 10 mg of anhydrous metoclopramide hydrochloride to 500 ml with 0.01 M hydrochloric acid.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 273 nm and 309 nm.

B. To a volume containing 50 mg of anhydrous metoclopramide hydrochloride add 5 ml of water and 5 ml of a 1 per cent w/v solution of 4-dimethylaminobenzaldehyde in 1 M hydrochloric acid; a yellow-orange colour is produced.

C. Gives reaction A of chlorides (2.3.1).

**Tests**

**pH** (2.4.24). 3.0 to 5.0.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel HF254.

**Mobile phase.** A mixture of 90 volumes of dichloromethane, 14 volumes of methanol, 10 volumes of dioxan and 2 volumes of strong ammonia solution.

**Test solution.** Dilute a volume of the injection with methanol, if necessary, to contain the equivalent of 0.5 per cent w/v of anhydrous metoclopramide hydrochloride.

**Reference solution (a).** Dilute 1 volume of the test solution to 200 volumes with methanol.

**Reference solution (b).** A 0.0025 per cent w/v solution of N,N-diethylhexylenediamine in methanol.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a). Spray the plate with dimethylaminobenzaldehyde reagent and allow it to dry in air. Any secondary spot in the chromatogram obtained with the test solution that has not been visualised in ultraviolet light at 254 nm is not more intense than the spot in the chromatogram obtained with reference solution (b).

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** Dilute an accurately measured volume containing about 10 mg of anhydrous metoclopramide hydrochloride to 100.0 ml with water. To 20.0 ml of this solution add 15 ml of 1.25 M sodium hydroxide and extract with three quantities, each of 30 ml, of chloroform, dry each extract with anhydrous sodium sulphate and filter. Dilute the combined extracts to 100.0 ml with chloroform and mix. Measure the absorbance of the resulting solution at the maximum at about 305 nm (2.4.7). Calculate the content of C14H22ClN3O2.HCl, taking 265 as the specific absorbance at 305 nm.

**Storage.** Store protected from light.

**Labelling.** The label states the strength in terms of the equivalent amount of anhydrous metoclopramide hydrochloride in a suitable dose-volume.

### Metoclopramide Syrup

Metoclopramide Hydrochloride Syrup

Metoclopramide Syrup contains Metoclopramide Hydrochloride in a suitable flavoured vehicle.

Metoclopramide Syrup contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous metoclopramide hydrochloride, C14H22ClN3O2.HCl.
Identification
To 50 ml add 5 M sodium hydroxide till the solution becomes alkaline and extract with three quantities, each of 40 ml, of chloroform, dry each extract with anhydrous sodium sulphate. Evaporate the combined extracts to dryness on a water-bath. The residue complies with the following tests.

A. Dissolve 10 mg of the residue in 0.01 M hydrochloric acid and dilute to 500 ml with 0.01 M hydrochloric acid.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 273 nm and 309 nm.

B. To 25 mg of the residue add 2.5 ml of 1 per cent w/v solution of 4-dimethylaminobenzaldehyde in 1 M hydrochloric acid; a yellow-orange colour is produced.

C. To 25 mg of the residue add 2.5 ml of water and 2.5 ml of a 1 per cent w/v solution of 4-dimethylaminobenzaldehyde in 1 M hydrochloric acid; a yellow-orange colour is produced.

D. Dissolve about 2 mg in 2 ml of water. The solution gives the reaction of primary aromatic amines (2.3.1).

Tests
pH (2.4.24). 2.0 to 4.0.

Other tests. Complies with the tests stated under Oral liquids.

Assay. Dilute an accurately measured volume containing about 10 mg of anhydrous metoclopramide hydrochloride to 100.0 ml with water. To 20.0 ml of this solution add 15 ml of 1.25 M sodium hydroxide and extract with three quantities, each of 30 ml, of chloroform, dry each extract with anhydrous sodium sulphate and filter. Dilute the combined extracts to 100.0 ml with chloroform and mix. Measure the absorbance of the resulting solution at the maximum at about 305 nm (2.4.7). Calculate the content of \( C_{14}H_{22}ClN_3O_2 \cdot HCl \), taking 265 as the specific absorbance at 305 nm.

Storage. Store protected from light and moisture.

Labelling. The label states the strength in terms of the equivalent amount of anhydrous metoclopramide hydrochloride in a suitable dose-volume.

Metoclopramide Tablets
Metoclopramide Hydrochloride Tablets

Metoclopramide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous metoclopramide hydrochloride, \( C_{14}H_{22}ClN_3O_2 \cdot HCl \).

Identification
A. Shake a quantity of the powdered tablets containing 10 mg of anhydrous metoclopramide hydrochloride with 50 ml of 0.01 M hydrochloric acid and heat at 70° for 15 minutes with frequent shaking. Cool, dilute to 100 ml with 0.01 M hydrochloric acid, filter and dilute 10 ml of the filtrate to 50 ml with the same solvent. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 273 nm and 309 nm.

B. Shake a quantity of the powdered tablets containing 50 mg of anhydrous metoclopramide hydrochloride with 5 ml of water, filter and add to the filtrate 5 ml of a 1 per cent w/v solution of 4-dimethylaminobenzaldehyde in 1 M hydrochloric acid; a yellow-orange colour is produced.

Tests
Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel HF254.

Mobile phase. A mixture of 90 volumes of dichloromethane, 14 volumes of methanol, 10 volumes of dioxan and 2 volumes of strong ammonia solution.

Test solution. Shake a quantity of the powdered tablets containing 0.1 g of anhydrous metoclopramide hydrochloride with 20 ml of methanol for 5 minutes and filter.

Reference solution (a). Dilute 1 volume of the test solution to 200 volumes with methanol.

Reference solution (b). A 0.0025 per cent w/v solution of \( N,N' \)-diethylethylenediamine in methanol.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a). Spray the plate with dimethylaminobenzaldehyde reagent and allow it to dry in air. Any secondary spot in the chromatogram obtained with the test solution that has not been visualised in ultraviolet light at 254 nm is not more intense than the spot in the chromatogram obtained with reference solution (b).

Uniformity of content. Comply with the test stated under Tablets.

Powder one tablet and carry out the Assay beginning at the words “add 50 ml of 0.1 M hydrochloric acid,...".

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 10 mg of anhydrous metoclopramide hydrochloride, add 50 ml of 0.1 M hydrochloric acid, heat on a water-bath at 70° for 15 minutes, cool, dilute to 100.0 ml with water and filter. To 20.0 ml of this solution add 15 ml of 1.25 M sodium hydroxide and extract with three quantities, each of 30 ml, of chloroform, dry each
extract with anhydrous sodium sulphate and filter. Dilute the combined extracts to 100.0 ml with chloroform and mix. Measure the absorbance of the resulting solution at the maximum at about 305 nm (2.4.7). Calculate the content of C_{14}H_{22}ClN_{3}O_{2},HCl, taking 265 as the specific absorbance at 305 nm.

**Storage.** Store protected from light and moisture.

**Labelling.** The label states the strength in terms of the equivalent amount of anhydrous metoclopramide hydrochloride.

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**Metoprolol Tartrate**

\[
\begin{align*}
\text{Metoprolol Tartrate} & = (\text{RS})-1\text{-isopropylamino-3-}(2\text{-methoxyethyl})\text{phenoxypropan-2-ol (2R,3R)-tartrate.} \\
\text{Mol. Wt.} & = 684.8
\end{align*}
\]

Metoprolol Tartrate contains not less than 99.0 per cent and not more than 101.0 per cent of (C_{15}H_{25}NO_{3})_{2},C_{4}H_{6}O_{6}, calculated on the dried basis.

**Description.** A white, crystalline powder or colourless crystals.

**Identification**

A. To 25 ml of a 0.4 per cent w/v solution add 2 ml of 5 M ammonia, extract with 20 ml of dichloromethane, filter the lower layer through anhydrous sodium sulphate and evaporate to dryness. Place in a freezer for a few minutes to congeal the residue and allow to warm to room temperature. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with metoprolol tartrate RS treated in the same manner or with the reference spectrum of metoprolol.

B. A 5 per cent w/v solution gives reaction B of tartrates (2.3.1).

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**Tests**

**Appearance of solution.** A 2.0 per cent w/v solution is clear (2.4.1), and not more intensely coloured than reference solution BS8 (2.4.1).

**pH** (2.4.24). 6.0 to 7.0, determined in a 2.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +7.0° to +10.0°, determined at 20° in a 2.0 per cent w/v solution.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

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**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.3 g, dissolve in 30 ml glacial acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.03424 g of (C_{15}H_{25}NO_{3})_{2},C_{4}H_{6}O_{6}.

**Storage.** Store protected from light.

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**Metoprolol Tablets**

**Metoprolol Tartrate Tablets**

Metoprolol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of metoprolol tartrate, (C_{15}H_{25}NO_{3})_{2}, C_{4}H_{6}O_{6}.

**Identification**

Transfer a quantity of the powdered tablets containing about 40 mg of Metoprolol Tartrate to a separator, add 25 ml of water and 4 ml of 5 M ammonia, extract with 20 ml of...
dichloromethane, filter the lower layer through anhydrous sodium sulphate and evaporate to dryness. Place in a freezer for a few minutes to congeal the residue and allow to warm to room temperature.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with metoprolol tartrate RS treated in the same manner or with the reference spectrum of metoprolol.

**Tests**

**Dissolution** (2.5.2).

Apparatus. No 2

Medium. 900 ml of simulated gastric fluid (without enzyme)

Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the solution and filter. Dilute appropriately with the dissolution medium if necessary. Measure the absorbance (2.4.7) of the solution at the maximum at about 275 nm.

Calculate the content of (C₁₅H₂₅NO₃)₂, C₄H₆O₆ in the medium from the absorbance obtained from a solution of known concentration of metoprolol tartrate RS.

D: Not less than 80 per cent of the stated amount of (C₁₅H₂₅NO₃)₂, C₄H₆O₆

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.12 g of Metoprolol Tartrate, transfer to a 100-ml volumetric flask, add about 75 ml of ethanol (95 per cent), mix and filter. Dilute 5.0 ml of the filtrate to 50.0 ml with ethanol (95 per cent). Measure the absorbance of the resulting solution at the maximum at about 275 nm (2.4.7). Calculate the content of (C₁₅H₂₅NO₃)₂, C₄H₆O₆ from the absorbance obtained by repeating the operation using metoprolol tartrate RS in place of the substance under examination.

**Metronidazole**

![Metronidazole structure](image)

C₆H₉N₃O₃ Mol. Wt. 171.2

Metronidazole is 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanol.

Metronidazole contains not less than 99.0 per cent and not more than 101.0 per cent of C₆H₉N₃O₃, calculated on the dried basis.

