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Review Article

A REVIEW ON CHROMATOGRAPHY TECHNIQUES

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ABSTRACT

Chromatography techniques like HPLC, Gas chromatography, Paper chromatography, TLC, etc and their working principle and applications for various samples. Different physical and chemical properties of wide ranges of the test samples to be seprated by the specific chromatographic methods selected which enumerate the seprations, identifications and analysis with optimum output for particular samples. This review deals with discussion of the conventional as well as sophisticated of various efforts for particular samples. The study signifies the appliance of chromatography at various stages of drug discovery and development

***Keywords**: HPLC, Gas Chromatography, TLC, Drug discovery, Chromatography.*

INTRODUCTION

Chromatography is a physicochemical method for separation of complex mixtures wasdiscovered at the very beginning of the twentieth century by Russian–Italian botanist M.S. Tswett. [1]. In his paper "On the new form of adsorption phenomena and itsapplication in biochemical analysis" presented on March 21, 1903 at the regular meetingof the biology section of the Warsaw Society of Natural Sciences, Tswett gave a verydetailed description of the newly discovered phenomena of adsorption-based separationof complex mixtures, which he later called "chromatography" as a transliteration fromGreek "color writing" [2]. Serendipitously, the meaning of the Russian word "tswett"actually means color. Although in all his publications Tswett mentioned that the origin ofthe name for his new method was based on the colorful picture of his first separation ofplant pigments , he involuntarily incorporated his own name in the name of themethod he invented. The chromatographic method was not appreciated among thescientists at the time of the discovery, as well as after almost 10 years when L. S. Palmer[3] in the United States and C. Dhere in Europe independently published the descriptionof a similar separation processes. In the late 1970's, new methods including reverse phase liquid chromatography allowed for improved separationbetween very similar compounds. By the 1980's HPLC was commonly used for theseparation of chemical compounds. New techniques improved separation, identification,purification and quantification far above the previous techniques. Computers andautomation added to the convenience of HPLC. Improvements in type of columns andthus reproducibility were made as such terms as micro-column, affinity columns, andFast HPLC began to immerge.

By the 2000 very fast development was undertaken in the area of column material withsmall particle size technology and other specialized columns. The dimensions of theGeneral Introduction typical HPLC

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column are 100-300 mm in length with an internaldiameter between 3-5 mm. The usual diameter of micro-columns, or capillary columns,ranges from 3 µm to 200 µm[9]. In this decade, sub 2 micron particle size technology(column material packed with silica particles of < 2µm size) with modified or improvedHPLC instrumentation becomes popular with different instrument brand name like UPLC(Ultra Performance Liquid Chromatography) of Waters and RRLC (Rapid ResolutionLiquid Chromatography) of Agilent.Today, chromatography is an extremely versatile technique; it can separate gases, andvolatile substances by GC, in-volatile chemicals and materials of extremely highmolecular weight (including biopolymers) by LC and if necessary very inexpensively byTLC. All three techniques, (GC), (LC) and TLC have common features that classify themas chromatography systems. Chromatography has been defined as follows,"Chromatography is a separation process that is achieved by distributing the components ofa mixture between two phases, a stationary phase and a mobile phase. Those componentsheld preferentially in the stationary phase are retained longer in the system than those thatare distributed selectively in the mobile phase. As a consequence, solutes are eluted from thesystem as local concentrations in the mobile phase in the order of their increasingdistribution coefficients with respect to the stationary phase; ipso facto a separation isachieved" [10].

Various Types of Chromatography

Chromatography can be classified by various ways (I) On the basis of interaction ofsolute to the stationary phase [11], (II) On the basis of chromatographic bed shape [12,13], (III) Techniques by physical state of mobile phase

On The Basis of Interaction of Solute To Stationary phase

- Adsorption Chromatography
- Partition Chromatography
- Ion Exchange Chromatography
- Molecular Exclusion Chromatography

On The Basis of Chromatographic Bed Shape

- Column Chromatography
- Planar Chromatography
- Paper Chromatography
- Thin Layer Chromatography
- Displacement Chromatography

Techniques by Physical State of Mobile Phase

- Gas Chromatography
- Liquid Chromatography
- Affinity Chromatography

Techniques of Chromatography

HPLC: High Pressure Liquid Chromatography

High performance liquid chromatography (HPLC) is a chromatographic technique used to separate a mixture of compounds in analytical chemistry and biochemistry with the purpose of identifying, quantifying or purifying the individual components of the mixture.

