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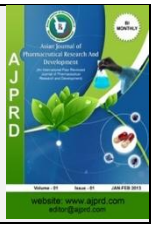

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Research Article

Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Salbutamol Sulphate and Ambroxol Hydrochloride in Oral Liquid Dosage Form

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A B S T R A C T

Pharmaceutical analysis is imported branch of science deals with to check the identity, strength, quality and purity of chemical and herbal compounds. Qualitative analysis of drugs and pharmaceutical compounds. Quantitative analysis is of much importance and done by various methods. One of the most accurate and precise methods is spectrophotometry which comprises the measurement of intensity of electromagnetic radiation emitted or absorbed by the compound. Another most popular method of for quantitative analysis is high performance liquid chromatography (HPLC). In the present article a RP-HPLC method was developed for the simultaneous determination of salbutamol sulphate and ambroxol hydrochloride in oral liquid doses form and validation of the developed method. Developed methods include selection of mobile phase, chromatographic method, and wavelength whereas validation involves the system suitability, accuracy, precision, linearity, reproducibility, robustness, specificity and solution stability of the developed method. The result showed that the developed method is the best suited to the simultaneous determination of salbutamol sulphate and ambroxol hydrochloride and validated as per the standards.

Keyword: Spectrophotometry, RP-HPLC, validation.

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INTRODUCTION

Pharmaceutical Analysis may be defined as the application of analytical procedures used to determine the purity, safety and quality of drugs and chemicals. The pharmaceutical analysis comprises the procedures necessary to determine the "identity, strength, quality and purity" of such compounds. It also includes the analysis of raw material and intermediates during manufacturing process of drugs. [1].Pharmaceutical analysis includes both qualitative and quantitative of drugs and pharmaceutical substances. There are various methods used for quantitative analysis of mixtures. One of them is spectrophotometry, which utilizes the measurement of intensity of electromagnetic radiation emitted or absorbed by the analytes. Another technique which has gained large popularity during last decade is high performance liquid chromatography. It permits simultaneous separation and determination of components of mixture. Chromatography is a technique for analyzing mixtures of gases, liquids or solutes by exploiting differences in their distribution between a stationary and a mobile phase. Chromatography is a fundamental technique in the detection, identification and quantization of chemical species. The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one. It becomes necessary, therefore to develop newer analytical methods for such drugs. Most of the drugs in multi-component dosage forms can be analyzed by HPLC method because of the several advantages like rapidity, specificity, accuracy, precision and ease of automation in this method. [2] HPLC method eliminates tedious extraction and isolation procedures. In the normal phase mode, the stationary phase is a polar substance such as polyethylene glycol or the untreated silica surface itself, and the mobile

phase is non polar (e.g. hexane), under these circumstances polar compounds retarded preferentially and non polar substances elute more quickly.

In reversed phase mode, the stationary phase is non polar (e.g. ODS) and the mobile phase is polar, usually a mixture of water, methanol and/or acetonitrile. Non polar compounds are retained more strongly, while polar solutes elute first. Reversed phase separations are the most frequently used methods in HPLC. The sample or solute is analyzed quantitatively in HPLC by either peak height or peak area measurements. Peak areas are proportional to the amount of constant rate. Peak heights are proportional to the amount of material only when peak width are constant and are strongly affected by the sample injection techniques. Once the peak height or the peak areas are measured, there are five principle evaluation methods for quantifying the solute [3,5]. Method validation can be defined as establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics.

The extensive literature survey carried out revealed few methods have been reported for simultaneous estimation of Salbutamol sulphate and Ambroxol hydrochloride and in combination with other drugs. However there is no method reported for the simultaneous estimation of Salbutamol and Ambroxol hydrochloride in oral liquid dosage form. So it was felt that there is a need to develop RP-HPLC method for the determination of Salbutamol sulphate and Ambroxol hydrochloride simultaneously in single step process. Hence the present work is aimed to develop reverse phase HPLC method for the simultaneous determination of Salbutamol sulphate and Ambroxol hydrochloride in oral liquid dosage form and validation of the developed method. [4]

MATERIALS AND METHODS

Drug samples

Salbutamol sulphate, and Ambroxol hydrocloride raw materials were obtained as gift samples from Bioplus Life Sciences Pvt, Ltd, Hosur.