**Description.** A white or yellowish, crystalline powder.

**Identification**

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with metronidazole RS or with the reference spectrum of metronidazole.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in 0.1 M hydrochloric acid shows an absorption maximum at about 277 nm and a minimum at about 240 nm; absorbance at about 277 nm, between 0.365 and 0.395.

C. Heat about 10 mg in a water-bath with 10 mg of zinc powder, 1 ml of water and 0.25 ml of 2 M hydrochloric acid for 5 minutes and cool. The solution gives the reaction of primary aromatic amines (2.3.1).

**Tests**

**Appearance of solution.** A 5.0 per cent w/v solution in 1 M hydrochloric acid is not more opalescent than opalescence standard OS2 (2.4.1), and not more intensely coloured than reference solution GYS4 (2.4.1).

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 80 volumes of chloroform, 10 volumes of diethylamine, 10 volumes of ethanol (95 per cent) and 1 volume of water.

**Test solution.** Dissolve 0.1 g of the substance under examination in 10 ml of acetone.

**Reference solution.** A 0.003 per cent w/v solution of the substance under examination in acetone.

**Apply to the plate** 20 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

**Assay.** Weigh accurately about 0.15 g, dissolve in 50 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid.
acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.01712 g of C₆H₉N₃O₃.

Storage. Store protected from light and moisture.

Metronidazole Benzoate

Benzoylmetronidazole

\[
\text{O} \quad \text{O} \\
\begin{array}{c}
\text{O} \\
\text{N} \\
\text{N} \\
\text{CH}_3
\end{array}
\]

C₁₃H₁₃N₃O₄ \quad \text{Mol. Wt. 275.3}

Metronidazole Benzoate is 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl benzoate.

Metronidazole Benzoate contains not less than 98.0 per cent and not more than 101.0 per cent of C₁₃H₁₃N₃O₄, calculated on the dried basis.

Description. A white or cream-coloured, crystalline powder or flakes.

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in ethanol shows an absorption maximum only at about 309 nm; absorbance at about 309 nm, about 0.3.

B. Gives reaction B of benzoates (2.3.1).

C. Melting range 98° to 102° (2.4.21).

Tests

pH (2.4.24). 5.0 to 7.0, determined in a 2.0 per cent w/v suspension.

Free benzoic acid. Not more than 0.2 per cent, determined by the following method. Dissolve 0.5 g in 25 ml of ethanol (95 per cent) and titrate with 0.01 M sodium hydroxide using phenol red solution as indicator. Carry out a blank titration.

1 ml of 0.01 M sodium hydroxide is equivalent to 0.001221 g of C₇H₆O₂.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel HF254.

Mobile phase. A mixture of 80 volumes of chloroform and 20 volumes of acetone.

Test solution. Dissolve 0.6 g of the substance under examination in 10 ml of equal volumes of chloroform and methanol.

Reference solution. A 0.02 per cent w/v solution of the substance under examination in a mixture of equal volumes of chloroform and methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine it in ultraviolet light at 254 nm. Any secondary spots in the chromatogram obtained with the test solution are not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying at in an oven at 60° at a pressure not exceeding 0.7 kPa.

Assay. Weigh accurately about 0.25 g and dissolve in 50 ml of acetone. Add 10 ml of acetic anhydride. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02753 g of C₁₃H₁₃N₃O₄.

Storage. Store protected from light and moisture.

Metronidazole Benzoate Oral Suspension

Benzoylmetronidazole Oral Suspension

Metronidazole Benzoate Oral Suspension is a suspension of Metronidazole Benzoate in a suitable aqueous vehicle. It may contain suitable colouring, flavouring, sweetening, buffering, suspending and antimicrobial agents.

Metronidazole Benzoate Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of metronidazole, C₆H₉N₃O₃.

Identification

Extract a quantity of the suspension containing 0.5 g of metronidazole with chloroform, filter and evaporate the filtrate to dryness. The residue complies with the following tests.

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in ethanol shows an absorption maximum only at about 309 nm; absorbance at about 309 nm, about 0.3.

B. Gives reaction B of benzoates (2.3.1).

Tests

Other tests. Complies with the tests stated under Oral Liquids.
Assay. Weigh accurately a quantity containing about 200 mg of metronidazole, add 10 ml of water and extract with four quantities, each of 25 ml, of chloroform. Combine the chloroform extracts and wash with two quantities, each of 5 ml, of water. Wash the aqueous solution with 5 ml of acetone and 10 ml of acetic anhydride. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.01712 g of C₆H₉N₃O₃.

Determine the weight per ml of the suspension (2.4.29), and calculate the content of metronidazole, weight in volume.

Storage. Store protected from light and moisture.

Labelling. The label states the strength in terms of the equivalent amount of metronidazole in a suitable dose-volume.

Metronidazole Injection

Metronidazole Intravenous Infusion

Metronidazole Injection is a sterile isotonic solution of Metronidazole in Water for Injections. It may contain suitable buffering agents.

Metronidazole Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of metronidazole, C₆H₉N₃O₃.

Description. An almost colourless to pale yellow solution.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 70 volumes of chloroform, 28 volumes of methanol, 4 volumes of water and 2 volumes of strong ammonia solution.

Test solution. Dilute a suitable volume of the injection with sufficient mobile phase to produce a solution containing 5 mg of Metronidazole per ml.

Reference solution. A 0.5 per cent w/v solution of metronidazole RS in mobile phase.

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of solvent is no longer detectable and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Heat 2 ml of the injection in a water-bath for 5 minutes with 10 mg of zinc powder and 0.25 ml of 2 M hydrochloric acid for 5 minutes and cool in ice. The solution gives the reaction of primary aromatic amines (2.3.1).

Tests

pH (2.4.24). 4.5 to 7.0.

Bacterial endotoxins (2.2.3). Not more than 0.35 Endotoxin Unit per mg of metronidazole.

Other tests. Complies with the tests stated under Parenteral Preparations (Intravenous Infusions).

Assay. Dilute a suitable volume with sufficient 0.1 M hydrochloric acid to produce a solution containing 0.001 per cent w/v of Metronidazole. Measure the absorbance of the resulting solution at the maximum at about 277 nm (2.4.7), using as the blank a solution prepared in the same manner omitting the substance under examination.

Calculate the content of C₆H₉N₃O₃ from the absorbance obtained by repeating the operation using metronidazole RS in place of the substance under examination.

Storage. Store protected from light, in single dose containers.

Labelling. The label states that the contents should not be used if they contain any visible solid particles.

Metronidazole Tablets

Metronidazole Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of metronidazole, C₆H₉N₃O₃. The tablets may be coated.

Identification

A. Shake a quantity of the powdered tablets containing 0.1 g of Metronidazole with 40 ml of chloroform for 15 minutes, filter and evaporate the filtrate to dryness.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with metronidazole RS or with the reference spectrum of metronidazole.

B. Heat a quantity of the powdered tablets containing 10 mg of Metronidazole in a water-bath with 10 mg of zinc powder, 1 ml of water and 0.25 ml of 2 M hydrochloric acid for 5 minutes and cool in ice. The solution gives the reaction of primary aromatic amines (2.3.1).

C. Shake a quantity of the powdered tablets containing about 0.2 g of Metronidazole with 4 ml of 0.5 M sulphuric acid and filter. To the filtrate add 10 ml of picric acid solution and allow to stand for 1 hour; the precipitate, after washing with cold
water under suction and drying at 105°, melts at about 150° (2.4.21).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 80 volumes of chloroform, 20 volumes of dimethylformamide and 5 volumes of a 90 per cent v/v solution of formic acid.

Test solution. Shake a quantity of the powdered tablets containing 0.2 g of Metronidazole with 5 ml of a mixture of equal volumes of chloroform and methanol for 5 minutes and filter.

Reference solution. A 0.02 per cent w/v solution of 2-methyl-5-nitroimidazole in a mixture of equal volumes of chloroform and methanol.

Apply to the plate 20 µl of each solution. After development, dry the plate in air, allow the solvent to evaporate and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Dissolution (2.5.2).

Apparatus. No 1
Medium. 900 ml of 0.1 M hydrochloric acid
Speed and time. 100 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 277 nm (2.4.7). Calculate the content of C₆H₉N₃O₃ from the absorbance obtained by repeating the operation using metronidazole RS instead of the substance under examination.

D. Not less than 85 per cent of the stated amount of C₆H₉N₃O₃.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.2 g of Metronidazole, transfer to a sintered-glass crucible and extract with six quantities, each of 10 ml, of hot acetone. Cool, add to the combined extracts 50 ml of acetic anhydride. Titrate with 0.1 M perchloric acid, using 0.1 ml of a 1 per cent w/v solution of brilliant green in anhydrous glacial acetic acid as indicator to a yellowish-green end-point. Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.01712 g of C₆H₉N₃O₃.

Mexiletine Hydrochloride

\[
\text{C}_{11}\text{H}_{17}\text{NO}_3 \cdot \text{HCl} \quad \text{Mol. Wt. 215.7}
\]

Mexiletine Hydrochloride is (RS)-1-methyl-2-(2,6-xyloloxly)ethylamine hydrochloride.

Mexiletine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of C₁₁H₁₇NO₃HCl, calculated on the anhydrous basis.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with mexiletine hydrochloride RS.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.04 per cent w/v solution in 0.01 M hydrochloric acid shows an absorption maximum at about 260 nm; absorbance at 260 nm, about 0.46.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

D. Dissolve 0.1 g in 3 ml of 0.02 M hydrochloric acid and add a few crystals of sodium nitrite. Nitrogen is evolved and a yellow colour may be produced slowly.

E. Gives reaction A of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution is clear (2.4.1), and colourless (2.4.1).

pH (2.4.24). 4.0 to 5.5, determined in a 10.0 per cent w/v solution.

2,6-Dimethylphenol. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 85 volumes of chloroform, 14 volumes of methanol and 1 volume of strong ammonia solution.

Test solution. Dissolve 0.5 g of the substance under examination in 10 ml of methanol.

Reference solution. A 0.01 per cent w/v solution of 2,6-dimethylphenol in methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with a 0.1 per cent w/v solution of fast blue BB salt in methanol, dry at about 90° and then spray
with 3 M methanolic potassium hydroxide. Any spot corresponding to 2,6-dimethylphenol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 85 volumes of chloroform, 14 volumes of methanol and 1 volume of strong ammonia solution.