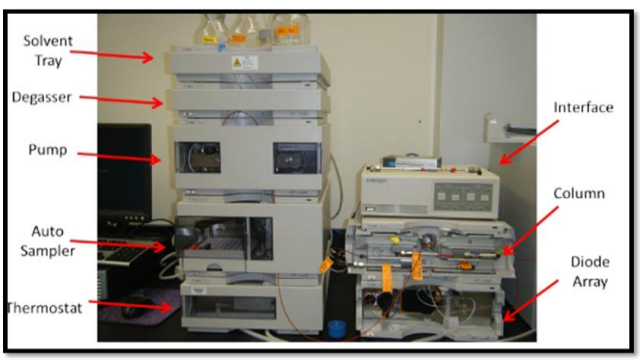

Fig.1 Labeled Agilent 1100 Series HPLC at UAF.

GAS CHROMATOGRAPHY

Gas-liquid chromatography utilizes carrier gas flowing through an injector (sample entry point), a glass or metal column in a temperature-controlled oven, and a detector with an electronically interfaced recorder/recording system . The basic components of GLC systems have not substantially changed since the tech-

Carrier Gas

The choice of a practical carrier gas is simple: nitrogen or helium. Air may be used as a carrier gas under certain conditions with portable or on-site chromatographs but this is uncommon with laboratory-scale instruments.

Sample Inlets

The chromatographic process begins when sample is introduced into the column, ideally without disrupting flows in the column. The chromatographic results will be reproducible inasmuch as this is accomplished with a minimum of change in pressure or flow of the carrier gas or mobile phase. Also, the injection step establishes the initial (and best possible) peak width for the GC measurement. Thus, delivery of sample into the column should be controlled, reproducible, and rapid.

Detectors and Data System

The subject of detectors inGC is a pivotal theme since the separation processes will have been wasted if the analyte cannot be detected. Excellent primers on GC detectorsare available and any general text on instrumental analysis will have introductory material on the common detectors. Abiennial review contains an extensive section on developments of Detectors and can serve as a guide to primary literature.

Column Chromatography

The column chromatography demonstrates the typical features found in this analytical technique. The diagram shows an experiment where a two-component mixture is subjected to column chromatography. The column is packed with a solid material called the stationary phase. A liquid solvent or eluting solution is poured into the column and completely wets the solid packing material. Then the mixture is loaded onto the top of the wet column and more eluent is added. Gravity pulls the mobile phase down through the stationary phase and the components in the mixture start to move through the column at different rates. In the diagram, component A moves faster than component B; thus component B is retained on the column for a longer time than component A. Usually this is due to a difference in solubility of the two compounds in the solvent and/or to a difference in attraction to the solid packing material. As more eluent is added to the top of the column, the components will eventually exit the column separately. The time taken to exit the column, called retention time, will be reproducible for each component under the given set conditions mobile and stationary phase identities, temperature and column width. Once the components exit the column, the solvent can be removed by evaporation and the pure components can be further analyzed or identified.

