

A Review on Different Type of Chromatographic Technique

Miss. Vaishnavi Rajesh Dhage, Satish D. Dukare, Dr. Avinash S. Jiddewar

Pragati Raju Gargelwar, Yogesh Prashant Rodge

NSPM College of Pharmacy, Darwha, Yavatmal

Abstract: *These days, chromatographic techniques identify and Quantify the components of the mixture. Every research lab, universities, pharmaceutical firms, and others For separation science, use mobile and stationary phases. The mixture's components are dispersed in a liquid solution called the mobile phase, which interacts with a stationary phase. Chromatography aims to separate components based on their partitioning between these phases, ultimately determining the qualitative and quantitative chemical composition of a sample for purification and extraction. This piece aids the writers in understanding chromatography. Technology that enables drug development is necessary. Scientists precisely investigate drug molecules, accurately and effortlessly. Chromatography makes use of To separate a mixture, use both Chromatographic techniques employed in the creation of drugs. Proper analysis of herbal products requires understanding the chemistry of phytochemicals, including their isolation, structural identification, and quantification. This research addresses Chromatographic techniques, TLC, Gas chromatography, HPTLC and HPLC utilized to examine pharmaceutical products.*

Keywords: TLC (Thin layer chromatography), HPTLC (High performance thin layer Chromatography), Paper Chromatography, HPLC (High performance liquid Chromatography), CC (column chromatography), GC gas chromatography, Ion exchange chromatography, Principal, Advantage, Disadvantages

I. INTRODUCTION

The term "chromatography" comes from Greek terms for write and color. Chromatography is the division of a mixture into separate components with mobile and stationary phases stage. It is a differential-based physical method. pattern of migration^[1]

Chromatography is a physical separation technique that divides elements into two phases: a mobile phase having a stationary phase and flowing in a particular direction. The molecular characteristics linked to this separation process determine its effectiveness. with changes in molecular weight, adsorption (liquid-solid), partition (liquid-solid), or affinity. The mixture's characteristics play a essential part in accomplishing efficient molecular separation. Partition-based chromatography methods are skilled at effectively identifying and separating small molecules such as fatty acids, carbohydrates, and amino acids. In contrast, larger entities Proteins and macromolecules like nucleic acids are more successfully separated using affinity chromatography, also referred to as like ion-exchange chromatography^[2]

The most popular method of separation in labs is chromatography, which can be investigated for purification, isolation, and analysis. Chromatography began as a straight forward method for dividing pigments into intricate processes involved in resolving The most challenging analytical and purification problems in separation science, such as Phytochemistry throughout the previous century.^[3]

Chromatographic methods are quick, easy, and require little equipment. Furthermore, handling complex mixtures is relatively simple.

Chromatographic separation, like fractional distillation or countercurrent distribution, depends on the relative movement of two phases. However, in chromatography, there are two phases: the stationary phase, which is fixed, and the mobile phase, which is mobile. Components of the mixture are transported by the mobile phase at varying speeds in



the direction of the mobile phase flow as it crosses the stationary phase. The components' varying affinities for a stationary phase and a mobile phase cause the components to separate.^[4]

Chromatography is a versatile technique used to separate complex mixtures.

This method works by passing a mixture through two phases: a stationary phase (a solid or liquid coating) and a mobile phase (a liquid or gas). The components in the mixture separate because they interact differently with these two phases. When the mobile phase is a gas, it's called Gas Chromatography (GC), which is perfect for analyzing volatile substances. When the mobile phase is a liquid, it's called Liquid Chromatography (LC), ideal for non-volatile or heat-sensitive materials. Different types of chromatography are suited for specific jobs: Paper chromatography helps study proteins, Gas-Liquid Chromatography separates substances like lipids and alcohols, and using Agarose gel allows for the purification of large particles like DNA, RNA, and viruses. Ultimately, chromatography is used not just for separation, but also for quantitative analysis—determining exactly how much of each component is present.^[5]