**Test solution (a).** Dissolve 0.5 g of the substance under examination in 10 ml of methanol.

**Test solution (b).** Dissolve 0.2 g of the substance under examination in 100 ml of methanol.

**Reference solution (a).** A 0.025 per cent w/v solution of the substance under examination in methanol.

**Reference solution (b).** A 0.2 per cent w/v solution of mexiletine hydrochloride RS in methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with ninhydrin solution and heat at 110° for 15 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Heavy metals (2.3.13).** 2.0 g complies with the limit test for heavy metals, Method C (10 ppm). Use 2 ml of lead standard solution (10 ppm Pb) to prepare the standard.

**Sulphated ash (2.3.18).** Not more than 0.1 per cent.

**Water (2.4.19).** Not more than 0.5 per cent, determined on 5 g.

**Assay.** Weigh accurately about 0.15 g, dissolve in 50 ml of a mixture of equal volumes of anhydrous glacial acetic acid and acetic anhydride, add 10 ml of mercuric acetate solution. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration. 1 ml of 0.1 M perchloric acid is equivalent to 0.02157 g of C₁₁H₁₇NO,HCl.

**Storage.** Store protected from light and moisture.

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**Mexiletine Capsules**

**Mexiletine Hydrochloride Capsules**

Mexiletine Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of mexiletine hydrochloride C₁₁H₁₇NO,HCl.

**Identification**

A. Shake a quantity of the contents of the capsules containing about 0.5 g of Mexiletine Hydrochloride with 10 ml of methanol, filter, evaporate to dryness and dry the residue at 105°.

When examined in the range 230 nm to 360 nm (2.4.7), a 0.04 per cent w/v solution of the residue in 0.01 M hydrochloric acid shows an absorption maximum at 260 nm.

B. Dissolve 0.1 g of the residue obtained in test A in 3 ml of 0.02 M hydrochloric acid and add a few crystals of sodium nitrite; nitrogen is evolved and a yellow colour may be produced slowly.

C. In the test of Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

D. A 1 per cent w/v solution of the residue obtained in test A gives the reactions of chlorides (2.3.1).

**Tests**

**2,6-Dimethylphenol.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 85 volumes of chloroform, 14 volumes of methanol and 1 volume of strong ammonia solution.

**Test solution.** A 5 per cent w/v solution of the residue obtained in Identification test A in methanol.

**Reference solution.** A 0.01 per cent w/v solution of 2,6-dimethylphenol in methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with a 0.1 per cent w/v solution of fast blue BB salt in methanol, dry at about 90° and then spray with 3 M methanolic potassium hydroxide. Any spot corresponding to 2,6-dimethylphenol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

**Mobile phase.** A mixture of 85 volumes of chloroform, 14 volumes of methanol and 1 volume of strong ammonia solution.

**Test solution (a).** A 5 per cent w/v solution of the residue obtained in Identification test A in methanol.

**Test solution (b).** Dissolve 20 mg of the residue obtained in Identification test A in 10 ml of methanol.

**Reference solution (a).** A 0.025 per cent w/v solution of the residue obtained in Identification test A in methanol.

**Reference solution (b).** A 0.2 per cent w/v solution of mexiletine hydrochloride RS in methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with ninhydrin solution and heat at 110° for 15 minutes. Any secondary spot in the chromatogram
obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Dissolution (2.5.2).**

Apparatus. No 1
Medium. 900 ml of water
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter with an average pore size of 1.0 µm. Measure the fluorescence intensities using the maximum excitation wavelength at about 265 nm and the maximum emission wavelength at about 295 nm (2.4.5). Calculate the content of C_{11}H_{17}NO, HCl by comparing the fluorescence intensities obtained with a standard solution of a known concentration of mexiletine hydrochloride RS in water.

D. Not less than 80 per cent of the stated amount of C_{11}H_{17}NO, HCl.

**Assay.** Weigh accurately a quantity of the mixed contents of 20 capsules containing about 0.05 g of Mexiletine Hydrochloride, mix with 50 ml of 0.01 M hydrochloric acid, shake for 30 minutes, dilute to 100.0 ml with 0.01 M hydrochloric acid and centrifuge. Measure the absorbance of the supernatant liquid at the maximum at about 260 nm (2.4.7). Calculate the content of C_{11}H_{17}NO, HCl taking 11.6 as the specific absorbance at 260 nm.

**Storage.** Store protected from light.

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Mianserin Hydrochloride

Mianserin Hydrochloride is (RS)-2-methyl-1,2,3,4,10,14b-hexahydrodibenzo[c,f]pyrazino[1,2-a]azepine hydrochloride.

Mianserin Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of C_{18}H_{20}N_{2}, HCl, calculated on the dried basis.

**Description.** A white or almost white crystals or crystalline powder; odourless or almost odourless.

**Identification**

A. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (b).

B. A volume containing 2.5 mg of Mianserin Hydrochloride diluted to 2 ml gives reaction A of chlorides (2.3.1).

**Tests**

**pH** (2.4.24). 5.0 to 6.0.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.
A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with mianserin hydrochloride RS or with the reference spectrum of mianserin hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.01 per cent w/v solution shows an absorption maximum only at about 279 nm; absorbance at about 279 nm, 0.64 to 0.72.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 75 volumes of cyclohexane, 20 volumes of ether and 5 volumes of diethylamine.

Test solution. Dissolve 0.2 g of the substance under examination in 100 ml of dichloromethane.

Reference solution (a). A 0.2 per cent w/v solution of mianserin hydrochloride RS in dichloromethane.

Reference solution (b). A solution containing 0.2 per cent w/v each of mianserin hydrochloride RS and cyproheptadine hydrochloride RS in dichloromethane.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in a current of cold air for 5 minutes and expose to iodine vapour for 20 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 65° over phosphorus pentoxide at a pressure not exceeding 0.7 kPa for 5 hours.

Assay. Weigh accurately about 0.2 g, dissolve in a mixture of 50 ml of ethanol (95 per cent) and 5 ml of 0.01 M hydrochloric acid. Titrate with 0.1 M sodium hydroxide, determining the end-point potentiometrically (2.4.25). Note the volume added between the two points of inflection.

1 ml of 0.1 M sodium hydroxide is equivalent to 0.03008 g of C₁₈H₂₀N₂·HCl.

Storage. Store protected from light and moisture.

Mianserin Tablets

Mianserin Hydrochloride Tablets

Mianserin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of mianserin hydrochloride, C₁₈H₂₀N₂·HCl. The tablets may be coated.

Identification

A. Shake a quantity of the powdered tablets containing about 20 mg of Mianserin Hydrochloride with 10 ml of methanol, filter and evaporate the filtrate to dryness.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with mianserin hydrochloride RS or with the reference spectrum of mianserin hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with test solution (b) corresponds to the peak due to mianserin in the chromatogram obtained with the reference solution.

C. The residue obtained in test A gives the reactions of chlorides (2.3.1).

Tests

pH (2.4.24). 4.0 to 5.5, determined in a 1.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 90 volumes of dichloromethane and 10 volumes of methanol.

Test solution. Dissolve 0.02 g of the substance under examination in 10 ml of a mixture of 4 volumes of methanol and 1 volume of 10 M ammonia.

Reference solution (a). A 0.01 per cent w/v solution of the substance under examination in a mixture of 4 volumes of methanol and 1 volume of 10 M ammonia.

Reference solution (b). A 0.002 per cent w/v solution of the substance under examination in a mixture of 4 volumes of methanol and 1 volume of 10 M ammonia.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in a current of cold air for 5 minutes and expose to iodine vapour for 20 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Storage. Store protected from light and moisture.
Test solution. Triturate a quantity of the powdered tablets containing 40 mg of Mianserin Hydrochloride with 2 ml of a mixture of 4 volumes of methanol and 1 volume of strong ammonia solution and centrifuge.

Reference solution (a). Dilute 1 volume of the test solution to 200 volumes with the same solvent mixture.

Reference solution (b). Dilute 1 volume of the test solution to 500 volumes with the same solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of cold air for 5 minutes and expose to iodine vapour for 20 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by gas chromatography (2.4.13).

Test solution (a). Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing about 60 mg of Mianserin Hydrochloride with 30.0 ml of 0.2 M hydrochloric acid for 1 hour and filter. To 10.0 ml of the filtrate add 3.0 ml of 1 M sodium hydroxide and 10.0 ml of toluene, mix thoroughly, centrifuge and use the clear upper layer.

Test solution (b). To 10.0 ml of the filtrate obtained in test solution (a) add 3.0 ml of 1 M sodium hydroxide and 10.0 ml of toluene containing 0.2 per cent w/v of triphenylamine (internal standard), mix thoroughly, centrifuge and use the clear upper layer.

Reference solution. Add 3.0 ml of 1 M sodium hydroxide and 10.0 ml of toluene containing 0.2 per cent w/v of triphenylamine (internal standard) to 10.0 ml of a solution containing 0.2 per cent w/v of mianserin hydrochloride RS in 0.2 M hydrochloric acid, mix thoroughly, centrifuge and use the clear upper layer.

Chromatographic system
- a glass column 1.0 m x 4 mm, packed with acid-washed, silanised diatomeous support (80 to 100 mesh) coated with 3 per cent w/v of phenyl methyl silicone fluid (50 per cent phenyl) (such as OV-17),
- temperature: column 255°, inlet port and detector at 240°,
- flow rate. 30 ml per minute of the carrier gas.

Inject 1 µl of test solution (a), test solution (b) and the reference solution.

Calculate the content of \( \text{C}_{18}\text{H}_{20}\text{N}_{2}\), HCl in the tablets.

Storage. Store protected from light and moisture.

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Miconazole Nitrate

Miconazole Nitrate is \((RS)-1-[2-(2,4-ichlorophenylmethoxy)-2-(2,4-dichlorophenyl)ethyl]-1H-imidazole nitrate.\)

Miconazole Nitrate contains not less than 98.5 per cent and not more than 101.5 per cent of \( \text{C}_{18}\text{H}_{14}\text{Cl}_{4}\text{N}_{2}\text{O,HNO}_3 \), calculated on the dried basis.

Description. A white or almost white, crystalline or micro-crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with miconazole nitrate RS or with the reference spectrum of miconazole nitrate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.04 per cent w/v solution in a mixture of 90 volumes of 2-propanol and 10 volumes of 0.1 M hydrochloric acid shows absorption maxima at about 264 nm, 272 nm and 280 nm; ratio of the absorbance at the maximum at about 272 nm to that at the maximum at about 280 nm, 1.18 to 1.22.

C. In the test for Related substances, examine the chromatograms obtained in ultraviolet light at 254 nm before exposure to iodine vapour. The principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

D. Gives the reactions of nitrates (2.3.1).

Tests

Appearance of solution. A 1.0 per cent w/v solution in methanol is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 60 volumes of hexane, 30 volumes of chloroform, 10 volumes of methanol and 1 volume of strong ammonia solution.
Test solution (a). Dissolve 0.5 g of the substance under examination in 10 ml of a mixture of 90 volumes of methanol and 10 volumes of strong ammonia solution.