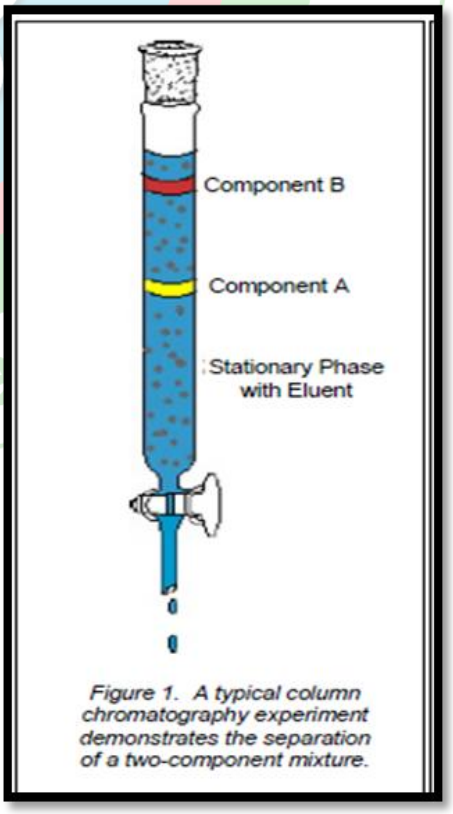

Figure 1. A typical column chromatography experiment demonstrates the separation of a two-component mixture.

Paper Chromatography

Most chemists and many other scientists must routinely separate mixtures and identify their components. The ability to qualitatively identify the substances found in a sample can be critical. For example, an environmental chemist investigating samples of polluted ground water will want to know which toxic ions might be present in a sample.

Chromatography is one of the first tools used in such situations. In this technique, many types of mixtures can be separated into the component pure substances; by comparison to a standard sample, each component substance can also be tentatively identified.

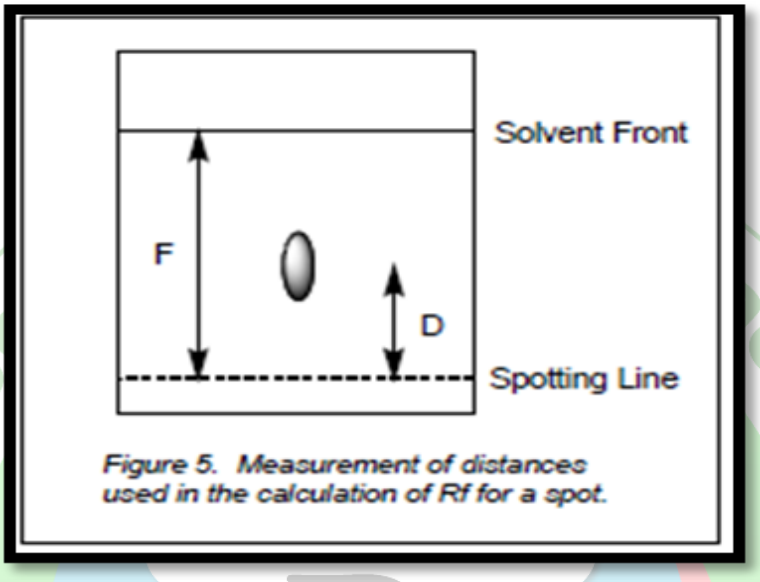

Figure 5. Measurement of distances used in the calculation of Rf for a spot.

Thin Layer Chromatography

Thin layer chromatography (TLC) is among the most useful tools for following the progress of organic chemical reactions and for assaying the purity of organic compounds. TLC requires only a few ng (yes that's right nano grams!) of sample for a successful analysis and can be accomplished in a matter of minutes. Like all chromatographic methods, TLC takes advantage of the different affinity of the analyte with the mobile and stationary phases to achieve separation of complex mixtures of organic molecules.

Stationary Phase

Silica gel, the most commonly used stationary phase, has the empirical formula SiO2. However, at the surface of the silica gel particles, the dangling oxygen atoms are bound to protons.

Mobile Phase

For silica gel chromatography, the mobile phase is an organic solvent or mixture of organic solvents. As the mobile phase moves past the surface of the silica gel it transports the analyte past the particles of the stationary phase. However, the analyte molecules are only free to move with the solvent if they are not bound to the surface of the silica gel. Thus, the fraction of the time that the analyte is bound to the surface of the silica gel relative to the time it spends in solution determines the retention factor of the analyte. The ability of an analyte to bind to the surface of the silica gel in the presence of a particular solvent or mixture of solvents can be viewed as a the sum of two competitive interactions. First, polar groups in the solvent can compete with the analyte for binding sites on the surface of the silica gel. Therefore, if a highly polar solvent is used, it will interact strongly with the surface of the silica gel and will leave few sites on the stationary phase free to bind with the analyte. The analyte will, therefore, move quickly past the stationary phase.