Formulation used

CRMS Syrup (100mL) containing 15 mg/5mL of Ambroxol and 1 mg/5mL of Salbutamol, manufactured by Pharmed Ltd., Whitefield road, Bangalore was procured from local market.

Equipments used

1. HPLC- agilent/ chemstation
2. Agilent sepration module 1200
3. Injector-auto injector
4. Column- Inertsil C8-3(250 x 4.6mm/5 µ)
5. Detector- agilent DAD or UV detector
6. pH meter- Adva AD 1020
7. Analytical balance- denver(10 mg- 200mg)
8. Hot air oven –techno lab, model BTZ

Reagents and Chemicals

1. Sodium dihydrogen phosphate (AR grade)
2. Triethyl amine(LR grade)
3. Ortho-phosphoric acid (AR grade)
4. Acetonitrile (HPLC grade)
5. Methanol (HPLC Grade)
6. Water (Milli Q)

Reference Standards

a. Ambroxol hydrochloride % Purity –99.8% w/w

b. **Salbutamol sulphate** % Purity –99.8% w/w

PREPARATION OF SOLUTIONS

Preparation of Phosphate buffer

Weighed 1.56 grams of NaH_2PO_4 into a 1000ml beaker, dissolved and to 1000 mL with water, add 3 mL of TEA and adjusted the pH to 3.0 with Ortho-phosporic acid.

Preparation of mobile phase

Mix a mixture of above buffer 650 mL (65%), 100 mL of Acetonitrile (10%) and 250 mL of Methanol (25%) degassed in ultrasonic water bath for 5 minutes. Filtered through 0.45µ filter under vacuum filtration.

Standard solution

Preparation of Salbutamol and Ambroxol stock solution:

Accurately weighed quantity of 150 mg Ambroxol and 25 mg of Salbutamol was transferred to three different 100 mL volumetric flask, dissolved in 25 mL of mobile phase, sonicated for 5 min and the volume was made up with mobile phase.

Concentration of stock solution

Ambroxol hydrochloride	-	150 µg/mL
Salbutamol sulphate	-	10 µg/mL

Preparation of working standard

Working standard for Ambroxol and Salbutamol were prepared by pipetting, 10 mL and 4 mL respectively from each of the stock solution in a 100 mL volumetric flasks and the volume was made up with the mobile phase to give the following concentration.

Ambroxol hydrochloride -150 µg/mL

Salbutamol sulphate - 10 µg/mL

Sample preparation

Accurately measure, amount equivalent to 150 mg of Ambroxol and 25 mg of salbutamol from liquid formulation was accurately weighed and taken in three different 100 mL volumetric flask and 25 mL of mobile phase was added. The mixture was subjected to sonication for 15 min with intermediate shaking for complete dissolving of drugs. Cooled to room temperature and the solution were made up to the mark with mobile phase then filtered using 0.45µ filter. Then 10 µL of this solution was injected for HPLC analysis. [8]

Procedure

Separately blank, standard and test solutions were injected and the areas for major peaks were recorded for Guaifenesin, Ambroxol and Salbutamol and % assay was calculated by using the following formula.

$$\frac{AT \times WS \times DT \times P \times Avg.\ Wt \times 100}{AS \times DS \times WT \times 100 \times Label\ Claim}$$

Where:

AT = average area counts of sample preparation.

As = average area counts of standard preparation.

WS = Weight of working standard taken in mg.

WT = Weight of test taken in mg.

DS = Standard dilution

DT = Test dilution

P = Percentage purity of working standard

LC = label claim mg/mL.

METHOD VALIDATION

The method has been validated for Salbutamol sulphate and ambroxol hydrochloride (CRMS Syrup) by following validation parameters,

The method validation involves establishing of the following parameters:

a. System suitability
b. Accuracy
c. Precision
   i. System Precision/Repeatability
   ii. Method Precision
   iii. Intermediate Precision/Ruggedness
a. Linearity
b. Robustness
c. Specificity
d. Solution stability

RESULTS

Development and Optimization of Chromatographic Parameters

Solubility

According to literature review, Salbutamol sulphate, and Ambroxol hydrochloride are freely soluble in methanol. Therefore the solubility of the drugs was checked with different dilutions of phosphate buffer (pH-3), methanol and acetonitrile. Finally buffer: acetonitrile: methanol in the ratio 65:10:25 was chosen as solvent for present work.

