A DETAILED ANALYSIS OF THE METHODS USED IN CHROMATOGRAPHY

Ajay Shankar Rao Dongarwar
Research Scholar
Department of Pharmacy
Sunrise University, Alwar, Rajasthan.
ajaydongarwr@ gmai.com

Dr. Sanjay kisanraobais
Research Guide
Department of Pharmacy
Sunrise University, Alwar, Rajasthan.

ABSTRACT
Various chromatography methods, including HPLC, Gas chromatography, Paper chromatography, TLC, etc., and their applicability for different substances. Different physical and chemical characteristics of a variety of test samples must be separated by the chosen chromatographic techniques, which count the separations, identify the samples, and analyze them with the best results for each sample. The conventional as well as the sophisticated of numerous attempts for specific samples are discussed in this study. The research shows how chromatography is used at different phases of drug discovery and development.
***Keywords**: HPLC, Gas Chromatography, TLC, Drug discovery, Chromatography.*

INTRODUCTION
The Russian-Italian botanist M.S. Tswett devised chromatography, a physicochemical technique for separating complicated mixtures, at the very beginning of the 20th century. [1]. Tswett provided a very detailed description of the newly discovered phenomenon of adsorption-based separation of complex mixtures in his paper titled "On the new form of adsorption phenomena and its application in biochemical analysis," which was presented on March 21, 1903 at the regular meeting of the biology section of the Warsaw Society of Natural Sciences [2]. He later referred to this phenomenon as "chromatography" as a transliteration from the Greek word for "color writing." Fortunately, the Russian term "tswett" denotes color in actuality. Even though Tswett said in all of his writings that the name of his new technique was derived from the vivid image of his initial separation of plant pigments, he unintentionally included his own name into the name of the method he devised. Scientists at the time of the discovery and over ten years later, when L. S. Palmer[3] in the United States and C. Dhere in Europe separately published the description of a comparable separation procedure, did not understand the chromatographic approach. In the late 1970s, enhanced separation of chemically identical molecules was made possible by new techniques, such as reverse phase liquid chromatography. By the 1980s, chemical compound separation was often accomplished using HPLC. New methods significantly outperformed older ones in terms of separation, identification, purification, and quantification. Computers and automation boosted HPLC's convenience. As words like micro-column, affinity columns, and fast HPLC became more popular, improvements in the kind of columns and therefore repeatability were created.

By the year 2000, extremely quick progress had been made in the field of specialty columns and column materials with tiny particle sizes. The typical HPLC column is between 100 and 300 mm in length and has an internal diameter

between 3 and 5 mm. Micro-columns, also known as capillary columns, typically have diameters between 3 to 200 m[9]. Sub-2 micron particle size technology—column material packed with silica particles less than 2 microns in size—as well as modified or improved HPLC instrumentation are becoming more and more popular in this decade. Popular instrument brands include Waters' UPLC and Agilent's RRLC (Rapid Resolution Liquid Chromatography).In today's world, chromatography is a highly flexible technology that can separate gases, volatile compounds, in-volatile chemicals, and materials with exceptionally high molecular weight (including biopolymers) using GC, LC, and, if required, TLC, at a very low cost. The three methods of chromatography—GC, LC, and TLC—all have characteristics that categorize various chromatography systems. The definition of chromatography is as follows:"A stationary phase and a mobile phase are used in the separation process known as chromatography to distribute the components of a mixture. Comparatively to components that are dispersed selectively in the mobile phase, those held preferentially in the stationary phase are kept in the system for a longer period of time. A separation is thus obtained as a result of the solutes being eluted from the system as local concentrations in the mobile phase in the order of their increasing distribution coefficients with respect to the stationary phase.

Various Types of Chromatography

Chromatography may be categorized in a number of ways. (I) On the basis of the solvent's interaction with the stationary phase [11], (II) on the basis of the form of the chromatographic bed, Techniques based on the physical state of the mobile phase

On The Basis of Interaction of Solute ToStationary phase

Adsorption Chromatography Partition Chromatography

Ion Exchange Chromatography Molecular Exclusion Chromatography

On The Basis of Chromatographic Bed Shape

Chromatography in a column Chromatography on a plane Chromatography on paper Displacement in Thin Layer Chromatography Chromatography

Techniques by Physical State of Mobile Phase

Gas Chromatography Liquid Chromatography Affinity Chromatography

Techniques of Chromatography

HPLC: High Pressure Liquid Chromatography

In analytical chemistry and biochemistry, high performance liquid chromatography (HPLC) is a chromatographic method used to separate a mixture of substances with the aim of identifying, measuring, or purifying the individual components of the mixture.