The investigation of the compounds known as Phytochemicals found in plants and in charge of several biological processes, including antioxidant, antibacterial, and anti-inflammatory. Phytochemical analysis is the term for these abilities. nutritional supplements, herbal remedies, and other. The term "completed product" refers to goods manufactured from plant materials. Chromatographic techniques are widely used for these products' phytochemical analysis due to their excellent sensitivity, precision, and ability to distinguish complex combinations. The importance of phytochemical analysis, commonly employed chromatographic methods, the associated processes, and This essay discusses both their advantages and disadvantages.^[6]

II. HISTORY

The chromatographic principle was initially discovered by a Russian botanist. Michael Tswett (1906) Used petroleum ether and a glass column of calcium carbonate to extract chlorophyll pigments from Plants. After the pigments resolved into various colored zones according to their adsorption patterns, he Separated and measured them.

Runge, who was fascinated by the nature of inorganic compounds, studied them on filter paper in the Eighteenth century, which marked the beginning of chromatography research. He observed that the Inorganic salts traveled to different degrees after being separated, forming a striking pattern. In 1898, Crude petroleum was pushed through a column of fuller's earth and limestone in the United States. He Saw that light hydrocarbons made up the first section, followed by unsaturated, aromatic hydrocarbons. High molecular weight heterocyclic hydrocarbons with elements such as sulfur and nitrogen.

In 1944, Martin, Consden, and Gorden invented paper chromatography by replacing silica gel columns With strips of filter paper. For this work, they were awarded the Nobel Prize in 1952.

Although Izmailov and Shralber made the initial discovery, Stahl and associates used silica gel on glass plates to advance thin layer chromatography. They demonstrated the efficiency of TLC in separating a Variety of substances. Reversed phase paper chromatography was then developed. Paper is Impregnated with a hydrophobic liquid in this process, and the mobile phase is an aqueous (or polar) Liquid. This technique is used to separate materials that are poorly soluble in water. One of the most modern and effective chromatographic techniques for examining complicated mixtures Is gas chromatography. In 1952, Martin and James introduced it. The components of the mixture Migrate at different rates when a gas is used as the mobile phase. This method has many advantages Over others in terms of speed, accuracy, sensitivity, and adaptability. Gas chromatography is now widely Used for routine compound separation and identification due to the rapid advancements in its Equipment.

Today's efficient, reliable, and sensitive chromatographic techniques are the result of continuous Improvements in chromatography, especially in methods, materials, and equipment needs. The most Recent development in chromatography is the HPTLC method.^[4]

III. IMPORTANT OF CHROMATOGRAPHY

Organic and other compounds can be separated from the mixture using a variety of techniques, including

- (a) Distillation by fraction
- (b) Crystallization



- (c) extraction
- (d) counter-current distribution
- (e) Distribution of fractions

Above all, techniques are very helpful for many compounds' separation, identification, and purification. However, of all these accessible techniques The following benefits make chromatography much more significant.

- 1) It is a very sensitive, quick, accurate, and gentle method.
- 2) A very small sample size is needed for the entire analysis.
- 3) This technique prevents compound breakdown, which is beneficial, particularly for biological goods.
- 4) Since the analysis is non-destructive, the sample can be recovered following the analysis.
- 5) Chemistry, biology, dyes, medicine, forensics, and other fields can all benefit from chromatography. Preclinical research, etc.