Selection of chromatographic method

The choice of the chromatographic method is based on the nature of the sample (ionic or neutral molecule), its molecular weight and solubility. As drugs are polar in nature, the reverse phase chromatographic technique was selected for the present study. [11]

Selection of wavelength (λmax)

In setting up the conditions for development of the assay method, the choice of detection wavelength was based on the scanned absorption spectrum for Salbutamol and Ambroxol. The UV- Spectrum of Salbutamol and Ambroxol were obtained separately. [15]

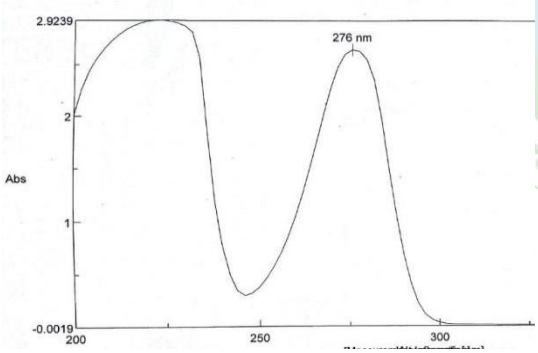

Figure 1: UV-Spectrum of Salbutamol sulphate

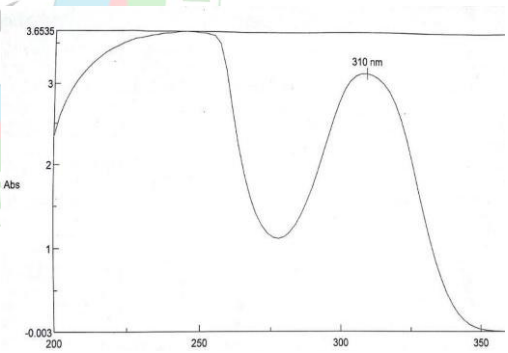

Figure 2: UV-Spectrum of Ambroxol hydrochloride

By scanning the sample solution Salbutamol and Ambroxol each 10µg/mL in methanol over the wavelength range 200-400 nm against blank. After thorough examination of the spectra, the wavelength 276 nm was chosen for further analysis.

METHOD DEVELOPMENT TRIALS

Sample preparation

Salbutamol and Ambroxol each 10µg/mL solution in methanol was prepared and used for trials.

Trial – 1

The trial 1 was performed with Water: Methanol in the ratio of 50:50%v/v with flow rate 1 mL/min.

Only one peak was identified.

Trial – 2

The trial 2 was performed with Water: Methanol: Acetonitrile in the ratio of 40:30:30%v/v with flow rate 1 mL/min.

Only one peak was obtained.

In Water: Methanol and water: Acetonitrile only two peaks were identified. So, instead of water, next trails were performed with Sodium dihydrogen Phosphate buffer (pH-3.0).

Trial – 3

The trial 3 was performed by using Buffer: Acetonitrile: Methanol in the ratio of 50:25:25%v/v with flow rate 1 mL/min.

Only one peak was eluted (i.e.) Salbutamol .

Trial – 4

The trial 4 was performed by using Buffer: Acetonitrile: Methanol in the ratio of 80:10:10%v/v with flow rate 1 mL/min.

Only one peak was eluted (i.e) Ambroxol.

Trial – 5

The trial 5 was performed by using Buffer: Acetonitrile: Methanol in the ratio of 40:30:30%v/v with flow rate 1 mL/min.

Only one peak was eluted (i.e) Salbutamol .

Trial – 6

The trial 6 was performed by using Buffer: Acetonitrile: Methanol in the ratio of 60:5:30%v/v with flow rate 1 mL/min.

Only one peak was eluted (i.e) Salbutamol.

Trial – 7

The 7 trial was performed by using Buffer: Acetonitrile: Methanol in the ratio of 65:10:25%v/v with flow rate 1 mL/min.

The retention time of Salbutamol and Ambroxol was found to be 3.2, 9.8 and 11.8mins respectively.