GAS CHROMATOGRAPHY

For gas-liquid chromatography, a carrier gas is passed via an injector (the point where the sample is introduced), a glass or metal column is placed in a temperature-controlled oven, and a detector is connected to an electronic recorder and recording system. Since the beginning, the fundamental parts of GLC systems have not altered much.

Carrier Gas

Simple options for useful carrier gases are helium and nitrogen. With portable or on-site chromatographs, air may be employed as a carrier gas under specific

circumstances, although this is unusual with laboratory-scale devices.

Sample Inlets

The introduction of the sample into the column, preferably without interfering with column flows, starts the chromatographic process. Insofar as this is done with a little variation in carrier gas or mobile phase pressure or flow, the chromatographic findings will be repeatable. The initial (and best feasible) peak width for the GC measurement is also established during the injection process. As a result, sample delivery into the column should be managed, repeatable, and quick.

Detectors and Data System

The topic of detectors in GC is crucial because if the analyte cannot be detected, the separation procedures would have been for nothing. There are excellent GC detector primers available, and any basic instrumental analysis literature will include an introduction to the most used detectors. An full section on detector advances may be found in the yearly review, which also acts as a roadmap to original sources.

Column Chromatography

The column chromatography exemplifies the common characteristics of this analytical method. The experiment, which involves subjecting a two-component mixture to column chromatography, is shown in the figure. The stationary phase, a solid substance, is tightly packed within the column. The solid packing material in the column is thoroughly moistened by the addition of a liquid solvent or eluting solution. More eluent is then added once the mixture is put into the wet column's top. The components of the mixture begin to move through the column at various speeds as gravity pushes the mobile phase down through the stationary phase. Component B is kept on the column for a longer period of time than Component A because Component B travels quicker in the diagram than Component A. The two chemicals' different solubilities in the solvent and/or different strengths of attraction to the solid packing material are often to blame for this. The components will finally depart the column independently when additional eluent is added to the column's top. Under the predetermined circumstances of mobile and stationary phase identities, temperature, and column width, the time it takes for a component to leave the column—also known as the retention time—will be repeatable for each component. The solvent may be eliminated by evaporation after the components have left the column, allowing for further analysis or identification of the pure components.

Paper Chromatography

The majority of chemists and many other scientists often need to separate mixtures and determine their constituent parts. It might be important to be able to qualitatively identify the compounds present in a sample. Toxic ions may be present in a sample, as in the case of an environmental scientist testing samples of contaminated ground water.

One of the first tools employed in such circumstances is chromatography. With this method, a variety of combinations may be broken down into their constituent pure substances. Each component material can also be roughly recognized by comparison to a reference sample.

Thin Layer Chromatography

One of the most effective methods for monitoring the development of organic

chemical processes and determining the purity of organic molecules is thin layer chromatography (TLC). For a successful analysis, TLC only needs a few ng (yep, that's right, nanograms!) of material, which may be completed in a matter of minutes. TLC, like other chromatographic techniques, uses the analyte's differing affinities for the mobile and stationary phases to separate complex organic compound mixtures.

Stationary Phase

The most popular stationary phase, silica gel, has the empirical formula SiO2. The oxygen atoms that are hanging off the silica gel particles' surface, however, are bonded to protons.

Mobile Phase

The mobile phase for silica gel chromatography is an organic liquid or combination of organic solvents. The analyte is transported through the stationary phase's particles as the mobile phase travels past the silica gel's surface. The analyte molecules can only travel freely with the solvent if they are not attached to the silica gel's surface. The retention factor of the analyte is therefore determined by the ratio of the amount of time the analyte spends attached to the silica gel surface to the amount of time it spends in solution. It is possible to think of an analyte's capacity to bind to the surface of silica gel in the presence of a certain solvent or combination of solvents as the result of two competing interactions. First, the analyte and polar groups in the solvent may fight for binding sites on the silica gel's surface. A highly polar solvent will thus interact significantly with the silica gel's surface if it is utilized, leaving few sites on the stationary phase free to bind the analyte. As a result, the analyte will pass through the stationary phase fast.

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 usedin preparative scale
- Simple,easy to maintain and inexpensive.