IV. DIFFERENT TYPES OF CHROMATOGRAPHY

- A] Thin layer Chromatography
- B] High performance thin layer Chromatography
- C] Paper chromatography
- D] High performance liquid chromatography
- E] Gas chromatography
- F] Column chromatography
- G] Ion exchange chromatography

A] Thin-layer chromatography (TLC):

Thin-layer chromatography (TLC) is a widely used technique in phytochemical research, which makes it is feasible to distinguish, identify, and sometimes measure the various chemicals present in plant extracts. [7]

TLC Principal :

The principle of separation is adsorption chromatography. One or more compounds are spotted on a thin layer of adsorbent coated on a chromatographic plate. The mobile phase solvent flow through via capillary action. The components move according to their affinity towards adsorbent. [3] The components with more affinity towards the stationary phase travels slower. The component with lesser affinity towards the stationary phase travels faster. [8]

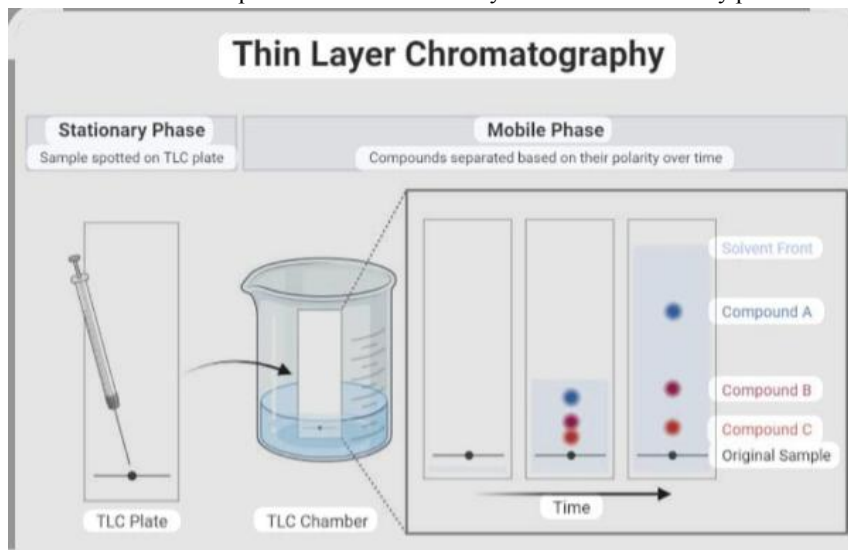


Fig.no.-1 Thin Layer Chromatography



Instrumentation of TLC:

- Selection of Plate
- Adsorbant
- Activation of Adsorbant
- Application of Sample
- Selection of mobile Phase
- Development of Chamber
- Development Technique
- Detecting Reagent
- Evaluation of Chromatogram

Advantages of TLC:

- Low Cost & Easy: The equipment is cheap, and the method is simple to perform.
- Very Fast: It's a rapid technique, much quicker than column chromatography.
- Small Samples: You only need milligrams (mg) of a substance to get a separation.
- Universal: It can analyze almost any kind of chemical compound.
- High Efficiency: It uses very fine powder (small particles) on the plate, which leads to excellent, clean separation of compounds.
- Easy Detection: Finding the separated spots is quick and straightforward.
- Versatile Use: You can easily change the layer thickness to use it for simple analysis or for separating and collecting larger amounts of purified substance (preparative use).
- Tough Plates: Strong, corrosive chemicals can be used to visualize the spots without damaging the plate.
- Saves Materials: It requires much less solvent, stationary phase, and time compared to column chromatography.^[9]

Disadvantages of TLC:

- It only applies to components of soluble mixtures.
- It is challenging to replicate the results obtained from TLC.
- Only qualitative analysis, not quantitative analysis, is feasible in TLC.
- The TLC process is not automatic.
- The results obtained from the experiment are hard to replicate.^[9]

B] High Performance Thin layer Chromatography:

Thin-layer chromatography (TLC) is unquestionably significant. There isn't another chromatographic method that makes it possible to display the outcome as an image. Additionally, TLC is the only technique where All of the sample's components are included in the chromatogram. However, not all of the components Because of their selectivity, HPLC and GC show the sample.^[10]

HPTLC principal:

HPTLC and TLC (adsorption chromatography) both employ the same physical principles. In other words, the basic unit of separation is adsorption. The solvent from the mobile phase passes through due to capillary action. Their affinities with the adsorbent indicate that the Parts move. Moving is the element more drawn to the stationary phase. slower. Less affinities for the immobile phase cause the components to move more swiftly. Consequently, the components are separated using a chromatographic plate.