Test solution (b). Dissolve 0.5 g of the substance under examination in 100 ml of a mixture of 90 volumes of methanol and 10 volumes of strong ammonia solution.

Reference solution (a). A 0.5 per cent w/v solution of miconazole nitrate RS in a mixture of 90 volumes of methanol and 10 volumes of strong ammonia solution.

Reference solution (b). A 0.0125 per cent w/v solution of miconazole nitrate RS in a mixture of 90 volumes of methanol and 10 volumes of strong ammonia solution.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of air for 15 minutes and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b). Expose the plate to iodine vapour until a brown spot appears in the chromatogram obtained with reference solution (b) and examine immediately in daylight. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

Assay. Weigh accurately about 0.4 g, dissolve in 50 ml of anhydrous glacial acetic acid, with slight heating if necessary. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.04791 g of C18H14Cl4N2O,HNO3.

Storage. Store protected from light and moisture.

Miconazole Cream

Miconazole Nitrate Cream

Miconazole Cream contains Miconazole Nitrate in a suitable basis.

Miconazole Cream contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of miconazole nitrate, C18H14Cl4N2O,HNO3.

Identification

A. Mix a quantity containing 40 mg of Miconazole Nitrate with 20 ml of a mixture of 4 volumes of methanol and 1 volume of 1 M sulphuric acid and shake with two quantities, each of 50 ml, of carbon tetrachloride, discarding the organic layers. Make the aqueous phase alkaline with 2 M ammonia and extract with two quantities, each of 40 ml, of chloroform. Evaporate the chloroform extracts, shake with 5 g of anhydrous sodium sulphate, filter and dilute the filtrate to 100 ml with chloroform. Evaporate 50 ml to dryness and dissolve the residue in 50 ml of a mixture of 90 volumes of methanol and 10 volumes of 0.1 M hydrochloric acid.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 264 nm, 272 nm and 280 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to miconazole nitrate in the chromatogram obtained with reference solution (a).

Tests

Other tests. Complies with the tests stated under Creams.

Assay. Determine by gas chromatography (2.4.13).

Test solution. Weigh accurately a quantity of the cream containing about 40 mg of Miconazole Nitrate, mix with 20 ml of a mixture of 4 volumes of methanol and 1 volume of 0.5 M sulphuric acid and shake with two quantities, each of 50 ml, of carbon tetrachloride. Wash each organic layer in turn with the same 10-ml quantity of a mixture of 4 volumes of methanol and 1 volume of 0.5 M sulphuric acid. Combine the aqueous phase and the washings, make alkaline with 2 M ammonia and extract with two quantities, each of 50 ml, of chloroform. To the combined extracts add 10.0 ml of a 0.3 per cent w/v solution of cetyl palmitate (internal standard) in chloroform and 5 g of anhydrous sodium sulphate, shake, filter, evaporate the filtrate to a low volume and add sufficient chloroform to produce 10.0 ml.

Reference solution (a). Weigh accurately about 40 mg of miconazole nitrate RS and mix with 10.0 ml of a 0.3 per cent w/v solution of the internal standard in chloroform and 0.2 ml of strong ammonia solution, add 1 g of anhydrous sodium sulphate, shake again and filter.

Reference solution (b). Prepare the solution in the same manner as reference solution (a) but omitting the addition of the internal standard solution.

Chromatographic system

- a glass column 1.5 m x 2 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) (such as 5 per cent OV-101), temperature:
  - column 270°,
  - inlet port and detector at 270°,
- flow rate: 30 ml per minute of the carrier gas.
Inject 1 µl of the test solution, reference solution (a) and reference solution (b).

Calculate the content of C_{18}H_{14}Cl_{4}N_{2}O.HNO_{3} in the cream.

**Storage.** Store protected from light and moisture at a temperature not exceeding 30°. If it is packed in aluminium tubes, the inner surfaces of the tubes should be coated with a suitable lacquer.

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**Miconazole Pessaries**

Miconazole Nitrate Pessaries; Miconazole Nitrate Vaginal Tablets; Miconazole Tablets

Miconazole Pessaries contain Miconazole Nitrate in a suitable basis.

Miconazole Pessaries contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of miconazole nitrate, C_{18}H_{14}Cl_{4}N_{2}O.HNO_{3}.

**Identification**

A. Mix a quantity of the crushed pessaries containing 40 mg of Miconazole Nitrate with 20 ml of a mixture of 4 volumes of methanol and 1 volume of 1 M sulphuric acid and shake with two quantities, each of 50 ml, of carbon tetrachloride, discarding the organic layers. Make the aqueous phase alkaline with 2 M ammonia and extract with two quantities, each of 40 ml, of chloroform. Combine the chloroform extracts, shake with 5 g of anhydrous sodium sulphate, filter and dilute the filtrate to 100 ml with chloroform. Evaporate 50 ml to dryness and dissolve the residue in 50 ml of a mixture of 90 volumes of methanol and 10 volumes of 0.1 M hydrochloric acid.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 264 nm, 272 nm and 280 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to miconazole nitrate in the chromatogram obtained with reference solution (a).

**Tests**

**Other tests.** Comply with the tests stated under Pessaries.

**Assay.** Determine by gas chromatography (2.4.13).

**Test solution.** Weigh accurately a quantity of the crushed pessaries containing about 40 mg of Miconazole Nitrate, mix with 20 ml of a mixture of 4 volumes of methanol and 1 volume of 0.5 M sulphuric acid and shake with two quantities, each of 50 ml, of carbon tetrachloride. Wash each organic layer in turn with the same 10-ml quantity of a mixture of 4 volumes of methanol and 1 volume of 0.5 M sulphuric acid. Combine the aqueous phase and the washings, make alkaline with 2 M ammonia and extract with two quantities, each of 50 ml, of chloroform. To the combined extracts add 10.0 ml of a 0.3 per cent w/v solution of cetyl palmitate (internal standard) in chloroform and 5 g of anhydrous sodium sulphate, shake, filter, evaporate the filtrate to a low volume and add sufficient chloroform to produce 10.0 ml.

**Reference solution (a).** Weigh accurately about 40 mg of miconazole nitrate RS and mix with 10.0 ml of a 0.3 per cent w/v solution of the internal standard in chloroform and 0.2 ml of strong ammonia solution, add 1 g of anhydrous sodium sulphate, shake again and filter.

**Reference solution (b).** Prepare the solution in the same manner as reference solution (a) but omitting the addition of the internal standard solution.

**Chromatographic system**

- a glass column 1.5 m x 2 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) (such as 5 per cent OV-101),
- temperature: column 270°, inlet port and detector at 300°,
- flow rate. 30 ml per minute of the carrier gas.

Inject 1 µl of the test solution, reference solution (a) and reference solution (b).

Calculate the content of C_{18}H_{14}Cl_{4}N_{2}O.HNO_{3} in the pessaries.

**Storage.** Store protected from light and moisture.

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**Microcrystalline Cellulose**

Microcrystalline Cellulose is purified, partially depolymerised cellulose prepared from alpha cellulose.

Microcrystalline Cellulose contains not less than 97.0 per cent and not more than 102.0 per cent of cellulose, calculated on the dried basis.

**Description.** A fine or granular, white or almost white powder; odourless.

**Identification**

A. To about 1 mg add 1 ml of phosphoric acid, heat on a water-bath for 30 minutes, add 4 ml of a 0.2 per cent w/v solution of catechol in phosphoric acid and heat for further 30 minutes; a red colour is produced.

B. To 50 mg add 2 ml of iodine solution, allow to stand for 5 minutes and remove the excess reagent with the aid of a filter
paper and add 1 or 2 drops of sulphuric acid (66 per cent v/v); a blue-purple colour is produced.

C. Mix 30 g with 270 ml of water, mix in a blender at 18,000 rpm for 5 minutes, transfer 100 ml of the mixture to a 100-ml graduated cylinder and allow to stand for 3 hours. A white, opaque, bubble-free dispersion is obtained that does not produce a supernatant liquid.

Tests

**pH** (2.4.24). 5.0 to 7.5, determined on the supernatant liquid obtained by shaking 2.0 g with 100 ml of carbon dioxide-free water for 5 minutes and centrifuging.

**Starch and dextrins.** Mix 0.1 g with 5 ml of water by vigorous shaking and add 2 to 3 drops of iodine solution; no blue or brownish-red colour is produced.

**Organic impurities.** Place 10 mg on a watch-glass and add 0.05 ml of a freshly prepared solution of 0.1 g of phloroglucinol in 5 ml of hydrochloric acid; no red colour is produced.

**Water-soluble substances.** Shake 5.0 g with about 80 ml of water for 10 minutes, filter through a filter paper (Whatman No 42 or equivalent) into a tared beaker and evaporate the filtrate to dryness and dry the residue at 105° for 1 hour. The residue weighs not more than 10 mg (0.2 per cent).

**Arsenic** (2.3.10). Mix 5.0 g with 3 g of anhydrous sodium carbonate, add 10 ml of bromine solution and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite and dissolve the cooled residue in a mixture of 15 ml of hydrochloric acid containing 0.15 ml of bromine solution and 45 ml of water. Add 2 ml of stannous chloride solution As$^T$. The resulting solution complies with the limit test for arsenic (2 ppm).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Loss on drying** (2.4.19). Not more than 6.0 per cent, determined on 0.5 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.125 g and transfer to a 500-ml conical flask with the aid of about 25 ml of water. Add 45 ml of 0.083 M potassium dichromate, mix, carefully add 100 ml of sulphuric acid and heat to boiling. Remove from heat, allow to stand at room temperature for 15 minutes, cool and transfer to a 250-ml volumetric flask. Dilute with water almost to volume, cool to 25°, dilute with water to volume and mix. Titrate 50.0 ml of the resulting solution with 1 M ferrous ammonium sulphate using 2 to 3 drops of ferroin sulphate solution as indicator. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of ferrous ammonium sulphate required.

1 ml of 0.1 M ferrous ammonium sulphate is equivalent to 0.000675 g of cellulose.

**Storage.** Store protected from light and moisture.

Microcrystalline Wax

Petroleum Wax (microcrystalline); Amorphous Wax

Microcrystalline Wax is a mixture of straight-chain, branched-chain and cyclic hydrocarbons, obtained by solvent fractionation of the still bottom fraction of petroleum by suitable dewaxing or de-oiling means.