Conclusion

Out of the 7 trials, the 7th trial was selected for further studies. When compared to the other trials the in the 7th trial has good plate count, Tailing factor, Symmetry and Resolution observed and peak shape was also good.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Stationary Phase : Inertsil C8-3 (250 mmx4.6 mm , 5 µ)

Separation module : Agilent 1200

Injector : Auto injector

Flow rate : 1.0 mL/min

Operating temperature : Ambient

Selected wave length : 276 nm

Mobile phase ratio : Buffer: Acetonitrile: Methanol (65:10:25 %v/v)

Diluents : Mobile Phase

Injection Volume : 10 µL

Run Time : 25 min

VALIDATION

System suitability studies

Salbutamol sulphate and Ambroxol hydrochloride solutions were prepared and injected. Then the system suitability parameters like Resolution, Retention Time, plate number (N), Peak asymmetry factor (Tailing) were evaluated with the help of standard chromatogram.

Table 1: Results for System suitability Parameters

Parameters	Salbutamol sulphate	Ambroxol hydrochloride	Acceptance criteria
Resolution	NA	4.69	NLT 2
Tailing factor	1.1	1.3	NLT 2
Number of theoretical plate	14133	9978	NLT 2000
Retention time	3.157	11.883	NA

Accuracy

Accuracy expresses the closeness of agreement between the value, which is accepted either as conventional true value or and accepted reference value (International Standard e.g. Pharmacopoeia Standard) and the value found (mean value) obtained by applying the test procedure a number of times.

The recovery study was carried out at 50%, 100% and 150% level and the contents were determined from the respective chromatogram. From the results obtained we can conclude that the method was accurate.

Table 2: Results for Recovery Study for Salbutamol

Accuracy Level	Amount of Salbutamol Added (µg)	Amount of Salbutamol Found (µg)	% Recovery	% Mean Recovery	SD	% RSD
Accuracy solution 50%-1	4.99	4.91	101.6	101.5	0.23	0.23
Accuracy solution 50%-2		4.93	101.2			
Accuracy solution 50%-3		4.91	101.6			
Accuracy solution 100%-1	9.98	9.87	101.1	101.3	0.15	0.15
Accuracy solution 100%-2		9.85	101.3			
Accuracy solution 100%-3		9.84	101.4			
Accuracy solution 150%-1	14.97	14.78	101.3	101.2	0.15	0.15
Accuracy solution 150%-2		14.82	101.0			
Accuracy solution 150%-3		14.79	101.2			

Acceptance Criteria: The % Recovery for each level should be between 98.0% to 102.0%.

Table 3: Results for Recovery Study for Ambroxol

Accuracy Level	Amount of Ambroxol hydrochloride Added (µg)	Amount of Ambroxol hydrochloride Found (µg)	% Recovery	% Mean Recovery	SD	% RSD
Accuracy solution 50%-1	74.85	74.15	100.6	100.5	0.25	0.25
Accuracy solution 50%-2		73.89	100.9			
Accuracy solution 50%-3		74.25	100.4			
Accuracy solution 100%-1	149.70	148.42	100.8	100.6	0.26	0.26
Accuracy solution 100%-2		148.62	100.7			
Accuracy solution 100%-3		149.12	100.3			
Accuracy solution 150%-1	224.55	222.58	100.9	100.7	0.15	0.15
Accuracy solution 150%-2		222.96	100.7			
Accuracy solution 150%-3		223.16	100.6			

Acceptance Criteria: The % Recovery for each level should be between 98.0% to 102.0%.

Precision

System Precision/Repeatability of Injection

The system precision was performed by injecting standard solution for five times on to the analytical column and the peak area was measured then %RSD for the area of five replicate injections was calculated.

Table 4: Results for System Precision of Salbutamol sulphate and Ambroxol hydrochloride

S. No	Area of Salbutamol sulphate	Area of Ambroxol hydrochloride
1	38.112	214.053
2	38.998	216.653
3	38.620	214.790
4	38.148	215.290
5	38.613	216.647
Mean	38.498	215.491
S.D	0.37	1.15
%RSD	1.0	0.5

Acceptance Criteria: The %R SD should be NMT 2.0%

Method Precision

The method precision was done by performing assay on six replicate determination of sample preparation at test concentration level (as per method of analysis) and the relative standard deviation of assay results was obtained. [17]

Table 5: Results for Method Precision of Salbutamol sulphate and Ambroxol hydrochloride