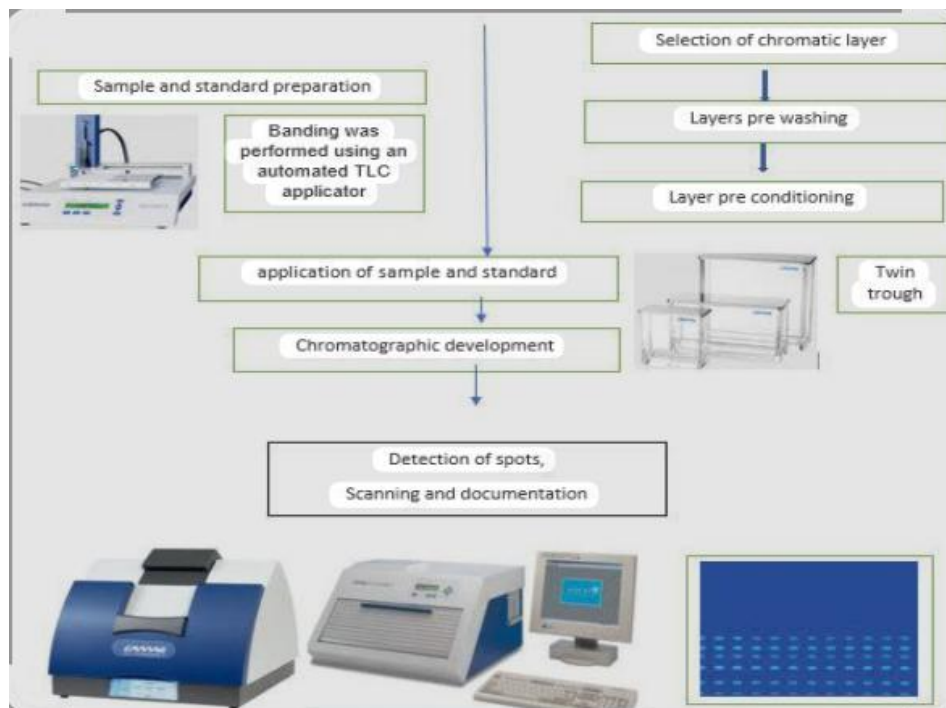


Fig.no.-2. High Performance Thin Layer Chromatography

Instrumentation of HPTLC:

1. Preparation of plates
2. Sample application
3. Chromatogram development
4. Derivatization
5. Chromatogram evaluation
6. Scanning and documentation

Advantage of HPTLC:

1. High Throughput
2. Cost-effective
3. Easy sample preparation
4. Flexibility in visualization

Disadvantages of HPTLC:

1. Reduce sensitivity
2. Revolution
3. qualitative analysis
4. Reproducibility

C] Paper Chromatography:

The paper Chromatography can be described as the method in which the unknown substance's separation is conducted primarily by the solvent flow on filter paper with a unique design. The fundamental idea.^[11]



Paper chromatography Principle:

The separation principle is primarily instead of adsorption, partition. A layer of cellulose in the moisture in the filter paper serves as stationary state. Organic buffers or solvents are utilized as a phase of mobility.^[12]