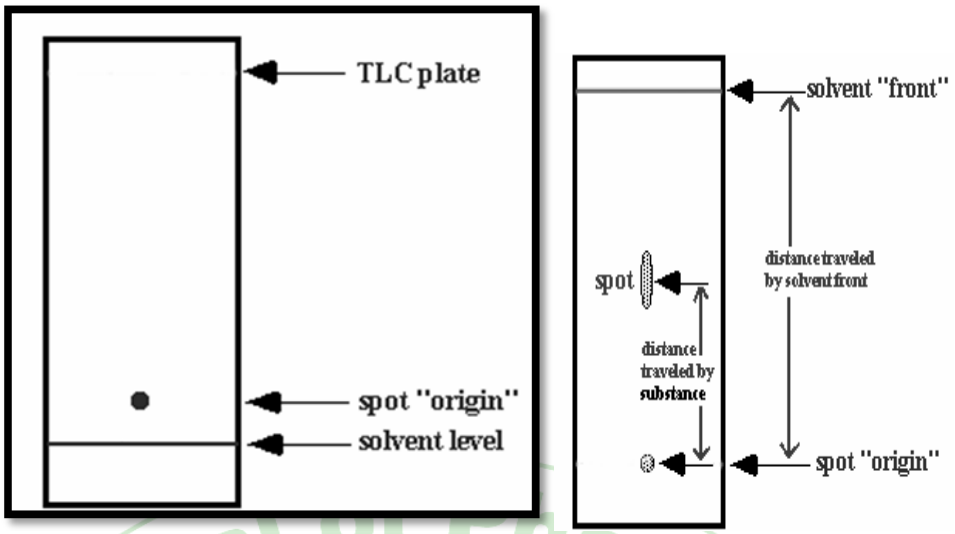

Figure- Position of the spot on a thin layer plate

TLC plate showing distances traveled by the spot and the solvent after solvent front nearly reached the top of the adsorbent.

$$R_f \text{ value} = \frac{\text{distance traveled by substance}}{\text{distance traveled by solvent front)}}$$

ADVANTAGES AND DISADVANTAGES

GAS CHROMATOGRAPHY

Advantage:

- Applicable to most compound
- Linearty is good
- The sample is not destroyed &hence used in preparative scale
- Simple,easy to maintain and inexpensive.

Disadvantage :

- Low sensitivity
- Affected by fluctuation in temperature and flow rate.
- The response is only relative and not absolute
- Biological sample can notanalysed

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Advatages:

- Need a small sample with a high accuracy and précis.
- Non-destructed sample during operation compared to GC..

Disadvantages:

- Need a skill to run the instruments.
- Solvent consuming.

THIN LAYER CHROMATOGRAPHY

Advantages

- Simple method and cost of the equipment is low.
- Rapid technique and not time consuming like column chromatography.
- Separation of µg of the substances can be achieved.
- Any type of compound can be analyzed.
- Detection is easy and not tedious.
- Capacity of the thin layer can be altered. Hence analytical and preparative separation can be made.
- Corrosive spray reagents can be used without damaging the plate.
- Needs less solvent, stationary phase and time for every separation when compared to column chromatography.

PAPER CHROMATOGRAPHY

Advantages:

- One of the major advantages of paper chromatography is the sensitivity with which compound can be located after separation. Amount as little as 0.1 µg (1µg is 1 millionth of gram) of some compound can be detected with routine reagents.
- The operation time small.

Disadvantages:

- Paper chromatography techniques can not be used in separation of volatile substance such as hydrocarbon and volatile fatty acid.
- The lower limit for the detection of most compound is 1-5 µg.