**Description.** A white or cream-coloured, waxy solid; odourless.

Tests

**Acidity or alkalinity.** Introduce 35.0 g into a 250-ml separating funnel, add 100 ml of boiling water and shake vigorously for 5 minutes. Draw off the separated water into a beaker, wash further with two quantities, each of 50 ml, of boiling water and add the washings to the liquid in the beaker. To the pooled washings add 0.05 ml of phenolphthalein solution and boil; the solution does not acquire a pink colour. Cool, add 0.1 ml of methyl orange solution; no red or pink colour is produced.

**Solidifying point** (2.4.10). 54° to 102°. Follow the method with the following modifications. Place in the inner test-tube sufficient of the melted substance to fill the tube to a depth of about 50 mm. Stir the substance gently and steadily, without scraping the side of the tube, while the tube and its contents are allowed to cool. The temperature at which the level of the mercury in the thermometer remains stationary for a short time is taken as the solidifying point.

**Colour.** Melt about 10.0 g on a water-bath and pour 5 ml of the liquid into a clear-glass (15 cm × 16 mm) bacteriological test-tube; the warm, melted liquid is not more intensely coloured than a solution prepared by mixing 3.8 ml of FCS and 1.2 ml of CCS (2.4.1), in a similar tube, the comparison being made in reflected light against a white background, the tubes being held directly against the background at such an angle that there is no fluorescence.

**Organic acids.** To 20.0 g add 100 ml of a 50 per cent v/v solution of ethanol neutralised to phenolphthalein solution and titrate rapidly with 0.1 M sodium hydroxide with vigorous agitation, to a sharp pink end-point. Not more than 0.4 ml of 0.1 M sodium hydroxide is required.

**Fats, fixed oils and rosin.** Digest 10.0 g with 10 ml of 5 M sodium hydroxide at 100° for 30 minutes. Separate the water layer and acidify with sulphuric acid; no oily or solid matter separates.
Ash (2.3.19). Not more than 0.1 per cent, determined on 2.0 g. It volatilises without emitting an acrid odour.

Storage. Store protected from light and moisture.

Minoxidil

\[
\text{C}_9\text{H}_{15}\text{N}_5\text{O} \quad \text{Mol. Wt. 209.3}
\]

Minoxidil is 2,4-diamino-6-piperidinopyrimidine-3-oxide.

Minoxidil contains not less than 98.5 per cent and not more than 101.0 per cent of C9H15N5O, calculated on the dried basis.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with minoxidil RS.

B. Dissolve about 20 mg in 0.1 M hydrochloric acid and dilute to 100 ml with the same solvent (solution A). Dilute 2 ml of solution A to 100 ml with 0.1 M hydrochloric acid (solution B) and dilute 2 ml of solution A to 100 ml with 0.1 M sodium hydroxide (solution C).

Measure the light absorption of solutions B and C in the range 220 nm to 360 nm (2.4.7). Solution B shows absorption maxima at about 230 nm, 262 nm and 288 nm; absorbance at about 230 nm, 0.406 to 0.408 and at about 281 nm, 0.424 to 0.468. Solution C shows absorption maxima at about 230 nm, 262 nm and 288 nm; absorbance at about 230 nm, 0.610 to 0.674, at about 262 nm, 0.194 to 0.214 and at about 288 nm, 0.222 to 0.242.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

Mobile phase. A mixture of 100 volumes of methanol and 1.5 volumes of strong ammonia solution.

Test solution. Dissolve 0.1 g of the substance under examination in 100 ml of methanol.

Reference solution. A 0.1 per cent w/v solution of minoxidil RS in methanol.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm.

The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

D. Dissolve about 10 mg in 1 ml of methanol, add 0.1 ml of cupric sulphate solution; a green colour develops. The solution becomes greenish-yellow on addition of 0.1 ml of 2 M hydrochloric acid.

Tests

Related substance. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 100 ml of the mobile phase.

Reference solution (a). Dilute 1 ml of the test solution to 100 ml with the mobile phase.

Reference solution (b). Dissolve 5 mg of deoxyminoxidil RS in the mobile phase and dilute to 20 ml with the mobile phase. To 2 ml of this solution add 2 ml of the test solution and dilute to 10 ml with the mobile phase.

Chromatographic system

- a stainless steel column 10 cm x 3 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 3.0 g of dioctyl sodium sulphosuccinate in a mixture of 10 ml of glacial acetic acid and 300 ml of water, adjusted to pH 3.0 with perchloric acid and add 700 ml of methanol,
- flow rate. 1 ml per minute,
- spectrophotometer set at 240 nm,
- a 10 µl loop injector.

Inject the test solution, reference solution (a) and reference solution (b). Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the sum of the areas of any secondary peaks is not greater than 1.5 times the area of reference solution (a). The test is not valid unless, in the chromatogram obtained with reference solution (b), the resolution between the peaks corresponding to minoxidil and deoxyminoxidil is at least 2.0. Ignore any peak with an area less than 0.1 per cent of that of the peak in the chromatogram obtained with reference solution (a) (0.001 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method C (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°C.

Assay. Weigh accurately about 0.15 g, dissolve in 50 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.
1 ml of 0.1 M perchloric acid is equivalent to 0.02093 g of C₉H₁₅N₅O.

**Storage.** Store protected from light and moisture.

**Minoxidil Tablets**

Minoxidil Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of minoxidil, C₉H₁₅N₅O.

**Identification**

A. Transfer a portion of the finely powdered tablets containing about 10 mg of Minoxidil to a separator, add 25 ml of water, and extract with three quantities, each of 15 ml, of chloroform. Combine the chloroform extracts and evaporate with the aid of stream of nitrogen. Wash the inside of the container with about 5 ml of ethanol (95 per cent) and evaporate under vacuum at 50° until dry. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with minoxidil RS.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel GF254.

**Mobile phase.** A mixture of 100 volumes of methanol and 1.5 volumes of strong ammonia solution.

**Test solution.** Shake a quantity of the powdered tablets containing 10 mg of Minoxidil with 10 ml of methanol, centrifuge and use the supernatant liquid.

**Reference solution.** A 0.1 per cent w/v solution of minoxidil RS in methanol.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

**Tests**

**Dissolution** (2.5.2).

Apparatus. No 1

Medium. 900 ml of phosphate buffer pH 7.2

Speed and time. 75 rpm and 15 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 231 nm (2.4.7), for tablets containing up to 10 mg Minoxidil; for tablets containing more than 10 mg of Minoxidil the wavelength used is about 287 nm. Similarly measure the absorbance of a solution of known concentration of minoxidil RS. Calculate the content of C₉H₁₅N₅O. D. Not less than 75 per cent of the stated amount of C₉H₁₅N₅O.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Weigh and powder 20 tablets. Shake a quantity of the powder containing about 5 mg of Minoxidil with 20 ml of a solution of medroxyprogesterone acetate (internal standard) in the mobile phase having a concentration of about 0.2 mg per ml (solution A) for 5 minutes and centrifuge.

**Reference solution.** Dissolve an accurately weighed quantity of minoxidil RS in solution A to obtain a solution having a known concentration of about 0.25 mg per ml.

**Chromatographic system**

- a stainless steel column 25 cm x 4 mm, packed with octadecysilane bonded to porous silica (5 µm),
- mobile phase: a solution prepared by adding 3.0 g of docusate sodium per litre to a mixture of 700 volumes of methanol, 300 volumes of water and 10 volumes of glacial acetic acid, adjusting to pH 3.0 with perchloric acid, filtering and degassing,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- a 10 µl loop injector.

Chromatograph not less than 4 replicate injections of reference solution and record the peak response as mentioned below. The relative standard deviation is not more than 2.0 per cent and the resolution between the internal standard and minoxidil is not less than 2.0.

Inject separately the test solution and the reference solution and measure the responses for the major peaks. The relative retention times are about 0.8 for the internal standard and 1.0 for minoxidil.

Calculated the content of minoxidil, C₉H₁₅N₅O in the tablets.

**Storage.** Store protected from light.

**Monothioglycerol**

Thioglycerol

\[
\text{HO}_3\text{SH}
\]

C₃H₈O₂S  
Mol. Wt. 108.2

Monothioglycerol is 3-mercaptopropane-1,2-diol.
Monothioglycerol contains not less than 97.0 per cent and not more than 101.0 per cent of C₃H₈O₂S, calculated on the anhydrous basis.

**Description.** A colourless to pale yellow, viscous liquid; odour resembling that of sulphides; hygroscopic.

**Tests**

- **pH** (2.4.24). 3.5 to 7.0, determined in a 10.0 per cent w/v solution.
- **Relative density** (2.4.29). 1.241 to 1.250.
- **Refractive index** (2.4.27). 1.521 to 1.526.
- **Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).
- **Sulphated ash** (2.3.18). Not more than 0.1 per cent.
- **Water** (2.3.43). Not more than 5.0 per cent, determined by Method C on 50.0 g.

**Assay**

Weigh accurately about 0.2 g, dissolve in 50 ml of water and titrate with 0.05 M iodine using 3 ml of starch solution, added towards the end of the titration, as indicator.

1 ml of 0.05 M iodine is equivalent to 0.01082 g of C₃H₈O₂S.

**Storage.** Store protected from light and moisture.

---

Morphine Sulphate

\[
\begin{align*}
\text{HO} & \quad \text{NCH₃} \\
\text{O} & \\
\text{HO}^\downarrow & \\
\text{H} & \\
\text{H₂SO₄, 5H₂O} &
\end{align*}
\]

(C₁₇H₁₉NO₃)₂H₂SO₄·5H₂O  Mol. Wt. 758.8

Morphine Sulphate is 7,8-didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol sulphate pentahydrate.

Morphine Sulphate contains not less than 98.0 per cent and not more than 102.0 per cent of (C₁₇H₁₉NO₃)₂H₂SO₄, calculated on the dried basis.

**Description.** White, acicular crystals or cubical masses or a white, crystalline powder; odourless.

**Identification**

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.015 per cent w/v solution shows an absorption maximum only at about 285 nm; absorbance at about 285 nm, about 0.65.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution in 0.1 M sodium hydroxide shows an absorption maximum only at about 298 nm; absorbance at about 298 nm, about 0.34.

C. Add a few mg of the powdered substance to a mixture of 1 ml of sulphuric acid and 0.05 ml of formaldehyde solution; a purple colour is produced.

D. Dissolve 5 mg in 5 ml of water and add 0.15 ml of dilute potassium ferricyanide solution and 0.05 ml of ferric chloride solution; a bluish green colour is produced immediately, which changes rapidly to blue.

E. Gives the reactions of sulphates (2.3.1).

**Tests**

- **Acidity.** Dissolve 0.2 g in 10 ml of freshly boiled and cooled water and titrate with 0.02 M sodium hydroxide using methyl red solution as indicator. Not more than 0.2 ml is required to change the colour of the solution.