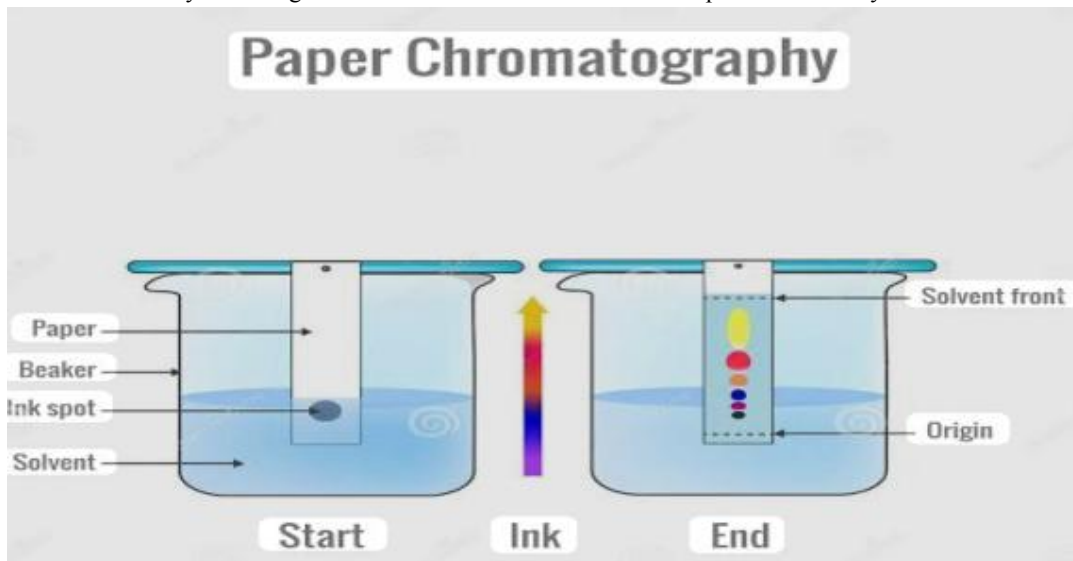


Fig.no.- 3 Paper Chromatography

Instrumentation Of Paper Chromatography:

1. selection of paper (stationary phase)
2. preparation of paper
3. preparation of sample
4. Application of sample
5. Selection of mobile phase
6. development of chromatographic chamber
7. Development techniques
8. Selection of mobile phase
9. Development chromatogram
10. Drying of chromatogram
11. Detection of spot
12. Quantitative analysis

Advantage of paper chromatography:

1. Minimal Sample Requirements: Paper chromatography only needs a tiny quantity of material. making it appropriate for small amounts of material.
2. Cost-Effective: Paper chromatography is comparatively less expensive than other chromatographic techniques. affordable, both in terms of consumables and equipment.
3. Unknown Substance Identification: The method works well for identifying unknown chemical and inorganic materials, offering qualitative insights into the constituents of the sample.
4. Space Efficiency: Compared to many other analytical methods, paper chromatography takes up less space. or tools, allowing it to be utilized in specific laboratory environments.^[13]



Disadvantages of paper chromatography:

1. Techniques for paper chromatography cannot be employed to separate volatile substances. such as volatile fatty acids and hydrocarbons acid.
2. The minimum threshold for identifying the majority of 1–5 μg of compound.^[12]

D] High Performance Liquid Chromatography (HPLC):

Additionally, high-performance liquid chromatography High-Pressure Liquid Chromatography, as it is known, is column chromatography type that is frequently employed in analysis and biochemistry to separate, Determine and measure the active ingredients. It's well-liked analytical method for sorting, recognizing, and measuring every component in a mixture. HPLC is a advanced technology for column liquid chromatography.^[14]

Principle of HPLC:

High-performance liquid chromatography (HPLC) is based on the principle that The sample is into a stationary phase (a column of porous material) and a mobile phase (a liquid phase).is expelled through the column at increased pressure. The adsorption of the solute in the stationary phase has been followed by the separation principle according to its affinity for the stationary phase. The primary application of column chromatography in their The mobile phase was pushed quickly through the column.^[15]