S.No	Area of Salbutamol sulphate	% Assay	Area of Ambroxol hydrochloride	%Assay
1	38.011	101.86	214.985	100.28
2	38.108	101.56	214.856	100.34
3	38.214	101.31	214.988	100.28
4	38.256	101.20	215.011	100.27
5	38.302	101.08	215.141	100.21
6	38.359	100.93	215.210	100.18
Mean	38.211	101.32	215.032	100.26
SD	0.13	0.34	0.12	0.06
%RSD	0.33	0.33	0.06	0.06

Acceptance Criteria: The %RSD should be NMT 2.0%

Intermediate Precision/Ruggedness

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

Table 6: Results for Intermediate Precision of Salbutamol sulphate and Ambroxol hydrochloride

S.No	Area of Salbutamol sulphate	% Assay	Area of Ambroxol hydrochloride	%Assay
1	38.154	101.47	214.654	100.44
2	38.210	101.33	214.852	100.35
3	38.245	101.23	214.741	100.39
4	38.311	101.06	214.998	100.28
5	38.345	100.97	215.014	100.27

6	38.352	100.95	214.961	100.29
Mean	38.269	101.17	214.870	100.34
SD	0.08	0.21	0.15	0.07
%RSD	0.21	0.31	0.07	0.07

Acceptance Criteria: The %RSD should be NMT 2.0

Linearity and Range

Preparation of Level – I

5mL and 2mL of stock solution for Salbutamol and Ambroxol were taken in 100mL of volumetric flask diluted up to the mark with mobile phase.

Preparation of Level – II

7.5mL and 3mL of stock solution for Salbutamol and Ambroxol were taken in 100mL of volumetric flask diluted up to the mark with mobile phase.

Preparation of Level – III

10mL and 4mL of stock solution for Salbutamol and Ambroxol were taken in 10mL of volumetric flask diluted up to the mark with mobile phase.

Preparation of Level – IV

12.5mL and 5mL of stock solution for Salbutamol and Ambroxol were taken in 10mL of volumetric flask diluted up to the mark with mobile phase.

Preparation of Level – V

15mL and 6mL of stock solution for Salbutamol and Ambroxol were taken in 10mL of volumetric flask diluted up to the mark with mobile phase.

Procedure

Solution of each level was injected into the chromatographic system and the peak area was measured. Salbutamol sulphate showed linearity in the range of 5-15 (µg/mL) and Ambroxol hydrochloride showed linearity in the range of 75-225 (µg/mL).The calibration graphs was plotted with peak area in the Y axis and concentration of standard solution in the X axis. The degree of linearity was estimated by calculating the correlation coefficient. The correlation coefficient values for Salbutamol sulphate and Ambroxol hydrochloride were found to be respectively. [12-20]

Table 7: Results for Linearity Data

Concentration of Salbutamol sulphate µg/mL)	Peak Area	Concentration of Ambroxol hydrochloride (µg/ml)	Peak Area
05	20.298	75	110.443
7.5	30.306	112.5	166.149
10	39.608	150	220.646
12.5	48.196	187.5	272.420
15	57.828	225	326.137
Correlation coefficient - 0.9994		Correlation coefficient - 0.9998	