Disadvantage :

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

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Advatages:

- Require a little sample that is very precise and accurate.
- Sample is not destroyed during operation, in contrast to GC.

Disadvantages:

- Operating the instruments requires competence.
- Consuming of solvent.

THIN LAYER CHROMATOGRAPHY

Advantages

- Simple procedure and inexpensive equipment.
- Quick approach that doesn't take as long as column chromatography.
- G of the compounds may be

successfully separated.

• Compounds of any kind may be examined. It is simple and quick to detect.

• The thin layer's capacity may be changed. So, it is possible to separate analytical from preparatory processes.

• You can use corrosive spray chemicals without harming the plate.

• Requires less time, stationary phase, and solvent per separation than column chromatography.

PAPER CHROMATOGRAPHY

Advantages:

The ability to find a chemical after separation is one of the key benefits of paper chromatography. With common reagents, a substance as small as 0.1 g (1 g is 1 millionth of a gram) may be identified.

Disadvantages:

The separation of volatile substances like hydrocarbons and volatile fatty acids cannot be done using paper chromatography methods.

Most compounds have a lower detection limit of 1–5 g.

APPLICATION OF CHROMATOGRAPHY

GAS CHROMATOGRAPHY

- Qualitative analysis
- Quantitative analyisis
- Isolation and identification of 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:

Comparing the retention times of the sample and the standard allows for the identification of a chemical.

Verifying a compound's purity: A compound's purity may be deduced. Additional peaks indicate the presence of impurities, which means the chemical is not pure. From the resultant peaks' perceptible area.

When compared to a reference standard reference material, the existence of extra peaks indicates the presence of contaminants. Peak areas may also be used to compute the impurity percentage.

• Quantitative analysis: Several techniques may be used to determine the amount of a component. Drug detection and isolation using multi-component analysis

• Separation and characterization of mixtures of natural or manufactured components

APPLICATUION OF ION EXCHANGE

Softening of water: Divalent and monovalent ions including sodium, potassium, calcium, and magnesium are removed.

Demineralisation or deionization of water: Demineralized water is obtained by removing certain ions.

removal of ionic contaminants from a solution by purification.

Organic separations: The majority of pharmacological compounds are either highly or moderately acidic or basic. As a result, a mixture of that molecule may be separated using ion exchange resin. Classes of compounds that can be separated include amino acid proteins, antibiotics, vitamins, fatty acids, and others.

APPLICATION PAPER CHROMATOGRAPHY AND THIN LAYER CHROMATOGRAPHY

separation of a drug combination from plant extracts, chemicals, or biological sources, etc.

separation of substances such as amino acids, glycosides, alkaloids, vitamins, and carbohydrates.

Drug identification Impurities' identification

Drug metabolite analysis in blood, urine, etc.

APPLICATION COLUMN CHROMATOGRAPHY

Column chromatography may be used to separate a variety of drug classes and their components, such as alkaloids, glycosides, amino acids, etc.

• Impurities may be eliminated from a substance via the purification process by utilizing the right stationary and mobile phases.

• Isolation of active constituents: from formulation, plant extract, or other crude extracts.

• Isolating metabolites from biological fluids, such as blood, plasma, or serum, for example, 17-ketosteroids from urine, cortisol, other drugs, etc.

• Calculating the amount of medicines in formulations or raw extracts

• Calculating the percentage by weight of strychnine in ferrous phosphate syrup with quinine and strychnine.

• Analyzing the major and secondary glycosides present in digitalis leaf.

• Diastereomer separation.

• The separation of inorganic ions such as nickel, copper, cobalt, etc.

CONCLUSION

The analysis as a whole suggests that HPLC is a flexible, repeatable chromatographic method for the quantification of pharmacological products. In terms of quantitative and qualitative estimate of active compounds, it has several applications in numerous domains.

Separation science has recently paid a lot of attention to the development of analytical techniques for drug identification, purity assessment, and quantification. The development and validation of GC methods are generally covered in this article. It was mentioned how to design a generic, extremely simple GC technique for the separation of chemicals. Chromatography has shown to

play a key part in the process of finding new drugs and developing existing ones. We may infer that chromatographic procedures are essential to the drug discovery process. If a competent chromatographic method is backed by an appropriate detection technology, depending on the type of the analyte, analysis is no longer difficult. The use of selective and specialized chromatographic techniques has reduced the time and expense of drug development from the discovery stage to the manufacturing stage.

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