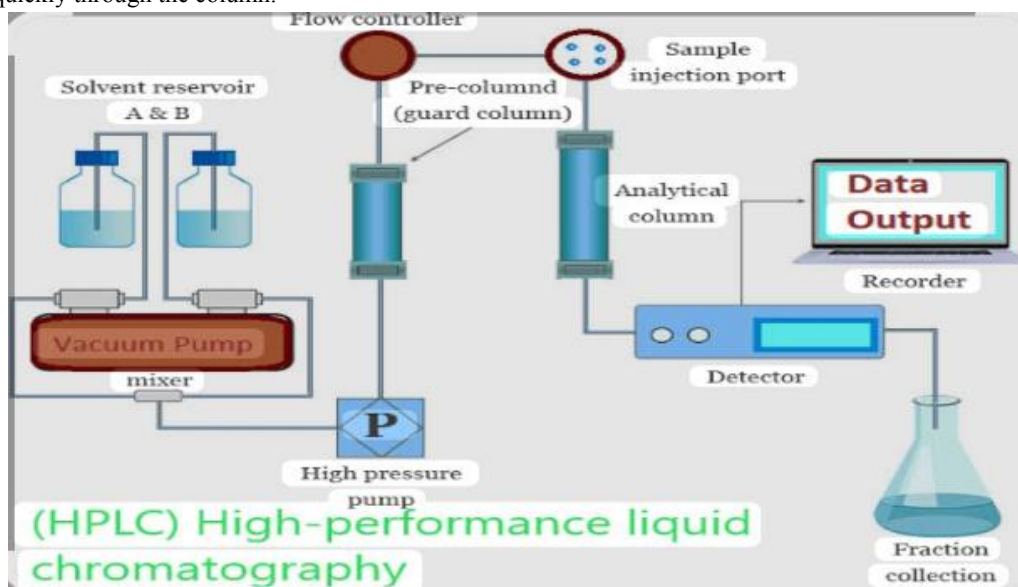


Fig. no.-4 High Performance Liquid Chromatography

Instrumentation of HPLC:

1. Solvent delivery system
2. Degasser (filtration, ultrasonication, Distillation)
3. Pump (Reciprocating pump, displacement pump, pneumatic pump)
4. Pre column
5. Analytical column
6. Detector
7. Recorder

Advantage of HPLC:

1. A small sample with high accuracy is required and specific.
2. Non-damaged sample while in use in contrast to GC.^[12]



Disadvantages of HPLC:

1. To operate the instruments, one must be skilled.
2. Consuming solvents.^[12]

E] Gas chromatography:

Gas chromatography is the process of separation in which the individual component from the mixture is separated by using gas as the mobile phase.^[4]

Principle of Gas chromatography:

The foundation of gas chromatography is the division of the analyte between a mobile phase that is gaseous and immobilized liquid phase on an inert surface strong. The separation of the organic compounds is caused by variations in the way they partition between the stationary phase and the mobile gas phase.^[16] The elements are divided based on their More soluble partition coefficients will The least soluble eluted out first. Gas carrier: ^[11] They serve as the mobile phase. in gas chromatography, where a combination of components that need to be separated are combined, such as argon, nitrogen, helium, and hydrogen.

Gas Chromatography

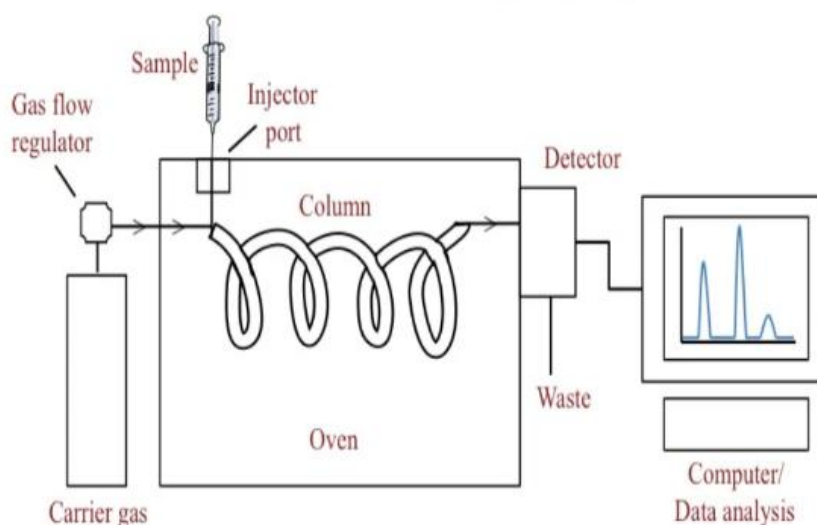


Fig no.-5 Gas Chromatography

Instrumentation of GC:

1. carrier gas
2. Flow rate and pressure
(Flow rate 25-50 ml/min. Pressure 10-50 psi)
3. Sample injector part
4. Column.
5. Stationary phase
6. Detector
(Thermal conductivity detector TCD , electron capture detector ECD ,flame ionization detector FID , flame photometric detector FPD)



Advantages of Gas Chromatography (GC):

1. Complicated mixtures can be broken down into their components because of its extremely high power for resolution.
2. Extremely sensitive to TCD.(thermal conductivity detector)
3. Because it's a micro method, even a tiny sample size is enough.
4. Analysis can be completed quickly because gas is a moving phase balance.
5. Quite good accuracy and precision.
6. Analysis, both qualitative and quantitative.^[17]

Disadvantages of GC:

1. GC's primary drawback is that only thermally stable and unpredictable composites can be Gas chromatography was used for separation.
2. Because the hydrogen gas used to make the honey is mostly ignitable, caution should be used when making use of it.
3. The sample's individual components cannot be recovered.^[18]

F] Column chromatography:

Led column chromatography is a type of chromatographic separation that is performed in a long tube that is filled with stationary material. In this technique, the mixture is poured at the top of a column filled with glass tubes to perform the separation. mobile Phase and stationary phase are permitted to flow through the column. The mixture's constituent parts separate as a result of distinct mixture components with varying affinities for the stationary and mobile phases, which Mature travel components move in the column at varying speeds.^[19]

Principle of CC:

Column chromatography is a clever separation technique that works by exploiting the different "preferences" components have for two different materials: the stationary phase (the packing material in the column) and the mobile phase (the solvent that moves through it). Think of it like a race: components that stick more strongly to the stationary phase move slower, while those that prefer the mobile solvent move faster. As the solvent carries the mixture down the column, the components are separated into distinct bands or zones based on these varying affinities, a process called selective adsorption. This difference in travel speed ensures each substance is collected individually, successfully separating the original mixture.^[19]

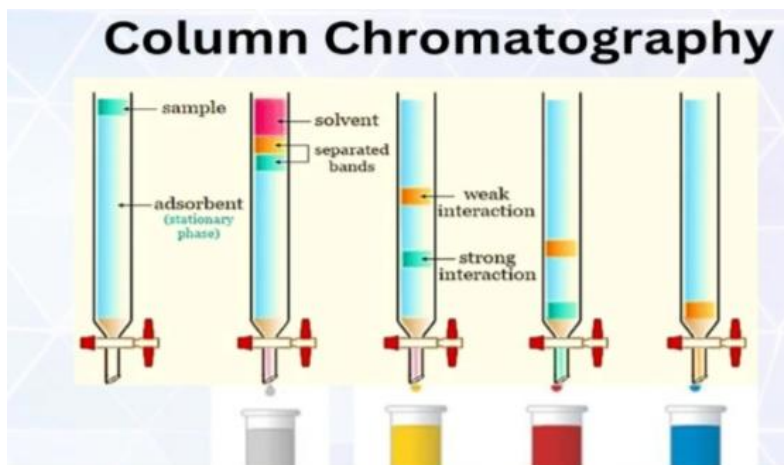


Fig.no.6- Column Chromatography

Instrumentation of CC:

- 1.Chromatographic Column (Apparatus)
- 2.Stationary Phase (Adsorbents)

Copyright to IJAR SCT
www.ijarsct.co.in



DOI: 10.48175/568



3. Preparation of column
4. Solvent Used (Mobile phase)
5. Development of Column
6. Detectors
(flame ionization detector, conductivity detector, optical detector)
7. Method of introducing the sample
8. Analysis