**Other alkaloids.** Not more than 1.5 per cent, calculated on the dried basis.

Transfer 0.5 g to a separating funnel, add 15 ml of water, 5 ml of 1 M sodium hydroxide and 10 ml of chloroform, shake, allow to separate and transfer the chloroform layer to another separating funnel. Repeat the extraction with two further quantities, each of 10 ml, of chloroform. Wash the mixed chloroform solutions with 10 ml of 0.1 M sodium hydroxide and then with two quantities, each of 5 ml, of water, evaporate to dryness on a water-bath and dry the residue to constant weight at 105°.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). 9.0 to 12.0 per cent, determined on 0.5 g by drying in an oven at 145° for 1 hour.

**Assay.** Weigh accurately about 0.5 g, dissolve in 30 ml of glacial acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.06688 g of (C₁₇H₁₉NO₃)₂H₂SO₄.

**Storage.** Store protected from light and moisture.

---

Morphine and Atropine Injection

Morphine Sulphate and Atropine Sulphate Injection

Morphine and Atropine Injection is a sterile isotonic solution in Water for Injections containing 1.0 per cent w/v of Morphine Sulphate and 0.06 per cent w/v of Atropine Sulphate.
Morphine and Atropine Injection contains not less than 0.90 per cent w/v and not more than 1.10 per cent w/v of morphine sulphate, \((C_{17}H_{19}NO_3)_2\cdot H_2SO_4\cdot 5H_2O\), and not less than 0.054 per cent w/v and not more than 0.066 per cent w/v of atropine sulphate, \((C_{17}H_{23}NO_3)_2\cdot H_2SO_4\cdot H_2O\).

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 100 volumes of methanol and 1.5 volumes of strong ammonia solution.

Test solution. Add 1 ml of 5 M ammonia to 1 ml of the injection, extract with two quantities, each of 5 ml, of chloroform, filter the combined extracts through anhydrous sodium sulphate, evaporate to dryness in a current of warm air and dissolve the residue in 0.5 ml of chloroform.

Reference solution (a). Treat 1 ml of a 0.06 per cent w/v solution of atropine sulphate RS in the same manner as for the test solution.

Reference solution (b). Treat 1 ml of a 1 per cent w/v solution of morphine sulphate RS in the same manner as for the test solution.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with dilute potassium iodobismuthate solution. The principal spots in the chromatogram obtained with the test solution correspond to the spots in the chromatograms obtained with reference solutions (a) and (b).

Tests

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. For atropine sulphate — To 10.0 ml add 10 ml of water and 5 ml of 1 M sodium hydroxide and extract successively with 15, 10 and 10 ml of chloroform and continue the extraction with 10-ml quantities of chloroform until complete extraction of alkaloids has been effected (2.6.4). Wash the chloroform extracts with the same 5 ml of water (preserve the aqueous solution and the washings for the Assay for morphine sulphate). Evaporate the chloroform, dissolve the residue in 2 ml of ethanol (95 per cent), add 2.0 ml of 0.025 M sulphuric acid, cool and titrate the excess of acid with 0.1 M sodium hydroxide using methyl red solution as indicator.

1 ml of 0.025 M sulphuric acid is equivalent to 0.01737 g of \((C_{17}H_{19}NO_3)_2\cdot H_2SO_4\cdot 5H_2O\).

Storage. Store protected from light.

Morphine Injection

Morphine Sulphate Injection

Morphine Injection is a sterile solution of Morphine Sulphate in Water for Injections.

Morphine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of morphine sulphate, \((C_{17}H_{23}NO_3)_2\cdot H_2SO_4\cdot 5H_2O\).

Identification

A. Evaporate a volume containing 5 mg of Morphine Sulphate to dryness on a water-bath. Dissolve the residue in 5 ml of water and add 0.15 ml of dilute potassium ferricyanide solution; a bluish green colour is produced immediately, which changes rapidly to blue.

B. Gives reaction A of sulphates (2.3.1).

Tests

pH (2.4.24). 2.5 to 6.0.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Measure accurately a volume containing about 25 mg of Morphine Sulphate and dilute with sufficient of the mobile phase to produce 100.0 ml, freshly prepared.

Reference solution. Weigh accurately about 25 mg of morphine sulphate RS and dissolve in sufficient of the mobile phase to produce 100.0 ml, freshly prepared.

Chromatographic system

- a stainless steel column 40 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 0.73 g of sodium heptane-sulphonate in 720 ml of water, add 280 ml of methanol and 10 ml of glacial acetic acid, mix and filter,
- flow rate. 1.5 ml per minute,
– spectrophotometer set at 280 nm,
– a 20 µl loop injector.

Inject the test solution and reference solution.

Calculate the content of $(C_{17}H_{19}NO_3)_2$, H$_2$SO$_4$, 5H$_2$O in the injection.

Storage. Store protected from light.

**Multiple Electrolytes And Dextrose Injection Type I**

Multiple Electrolytes and Dextrose Injection Type I is a sterile solution of Dextrose and suitable salts in Water for Injection to provide sodium, potassium, magnesium, acetate, phosphate and chloride ions. It may contain Hydrochloric Acid or Sodium Hydroxide used for adjusting the pH.

**Composition**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium acetate</td>
<td>0.32 g</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>0.13 g</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>0.026 g</td>
</tr>
<tr>
<td>Magnesium chloride</td>
<td>0.031 g</td>
</tr>
<tr>
<td>Dextrose</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Water for Injections to</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

Concentration of electrolytes in mmol / l

- Sodium 23.0
- Potassium 20.0
- Magnesium 1.5
- Acetate 23.0
- Chloride 20.0
- Phosphate 1.5

Multiple Electrolytes and Dextrose Injection Type I contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of sodium, Na, potassium, K, magnesium, Mg, acetate, C$_2$H$_2$O$_2$, and phosphate, PO$_4$. It also contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of chloride, Cl and not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dextrose, C$_6$H$_{12}$O$_6$. It contains no antimicrobial agent.

**Description.** A clear, colourless or faintly straw-coloured solution.

**Identification.**

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. 20 ml gives the reactions of acetates, chlorides phosphates, sodium salts, potassium salts and magnesium salts (2.3.1).

**Tests**

**pH** (2.4.24). 3.0 to 7.0.

**5-Hydroxymethylfurfural and Related substances.** Dilute a volume containing 1.0 g of Dextrose to 500.0 ml with water and measure the absorbance of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.25 (2.4.7).

**Bacterial endotoxins** (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.**

*For sodium* — Dilute suitably with water and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using *sodium solution FP* or *sodium solution AAS* respectively, suitably diluted with water for the standard solutions.

*For total potassium* — Dilute suitably with water and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using *potassium solution FP* or *potassium solution AAS* respectively, suitably diluted with water for the standard solutions.

*For magnesium* — To 50.0 ml add 50 ml of water and 5 ml of strong ammonia-ammonium chloride solution and titrate with 0.005 M disodium edetate using 50 mg of eriochrome black T mixture as indicator.

1 ml of 0.005 M disodium edetate is equivalent to 0.1215 mg of Mg.

*For acetate* — Determine by liquid chromatography (2.4.14).

**Test solution.** Dilute an accurately measured volume of the preparation under examination quantitatively with water to obtain a solution containing about 0.12 per cent w/v of Sodium Acetate.

**Reference solution.** Dissolve an accurately weighed quantity of *sodium acetate* in water to obtain a solution having a known concentration of about 0.12 per cent w/v of sodium acetate.

**Chromatographic system**

- a stainless steel column 30 cm x 7.8 mm, packed with strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form (7 µm) and a guard column 4 cm x 7.8 mm packed with the same column material,
- temperature. column 60°,
- mobile phase: 0.1 M sulphuric acid,
Multiple Electrolytes and Dextrose Injection Type II

Multiple Electrolytes and Dextrose Injection Type II is a sterile solution of Dextrose and suitable salts in Water for Injections to provide sodium, potassium, calcium, magnesium and chloride ions. It may contain Hydrochloric Acid or Sodium Hydroxide used for adjusting the pH.

**Composition**

- Sodium acetate: 0.33 g
- Sodium chloride: 0.088 g
- Potassium chloride: 0.12 g
- Calcium chloride dihydrate: 0.037 g
- Magnesium chloride: 0.031 g
- Dextrose: 5.0 g
- Water for Injections to produce 100 ml

Concentration of electrolytes in mmol / l

- Sodium: 40.0
- Potassium: 16.0
- Calcium: 2.5
- Chloride: 40.0
- Magnesium: 1.5
- Acetate: 24.0

Multiple Electrolytes and Dextrose Injection Type II contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of sodium, Na, potassium, K, calcium, Ca, magnesium, Mg, and acetate, C2H3O2. It contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of chloride, Cl. It also contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dextrose, C6H12O6. It contains no antimicrobial agent.

**Description.** A clear, colourless or faintly straw-coloured solution.

**Identification**

A. To 1 ml add 0.05 ml of potassium cupri-tartrate solution; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. 20 ml gives the reactions of acetates, chlorides sodium salts, potassium salts, calcium salts and magnesium salts (2.3.1).

**Tests**

**pH** (2.4.24). 3.0 to 7.0.

5-Hydroxymethylfurfural and Related substances. Dilute a volume containing 1.0 g of Dextrose to 500.0 ml with water.
and measure the absorbance of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.25 (2.4.7).

**Bacterial endotoxins** (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** For total sodium — Dilute suitably with water and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using sodium solution FP or sodium solution AAS respectively, suitably diluted with water for the standard solutions.

For potassium — Dilute suitably with water and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using potassium solution FP or potassium solution AAS respectively, suitably diluted with water for the standard solutions.

For calcium — Dilute suitably with water and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 422.7 nm and using calcium solution FP or calcium solution AAS respectively, suitably diluted with water for the standard solutions.

For magnesium — To 50.0 ml add 50 ml of water and 5 ml of strong ammonia-ammonium chloride solution and titrate with 0.005 M disodium-ammonium chloride solution using 50 mg of eriochrome black T mixture as indicator.

1 ml of 0.005 M disodium edetate is equivalent to 0.1215 mg of Mg.

For acetate — Determine by liquid chromatography (2.4.14).

**Test solution.** Dilute an accurately measured volume of the preparation under examination quantitatively with water to obtain a solution containing about 0.12 per cent w/v of Sodium Acetate.

**Reference solution.** Dissolve an accurately weighed quantity of sodium acetate in water to obtain a solution having a known concentration of about 0.12 per cent w/v of sodium acetate.

**Chromatographic system**
- a stainless steel column 30 cm × 7.8 mm, packed with strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form (7 µm) and a guard column 4 cm × 7.8 mm packed with the same column material,
- column temperature 60°,
- mobile phase: 0.1 M sulphuric acid,
- flow rate. 0.8 ml per minute,
- spectrophotometer set at 210 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject separately the test solution and the reference solution and measure the responses for the major peak and calculate the content of acetate in the preparation under examination.