Acceptance Criteria: The correlation coefficient should be NLT 0.99

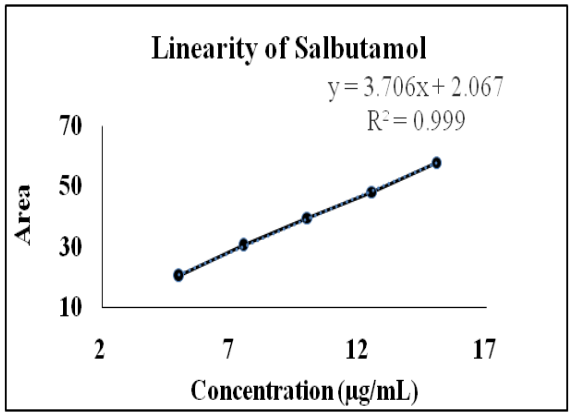

Figure 3: Linearity Plot for Salbutamol sulphate

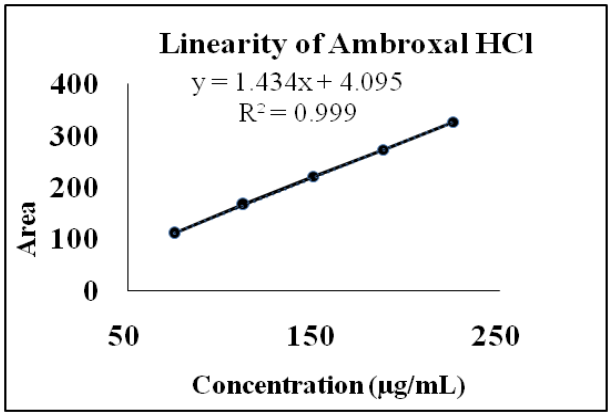

Figure 8 Linearity Plot for Ambroxol hydrochloride

Robustness

For demonstrating the robustness of the developed method, experimental conditions were purposely altered and evaluated. The method must be robust enough to withstand such slight changes and allow routine analysis of the sample. Following optimized conditions were slightly varied.

Effect of Flow rate

Robustness of assay method was carried out with variation of flow rate ±0.1mL/min of the set value i.e. 1mL/min. Standard solution was prepared and performed analysis as per test method and evaluated the system suitability parameters.

Table 8: Results for effect of Flow rate

Name	Flow rate mL/Min	RT	Plate count	Tailing	Resolution
Salbutamol sulphate	0.9	3.49	11482	0.8	NA
	1.0	3.15	14133	1.1	NA
	1.1	2.80	10403	0.8	NA
Ambroxol hydrochloride	0.9	12.86	10421	1.3	4.52
	1.0	11.88	9978	1.3	4.69
	1.1	10.93	9477	1.3	4.48

Table 9: Results for change in flow rate (0.9 ml/Min)

S. No	Area of Salbutamol sulphate	Area of Ambroxol hydrochloride
1	38.011	214.053
2	38.108	216.653
Mean	38.056	215.353
S.D	0.07	1.84
%RSD	0.18	0.85

Acceptance Criteria : %RSD should be NMT 2

Table 10: Results for change in flow rate (1.0 mL)

S. No	Area of Salbutamol sulphate	Area of Ambroxol hydrochloride
1	38.684	215.243
2	38.298	215.987
Mean	38.491	215.615
S.D	0.27	0.54
%RSD	0.71	0.24

Acceptance Criteria : %RSD should be NMT 2

Table 11: Results for change in flow rate (1.1 mL/Min)

S. No	Area of Salbutamol sulphate	Area of Ambroxol hydrochloride
1	37.854	213.458
2	38.024	213.853
Mean	37.939	213.655
S.D	0.12	0.28
%RSD	0.32	0.13

Acceptance Criteria : %RSD should be NMT 2

Effect of Wavelength

Robustness of assay method was carried out with variation of wavelength ±2nm of the set value i.e. 276nm. Standard solution was prepared and performed analysis as per test method and evaluated the system suitability parameters.

Table 12: Results for effect of Wavelength

Name	Wavelength (nm)	RT	Plate count	Tailing	Resolution
Salbutamol sulphate	274	3.56	11882	0.8	NA
	276	3.15	14133	1.1	NA
	278	2.86	11403	0.8	NA
Ambroxol hydrochloride	274	12.46	10521	1.3	4.82
	276	11.88	9978	1.3	4.69
	278	11.13	9677	1.3	4.28

Table 13: Results for change in wavelength (274nm)

S. No	Area of Salbutamol sulphate	Area of Ambroxol hydrochloride
1	39.021	216.687
2	39.254	216.458
Mean	39.138	216.573
S.D	0.16	0.16
%RSD	0.42	0.07

Acceptance Criteria : %RSD should be NMT 2

Table 14: Results for change in wavelength 276nm

S. No	Area of Salbutamol sulphate	Area of Ambroxol hydrochloride
1	38.698	215.124
2	38.386	215.354
Mean	38.542	215.239
S.D	0.22	0.16
%RSD	0.57	0.08

Acceptance Criteria : %RSD should be NMT 2

Table 15: Results for change in wavelength (278nm)

S. No	Area of Salbutamol sulphate	Area of Ambroxol hydrochloride
1	38.054	214.689
2	38.114	214.287
Mean	38.084	214.488
S.D	0.04	0.28
%RSD	0.11	0.13

Acceptance Criteria : %RSD should be NMT 2

Specificity

In case of simultaneous assay of Salbutamol sulphate and Ambroxol hydrochloride, demonstration of specificity requires that the procedure is unaffected by the presence of impurities or excipients. This comparison should include samples stored under relevant stress conditions.