Column chromatography

Advantages :

- Any type of mixture can be separated by column chromatography
- Any quantity of the mixture can be separated (µg to mg of substance)
- Wider choice of mobile phase
- In preparative type the sample can b e separated and reused
- Automation is possible

Disadvantages:

- Time consuming method
- More amount solvent are required which are expensive
- Automation makes the techniques more complicated and expensive

APPLICATION OF CHROMATOGRAPHY

GAS CHROMATOGRAPHY

- Qualitative analysis
- Quantitative analyisis
- Isolation and identificationof mixture of component like amino acid, plant extracts and volatileoils,etc.
- Petroleum industry: GCis used in analysis of crude petroleum product ,gasoline,wax, LPG and sulphur and nitrogen compounds

Food industry:

- Determinationof colour and flavor of food.
- Determination of residual solvent in spice, oleoresins and for pesticides in food.
- Pharmaceuticals:
- Determination of styrene monomer.
- Identification and determination of fatty acid of oil.
- Analysis of solvents.

Plastic industry:

GC is used in the identification of plastic, determination of esters in acrylic copolymers, long chain alcohol ester in acrylic copolymer,

- Miscellaneous:
- Analysis of organic functional groups.
- Analysis of gases.
- Analysis of fertilizers.

APPLICATION OF HPTLC:

Qualitative analysis:

Identification of a compound by comparing the retention time of the sample well as the standard.

- Checking the purity of a compound:the purity of the compound can be inferred. If additional peaks are obtained,impurities present and hence the compound is not pure. from the persense area of the peaks obtained.
- Presence of impurities:The presence additional peaks when compared with a reference standard reference material. The percentage of impurities may also be calculate from peak areas.
- Quantitative analysis : the quantity of a component can be determine by several method like, Multicomponent analysis, Isolation and identification of drugs
- Isolation and identification of mixture of component of natural or synthetic origin

APPLICATUION OF ION EXCHANGE

- **Softening of water:** Removal of monovalent and divalent ions like sodium, potassium, calcium, magnesium, etc.
- **Demineralisation or deionization of water:** Removal of different ions to get demineralized water.
- Purification of some solution to be free from ionic impurities.
- **Separation of inorganic ions:**cation and anions.
- **Organic separations:** most of the pharmaceutical compound either strongly or weakly acidic or basic in nature .hence a mixture of that compound can be separated by using ion exchange resin some classes of compound which can be separated are amino acid protein, antibiotics, vitamins, fatty acids, etc.

APPLICATION PAPER CHROMATOGRAPHY AND THIN LAYER CHROMATOGRAPHY

- Separation of mixture of drug of chemical or biological origin, plant extracts, etc
- Separation of carbohydrates, vitamin, antibiotic, protein alkaloids, glycoside,aminoacids, etc.
- Identification of drug
- Identification of impurities
- Analysis of metabolite of drug in blood ,urine etc.

APPLICATION COLUMN CHROMATOGRAPHY

- Separation of mixture of compounds: Column chromatography can be used for the separation of several classes of drug and constituents like alkaloids glycosides amino acid etc.
- Removal of impurities or purification process:impurities present in a compound can be removed by using appropriate stationary and mobile phase.
- Isolation of active constituents: from plant extract,from formulation or other crude extracts,active constituents.
- Isolation of metabolite from biological fluid: eg.17-ketosteroids from urine cortisol ,other drug etc. from biological fluids like blood,plasma or serum , etc.
- Estimation of drugs in formulation or crude extracts
- Determination of %w/w of stychine in syrup of ferrous phosphate with quinine and strychnine.
- Determination of primary and secondaryglycoside in digitalis leaf.
- Separation of diastereomers.
- Separation of inorganic ions like copper,cobalt, nickel ,etc.

CONCLUSION

It can be concluded from the entire review that HPLC is a versatile, reproducible chromatographic technique for the estimation of drug products. It has wide applications in different fields in term of quantitative and qualitative estimation of active molecules.

In recent years development of the analytical methods for identification, purity evaluation and quantification of drugs has received a great deal of attention in the field of separation science. This review describes GC method development and validation in general way. A general and very simple approach for the GC method development for the separation of compounds was discussed.

In the drug discovery and development process the chromatography has proven a crucial role. It may be concluded that drug discovery phenomenon is incomplete without chromatographic techniques. Depending on the nature of analyte if proper chromatographic method is supported with suitable detection technique, the analysis is no longer a challenge. Appliance of selective and specific chromatographic technique in the various steps of the drug discovery has declined the time and cost of drug research from discovery to manufacturing stage.

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