For total chloride — To 20.0 ml add 30 ml of water, 50.0 ml of 0.1 M silver nitrate and 2 ml of nitric acid. Filter, wash the precipitate slightly acidified with nitric acid and titrate the excess of silver nitrate with 0.1 M ammonium thiocyanate using ferric ammonium sulphate solution as indicator until a reddish yellow colour is produced. Carry out a blank titration.

1 ml of 0.1 M silver nitrate is equivalent to 0.003545 g of total chloride, calculated as Cl.

For dextrose — To an accurately measured volume containing 2 g to 5 g of Dextrose, add 0.2 ml of 3 M ammonia and sufficient water to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, C₆H₁₂O₆, in the volume taken for assay.

**Storage.** Store in single dose containers at a temperature not exceeding 30°.

**Labelling.** The label states (1) the content of each electrolyte in terms of millimoles in a given volume; (2) the amount of each ingredient in 100 ml; (3) the total osmolar concentration in mOsmol per litre; (4) that the preparation should not be used if it contains visible particles.

**Multiple Electrolytes and Dextrose Injection Type III**

Multiple Electrolytes and Dextrose Injection Type III is a sterile solution of Dextrose and suitable salts in Water for Injections to provide sodium, potassium, acetate, chloride and phosphate ions. It may contain Hydrochloric Acid or Sodium Hydroxide used for adjusting the pH.

**Composition**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium acetate</td>
<td>0.28 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.10 g</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>0.15 g</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>0.13 g</td>
</tr>
<tr>
<td>Dextrose</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Water for Injections</td>
<td>100 ml</td>
</tr>
</tbody>
</table>
Concentration of electrolytes in mmol/l

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>37.0</td>
</tr>
<tr>
<td>Potassium</td>
<td>35.0</td>
</tr>
<tr>
<td>Acetate</td>
<td>20.0</td>
</tr>
<tr>
<td>Chloride</td>
<td>37.0</td>
</tr>
<tr>
<td>Phosphate</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Multiple Electrolytes and Dextrose Injection Type III contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of sodium, Na, potassium, K, acetate, C₇H₁₀O₄, and phosphate, PO₄, and not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of chloride, Cl. It also contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dextrose, C₆H₁₂O₆. It contains no antimicrobial agent.

**Description.** A clear, colourless or faintly straw-coloured solution.

**Identification**

A. To 1 ml add 0.05 ml of potassium cupri-tartrate solution; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. 20 mg gives the reactions of acetates, chlorides phosphates, sodium salts and potassium salts (2.3.1).

**Tests**

**pH** (2.4.24). 3.0 to 7.0.

5-Hydroxymethylfurfural and Related substances. Dilute a volume containing 1.0 g of Dextrose to 500.0 ml with water and measure the absorbance of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.25 (2.4.7).

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** For total sodium — Dilute suitably with water and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using sodium solution FP or sodium solution AAS respectively, suitably diluted with water for the standard solutions.

For potassium — Dilute suitably with water and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using potassium solution FP or potassium solution AAS respectively, suitably diluted with water for the standard solutions.

For acetate — Determine by liquid chromatography (2.4.14).

**Test solution.** Dilute an accurately measured volume of the preparation under examination quantitatively with water to obtain a solution containing about 0.12 per cent w/v of Sodium Acetate.

**Reference solution.** Dissolve an accurately weighed quantity of sodium acetate in water to obtain a solution having a known concentration of about 0.12 per cent w/v of sodium acetate.

**Chromatographic system**

- a stainless steel column 30 cm x 7.8 mm, packed with strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form (7 µm) and a guard column 4 cm x 7.8 mm packed with the same column material,
- column, temperature 60°,
- mobile phase: 0.1 M sulphuric acid,
- flow rate. 0.8 ml per minute,
- spectrophotometer set at 210 nm,
- a 20 µl loop injector.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent. Inject separately the test solution and the reference solution and measure the responses for the major peak and calculate the content of acetate in the preparation under examination.

For phosphate — Dilute an accurately measured volume containing about 4 mg of phosphate with sufficient water to produce 50.0 ml. Transfer 2.0 ml of this solution to a test-tube. Add 1.0 ml of a 5 per cent w/v solution of ammonium molybdate in a cooled mixture of sulphuric acid and water (15:85) and allow to stand for 3 minutes. Add 1.0 ml of a freshly prepared 0.5 per cent w/v solution of hydroquinone containing 1 drop of sulphuric acid and 1.0 ml of a freshly prepared 20 per cent w/v solution of anhydrous sodium sulphite, mix and allow to stand for 30 minutes. Measure the absorbance of the resulting solution at the maximum at about 640 nm (2.4.7), using as the blank a solution prepared in the same manner by treating 2 ml of water instead of the solution of the preparation under examination. Calculate the content of phosphate from the absorbance obtained by simultaneously carrying out the operation using a known concentration of about 0.11 mg per ml of dipotassium hydrogen phosphate in water instead of the solution of the preparation under examination.

For total chloride — To 20.0 ml add 30 ml of water, 50.0 ml of 0.1 M silver nitrate and 2 ml of nitric acid. Filter, wash the precipitate with water slightly acidified with nitric acid and titrate the excess of silver nitrate with 0.1 M ammonium thiocyanate using ferric ammonium sulphate solution as indicator until a reddish yellow colour is produced. Carry out a blank titration.
1 ml of 0.1 M silver nitrate is equivalent to 0.003545 g of total chloride, calculated as Cl.

For dextrose — To an accurately measured volume containing 2 g to 5 g of Dextrose, add 0.2 ml of 5 M ammonia and sufficient water to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, C₆H₁₂O₆, in the volume taken for assay.

Storage. Store in single dose containers at a temperature not exceeding 30°.

Labelling. The label states (1) the content of each electrolyte in terms of millimoles in a given volume; (2) the amount of each ingredient in 100 ml; (3) the total osmolar concentration in mOsmol per litre; (4) that the preparation should not be used if it contains visible particles.

**Multiple Electrolytes and Dextrose Injection Type IV**

Multiple Electrolytes and Dextrose Injection Type IV is a sterile solution of Dextrose and suitable salts in Water for Injections to provide sodium, potassium, ammonium and chloride ions. It may contain Hydrochloric Acid or Sodium Hydroxide used for adjusting the pH.

**Composition**

- Sodium chloride 0.37 g
- Potassium chloride 0.13 g
- Ammonium chloride 0.37 g
- Dextrose 5.0 g
- Water for Injections to 100 ml

Concentration of electrolytes in mmol / l

- Sodium 63.0
- Potassium 17.0
- Ammonium 70.0
- Chloride 150.0

Multiple Electrolytes and Dextrose Injection Type IV contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of sodium, Na, potassium, K, and ammonium, NH₄ and not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of chloride, Cl₂. It also contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dextrose, C₆H₁₂O₆. It contains no antimicrobial agent.

**Description.** A clear, colourless or faintly straw-coloured solution.

**Identification**

A. To 1 ml add 0.05 ml of potassium cupri-tartrate solution; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. 20 ml gives the reactions of chlorides ammonium salts, sodium salts and potassium salts (2.3.1).

**Tests**

**pH** (2.4.24). 3.0 to 7.0.

5-Hydroxymethylfurfural and Related substances. Dilute a volume containing 1.0 g of Dextrose to 500.0 ml with water and measure the absorbance of the resulting solution at the maximum at about 284 nm: absorbance at about 284 nm, not more than 0.25 (2.4.7).

**Bacterial endotoxins** (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** For total sodium — Dilute suitably with water and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using sodium solution FP or sodium solution AAS respectively, suitably diluted with water for the standard solutions.

For potassium — Dilute suitably with water and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using potassium solution FP or potassium solution AAS respectively, suitably diluted with water for the standard solutions.

For ammonium — Transfer an accurately measured volume of the preparation under examination, containing about 63 mg of ammonium, to a 500-ml Kjeldahl flask, dilute to 200 ml with water, mix and add 50 ml of 40 per cent w/v solution of sodium hydroxide. Connect the flask immediately to a well-cooled condenser through a distillation trap. Let the delivery tube from the condenser dip into 40 ml of a 4.0 per cent w/v solution of boric acid contained in a suitable receiver. Heat to boiling and distil about 200 ml. Cool the liquid in the receiver, if necessary, and titrate with 0.05 M sulphuric acid using methyl red solution as indicator. Carry out a blank titration.

1 ml of 0.05 M sulphuric acid is equivalent to 1.804 mg of ammonium, NH₄.

For total chloride — To 20.0 ml add 30 ml of water, 50.0 ml of 0.1 M silver nitrate and 2 ml of nitric acid. Filter, wash the precipitate with water slightly acidified with nitric acid and titrate the excess of silver nitrate with 0.1 M ammonium thiocyanate using ferric ammonium sulphate solution as
indicate until a reddish yellow colour is produced. Carry out a blank titration.

1 ml of 0.1 M silver nitrate is equivalent to 0.003545 g of total chloride, calculated as Cl.

For dextrose — To an accurately measured volume containing 2 g to 5 g of Dextrose, add 0.2 ml of 5 M ammonia and sufficient water to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, C6H12O6 in the volume taken for assay.

Storage. Store in single dose containers at a temperature not exceeding 30°.

Labelling. The label states (1) the content of each electrolyte in terms of millimoles in a given volume; (2) the amount of each ingredient in 100 ml; (3) the total osmolar concentration in mOsmol per litre; (4) that the preparation should not be used if it contains visible particles.

Multiple Electrolytes and Dextrose Injection Type V

Multiple Electrolytes and Dextrose Injection Type V is a sterile solution of Dextrose and suitable salts in Water for Injections to provide sodium, potassium, calcium, magnesium, acetate, citrate and chloride ions. It may contain Hydrochloric Acid or Sodium Hydroxide used for adjusting the pH.

Composition

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium acetate</td>
<td>0.64 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.50 g</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>0.075 g</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>0.075 g</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>0.035 g</td>
</tr>
<tr>
<td>Magnesium chloride</td>
<td>0.031 g</td>
</tr>
<tr>
<td>Dextrose</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Water for Injections to</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

Concentration of electrolytes in mmol / l

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>140.0</td>
</tr>
<tr>
<td>Potassium</td>
<td>10.0</td>
</tr>
<tr>
<td>Calcium</td>
<td>2.5</td>
</tr>
<tr>
<td>Magnesium</td>
<td>1.5</td>
</tr>
<tr>
<td>Acetate</td>
<td>47.0</td>
</tr>
<tr>
<td>Chloride</td>
<td>103.0</td>
</tr>
<tr>
<td>Citrate</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Multiple Electrolytes and Dextrose Injection Type V contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of sodium, Na, potassium, K, and ammonium, NH4 and not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of chloride, Cl. It also contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dextrose, C6H12O6. It contains no antimicrobial agent.