Table 16: Results for Peak purity

Name	Retention Time		Peak Purity
	Salbutamol sulphate	Ambroxol hydrochloride	
Blank	Not detected	Not detected	NA
Placebo	Not detected	Not detected	NA
Standard	3.13	11.88	1.1
Control Sample	3.14	11.86	1.1

Acceptance Criteria : Peak purity should be not less than 1

Table 17: Results for %Assay

Name	Lable claim (mg)	Amount found (mg)	% Assay
Salbutamol sulphate	1	1.01	101.00
Ambroxol hydrochloride	15	14.98	99.87

Acceptance Criteria : % Assay should be between 98% to 102%

Solution Stability

Standard solution was prepared as per the test method and stored at room temperature (23^0C-27^0C). Solution stability study to be performed at different days, against freshly prepared standard solution.

Table 18: Results for Standard solution stability

Interval	Room Temperature (23^0C-27^0C)			
	Salbutamol Area	Ambroxol Area	Similarity factor	
			Salbutamol	Ambroxol
Initial	38.620	214.790	1.01	1.00
24 Hrs	37.557	213.358	1.00	1.01
36 Hrs	38.998	215.165	0.99	1.01
48 Hrs	40.356	216.639	1.01	1.00

Acceptance Criteria: The similarity factor should be 0.98-1.02

Sample solution was prepared as per the test method and stored at room temperature (23^0C-27^0C). Solution stability study to be performed at different days, against freshly prepared Sample solution.

Table 19: Results for Sample solution stability

Interval	Room Temperature (23^0C-27^0C)					
	SAB Area	AMB Area	% Assay		Absolute % Difference	
			SAB	AMB	SAB	AMB
Initial	38.256	215.011	101.58	100.88	NA	NA
24 Hrs	38.557	214.058	101.15	100.56	0.4	0.3
36 Hrs	39.987	214.665	100.96	100.05	0.2	0.5
48 Hrs	40.998	216.139	100.85	99.87	0.1	0.2

Acceptance Criteria: The absolute % difference should be NMT 1.0

DISCUSSION

Method development

Several mobile phase compositions were tried to get good optimum resolutions of Salbutamol sulphate and Ambroxol hydrochloride peaks. The mobile phase containing Sodium dihydrogen phosphate buffer (pH3.0): Acetonitrile: Methanol (65:10:25 %v/v) was selected because it gave sharp peaks with good resolution, minimum tailing and satisfactory retention time. Both the drugs having appreciable absorbance at 276nm and therefore 276nm were selected as the detection wavelength. The working standard solutions of Salbutamol sulphate and Ambroxol hydrochloride were injected separately. The retention time of Salbutamol sulphate and Ambroxol hydrochloride was found to be 3.157 min and 11.883 min respectively when injected as individual compounds.

Validation of the method

The system suitability parameters were studied from the chromatogram to ascertain the suitability of the proposed method. The number of theoretical plates was found to be 14133 for Salbutamol and 9978 for Ambroxol indicating the suitability of the method. The tailing factor was found to be 1.1 for Salbutamol and 1.3 for Ambroxol indicating good symmetry. The obtained resolution between Salbutamol was 4.69 indicating good and complete separation of three drugs (presented in table 1).

The accuracy of the method was determined by recovery experiments. The recovery study was carried out at 50%, 100% and 150% level. The percentage recovery and mean percentage of the recovery were calculated. The results showed percentage recovery of 100.5% to 101.6% for Salbutamol and 100.1% to 100.8% for Ambroxol was in agreement to the acceptance criteria 98% to 102%. From the data obtained, the recoveries of standard drugs were found to be accurate (presented in table 2&3).

The precision of system and method was determined by replicate injections of standard drug solution. In the system precision the %RSD of peak area was found to be 1.0 for Salbutamol and 0.5 for Ambroxol. In the method precision the %RSD of assay was found to be 0.3 for Salbutamol and 0.06 for Ambroxol. The values of %RSD for precision study obtained were well within the acceptance criteria less than 2%. Thus the method providing high degree of precision (presented in table 4&5). [21]

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions. The %RSD of peak area was found to be 0.2 for Salbutamol and 0.07 for Ambroxol (presented in table 6). Thus the results were found to be highly reproducible in spite of variations in the conditions.