Description. A clear, colourless or faintly straw-coloured solution.

Identification

A. To 1 ml add 0.05 ml of potassium cupri-tartrate solution; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. 20 ml gives the reactions of acetates, chlorides citrates, sodium salts, potassium salts, calcium salts and magnesium salts (2.3.1).

Tests

pH (2.4.24). 3.0 to 7.0.

5-Hydroxymethylfurfural and Related substances. Dilute a volume containing 1.0 g of Dextrose to 500.0 ml with water and measure the absorbance of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.25 (2.4.7).

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. For total sodium — Dilute suitably with water and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using sodium solution FP or sodium solution AAS respectively, suitably diluted with water for the standard solutions.

For potassium — Dilute suitably with water and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using potassium solution FP or potassium solution AAS respectively, suitably diluted with water for the standard solutions.

For calcium — Dilute suitably with water and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 422.7 nm and using calcium solution FP or calcium solution AAS respectively, suitably diluted with water for the standard solutions.
For magnesium — To 50.0 ml add 50 ml of water and 5 ml of strong ammonia-ammonium chloride solution and titrate with 0.005 M disodium edetate using 50 mg of eriochrome black T mixture as indicator.

1 ml of 0.005 M disodium edetate is equivalent to 0.1215 mg of Mg.

For acetate — Determine by liquid chromatography (2.4.14).

Test solution. Dilute an accurately measured volume of the preparation under examination quantitatively with water to obtain a solution containing about 0.12 per cent w/v of Sodium Acetate.

Reference solution. Dissolve an accurately weighed quantity of sodium acetate in water to obtain a solution having a known concentration of about 0.12 per cent w/v of sodium acetate.

Chromatographic system
- a stainless steel column 30 cm x 7.8 mm, packed with strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form (7 µm) and a guard column 4 cm x 7.8 mm packed with the same column material,
- column. temperature 60°,
- mobile phase: 0.1 M sulphuric acid,
- flow rate. 0.8 ml per minute,
- spectrophotometer set at 210 nm,
- a 20 µl loop injector.

Inject the reference solution containing 1.0 mg of anhydrous sodium citrate per ml. The test is not valid unless the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject separately the test solution and all the three preparations of reference solution and measure the responses for the major peak. Plot the responses of all the three preparations of reference solution versus concentration, in mg of anhydrous sodium citrate per ml, and draw the straight line best fitting the three plotted points. From the graph so obtained, calculate the content of citrate in mg equivalent to anhydrous sodium citrate per litre of the preparation under examination.

For total chloride — To 20.0 ml add 30 ml of water, 50.0 ml of 0.1 M silver nitrate and 2 ml of nitric acid. Filter, wash the precipitate with water slightly acidified with nitric acid and titrate the excess of silver nitrate with 0.1 M ammonium thiocyanate using ferric ammonium sulphate solution as indicator until a reddish yellow colour is produced. Carry out a blank titration.

1 ml of 0.1 M silver nitrate is equivalent to 0.003545 g of total chloride, calculated as Cl.

For dextrose — To an accurately measured volume containing 2 g to 5 g of Dextrose, add 0.2 ml of 5 M ammonia and sufficient water to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, C₆H₁₂O₆, in the volume taken for assay.

Storage. Store in single dose containers at a temperature not exceeding 30°.

Labelling. The label states (1) the content of each electrolyte in terms of millimoles in a given volume; (2) the amount of each ingredient in 100 ml; (3) the total osmolar concentration in mOsmol per litre; (4) that the preparation should not be used if it contains visible particles.

**Multiple Electrolytes Injection Type VI**

Multiple Electrolytes Injection Type VI is a sterile solution of suitable salts in Water for Injections to provide sodium, potassium, calcium, magnesium, acetate, citrate and chloride ions. It may contain Hydrochloric Acid or Sodium Hydroxide used for adjusting the pH.
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject separately the test solution and reference solution and measure the responses for the major peak and calculate the content of acetate in the preparation under examination.

For phosphate — Dilute an accurately measured volume containing about 4 mg of phosphate with sufficient water to produce 50.0 ml. Transfer 2.0 ml of this solution to a test-tube. Add 1.0 ml of a 5 per cent w/v solution of ammonium molybdate in a cooled mixture of sulphuric acid and water (15:85) and allow to stand for 3 minutes. Add 1.0 ml of a freshly prepared 0.5 per cent w/v solution of hydroquinone containing 1 drop of sulphuric acid and 1.0 ml of a freshly prepared 0.5 per cent w/v solution of anhydrous sodium sulphite, mix and allow to stand for 30 minutes. Measure the absorbance of the resulting solution at the maximum at about 640 nm (2.4.7), using as the blank a solution prepared in the same manner by treating 2 ml of water instead of the solution of the preparation under examination. Calculate the content of phosphate from the absorbance obtained by simultaneously carrying out the operation using a known concentration of about 0.11 mg per ml of dipotassium hydrogen phosphate in water instead of the solution of the preparation under examination.

For total chloride — To 20.0 ml add 30 ml of water, 50.0 ml of 0.1 M silver nitrate and 2 ml of nitric acid. Filter, wash the precipitate with water slightly acidified with nitric acid and titrate the excess of silver nitrate with 0.1 M ammonium thiocyanate using ferric ammonium sulphate solution as indicator until a reddish yellow colour is produced. Carry out a blank titration.

1 ml of 0.1 M silver nitrate is equivalent to 0.003545 g of total chloride, calculated as Cl.

For dextrose — To an accurately measured volume containing 2 g to 5 g of Dextrose, add 0.2 ml of 5 M ammonium and sufficient water to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, C₆H₁₂O₆ in the volume taken for assay.

Storage. Store in single dose containers at a temperature not exceeding 30°.

Labelling. The label states (1) the content of each electrolyte in terms of millimoles in a given volume; (2) the amount of each ingredient in 100 ml; (3) the total osmolar concentration in mOsmol per litre; (4) that the preparation should not be used if it contains visible particles.
Chromatographic system
- a stainless steel column 30 cm x 7.8 mm, packed with strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form (7 µm) and a guard column 4 cm x 7.8 mm packed with the same column material,
- column temperature 60°,
- mobile phase: 0.1 M sulphuric acid,
- flow rate. 0.8 ml per minute,
- spectrophotometer set at 210 nm,
- a 20 µl loop injector.

Inject the reference solution containing 1.0 mg of anhydrous sodium citrate per ml. The test is not valid unless the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject separately the test solution and all the three preparations of reference solution and measure the responses for the major peak. Plot the responses of all the three preparations of reference solution versus concentration, in mg of anhydrous sodium citrate per litre of the preparation under examination.

For total chloride — To 20.0 ml add 30 ml of water, 50.0 ml of 0.1 M silver nitrate and 2 ml of nitric acid. Filter, wash the precipitate with water slightly acidified with nitric acid and titrate the excess of silver nitrate with 0.1 M ammonium thiocyanate using ferric ammonium sulphate solution as indicator until a reddish yellow colour is produced. Carry out a blank titration.

1 ml of 0.1 M silver nitrate is equivalent to 0.003545 g of total chloride, calculated as Cl.

Storage. Store in single dose containers at a temperature not exceeding 30°.

Labelling. The label states (1) the content of each electrolyte in terms of millimoles in a given volume; (2) the amount of each ingredient in 100 ml; (3) the total osmolar concentration in mOsmol per litre; (4) that the preparation should not be used if it contains visible particles.

**Mustine Hydrochloride**

Nitrogen Mustard

\[
\text{Cl} - \text{N} - \text{Cl}, \text{HCl}
\]

C₅H₁₁Cl₂N.HCl, Mol Wt. 192.4

Mustine Hydrochloride is bis(2-chloroethyl)methylamine hydrochloride.

Mustine Hydrochloride contains not less than 98.0 per cent and not more than 101.0 per cent of C₅H₁₁Cl₂N.HCl.

**Description.** A white or almost white, crystalline powder or mass; hydroscopic; vesicant.

**Identification**

A. Dissolve 50 mg in 5 ml of water and add 1 ml of 5 M sodium hydroxide; oily globules are produced which dissolve on warming.

B. Dissolve 50 mg in 5 ml of water and add 0.02 ml of potassium mercuri-iodide solution; a cream-coloured precipitate is produced.

A. Melts at about 108° (2.4.21).

**Tests**

**Assay.** Weigh accurately about 0.2 g, add 15 ml of 1 M ethanolic potassium hydroxide and 15 ml of water and boil under a reflux condenser for 2 hours. Evaporate the solution to half its volume on water-bath, dilute to 150 ml with water, add 3 ml of nitric acid and 50.0 ml of 0.1 M silver nitrate. Shake vigorously and filter. Wash the residue with water and titrate the excess of silver nitrate in the combined filtrate and washings with 0.1 M ammonium thiocyanate solution as indicator.

1 ml of 0.1 M silver nitrate is equivalent to 0.006418 g of C₅H₁₁Cl₂N.HCl.

Storage. Store protected from light and moisture at a temperature not exceeding 30°.

Labeling. The label states that the contents of the container are strongly vesicant.

**Mustine Injection**

Mustine Hydrochloride Injection

Mustine Injection is a sterile material consisting of Mustine Hydrochloride with or without buffering agents and other excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections or Sodium Chloride Intravenous Infusion, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution deteriorates rapidly on storage and should be used immediately after preparation.
Mustine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of the stated amount of mustine hydrochloride, C₅H₁₁C₁₂N, HCl.

The contents of the sealed container complies with the tests stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

**Identification**

Dissolve about 20 mg in 1 ml of water and add 0.02 ml of potassium mercuri-iodide solution; a cream-coloured precipitate is produced.

**Tests**

**Assay.** Determine the weight of the contents of 10 containers. Weigh accurately a quantity of the mixed contents of the ten containers, containing 40 mg of Mustine Hydrochloride, dissolve in 10 ml of ethanol (95 per cent), previously neutralised to dilute phenolphthalein solution. Titrate with 0.01 M sodium hydroxide using dilute phenolphthalein solution as indicator.

1 ml of 0.01 M sodium hydroxide is equivalent to 0.001925 g of C₅H₁₁C₁₂N, HCl.

**Storage.** Store protected from moisture at a temperature not exceeding 30°.

**Labelling.** The label states (1) that the contents are strongly vesicant; (2) the amount of Mustine Hydrochloride in the container, (3) that the injection should be used immediately after preparation.