To evaluate the linearity of method, the standard drug solutions of varying concentrations ranging from 50 % to 150 % of the targeted level of the assay concentration were examined by the proposed method. The peak area and concentration were plotted to get a standard calibration curve. The correlation coefficient was found to be 0.9994 for Salbutamol and 0.9998 for Ambroxol. The linearity was obtained in the concentration range of 5-15 µg/mL Salbutamol and 75-125 µg/mL for Ambroxol (presented in table 7). The obtained data demonstrates that the methods have adequate sensitivity to the concentrations of the analytes. The range demonstrates that the method is linear outside the limits of expected use. [22]

The robustness of the method was studied by carrying out experiments by changing experimental conditions flow rate and wavelength ratio. No significant effect on chromatographic resolution was seen and hence the developed method is said to be robust (shown on table 8-15).

The specificity of the method was studied by the values obtained while sample stored under relevant stress

conditions. The procedure was unaffected by the presence of impurities (shown on table 16&17).

The solution stability of the method, the standard and sample solutions were prepared vand injected for several days and check the similarity factor for standard solution and absolute % difference for sample solution. The similarity factor for standard solution were found to be, Initially 1.01, after 24 Hrs 1.00, after 36 Hrs 0.99 and after 48 Hrs 1.01 for Salbutamol and Initially 1.00, after 24 Hrs 1.01, after 36 Hrs 1.01 and after 48 Hrs 1.00 for Ambroxol. The values for solution stability of standard were within the limit of 0.981.02. Thus, the solution stability of standard was passed (shown on table 18). The absolute % difference for sample solution were found to be, Initially NA, after 24 Hrs 0.4, after 36 Hrs 0.2 and after 48 Hrs 0.1 for Salbutamol and Initially NA, after 24 Hrs 0.3, after 36 Hrs 0.5 and after 48 Hrs 0.2 for Ambroxol. The values for solution stability of sample were within the limit. Thus, the solution stability of sample was also passed (shown on table 19).

Table 24: Validation data

Parameters		**Salbutamol Sulphate**	**Ambroxol hydrochloride**
Accuracy		% Recovery = 101.2	% Recovery = 100.5
Linearity		R^2 = 0.9994	R^2 = 0.9998
Range		05-15 µg/mL	75-225 µg/mL
System Precision		%RSD = 1.0	%RSD = 0.5
Method precision		%RSD = 0.3	%RSD = 0.06
Intermediate precision		%RSD=0.21	%RSD=0.07
Robustness (Effect of flow)		%RSD=0.71	%RSD=0.24
Robustness (Effect of wavelength)		%RSD=0.57	%RSD=0.08
Solution stability	Standard	Similarity factor=1.01	Similarity factor=1.01
	Sample	% difference=0.2	% difference=0.4

CONCLUSION

A RP-HPLC method was developed with mobile phase system Sodium dihydrogen phosphate buffer pH 3.0: Acetonitrile: Methanol in the ratio of 65:10:25 with the flow rate of 1 mL/min. Detection was carried out at 276 nm. Quantitation was done by external standard method with the above mentioned optimized chromatographic condition. This system produced sharp peaks with good resolution, minimum tailing and satisfactory retention times of Salbutamol sulphate and Ambroxol hydrochloride were found to be 3.157and 11.883 minutes respectively indicating the suitability of system.

The developed method was validated for various parameters accuracy, precision, linearity, robustness, specificity and solution stability as per ICH guidelines. The accuracy of the method was in agreement to the acceptance criteria. The results indicate satisfactory accuracy of the method. Precision of the developed method was studied under system precision, method precision and intermediate precision. The %RSD values for precision was found to be within the acceptable limit, which revealed that the developed method was precise. The linearity was obtained in the concentration range of 5-15 µg/mL for Salbutamol sulphate, and 75-225 µg/mL for Ambroxol. The correlation coefficient was found to be 0.9994 for Salbutamol, and 0.9998 for Ambroxol hydrochloride which indicates excellent correlation between response factor Vs concentration of standard solutions. The robustness of the method was studied. The results indicate that the method was robust and did not show significant effect on chromatographic resolution. The specificity of the method was studied by the values obtained while sample stored under relevant stress conditions. The procedure was unaffected by the presence of impurities. The method was applied for the assay of sample i.e. marketed tablet dosage form of Salbutamol sulphate and Ambroxol hydrochloride. The assay results conformed to the label claim of the dosage form.

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