Advantage of CC:

1. All types of complex mixtures can be separated using column chromatography.
2. Any quantity of the blend can be separated using chromatography in columns,
3. A variety of mobile stages.
4. In preparative type chromatography, analytes can be separated and repurposed.
5. Automation may be able to be implemented.
6. This approach is reliable.^[21]

Disadvantages of CC:

1. The compounds take longer to separate.
2. In comparison to advancement, column chromatography has a low separation power.
3. More costly solvents are required in larger quantities.
4. Automation increases complexity and expense.^[21]

G] Ion exchange chromatography:

This procedure makes it possible to separate polar molecules and ions according to their propensity to the exchanger of ions. It can be used on nearly any type of charged molecule, such as big proteins, tiny amino acids and nucleotides. Anions and cations can be divided using this technique.^[21]

Principle of ion exchange chromatography:

The reversible process causes the separations. inter-ion exchange between the ions in the ion exchange.^[22]

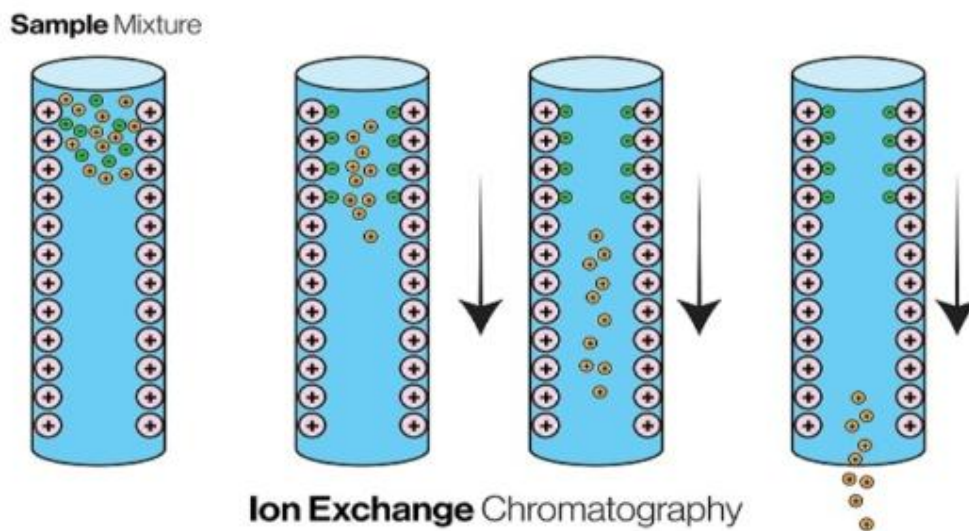


Fig.no.7- Ion Exchange Chromatography



Instrumentation of ion exchange chromatography:

1. Column (Glass, stainless steel)
- 2.type of ion exchange resin
3. Mobile phase
- 4.packaging of column
5. Application of sample
- 6.Elution
7. Analysis of elute

Advantage of ion exchange chromatography:

1. It is among the best techniques for separating charged particles.
2. It works with practically all charged molecules, including big amino acids, proteins, and tiny nucleotides.
3. Ion exchange serves both preparatory and analytical functions in the laboratory, with analytical applications being more prevalent.
4. Ion-exchange chromatography is another method for separating inorganic ions.

Disadvantages of ion exchange chromatography:

1. Separation is only possible for charged molecules.
2. Buffer requirement.

V. CONCLUSION

- 1.In this review, we look at the drug development procedure that is founded on the methods of analysis. These days, Chromatography is acknowledged as a very method of separation that is both sensitive and efficient. Regarding the term of technological developments, chromatography is regarded as one of the most significant developments in the previous few days. The HPLC procedure is limited to analysts, but is currently extensively carried out by biologists, chemists, students, workers in production, as well as additional research and quality control labs.
2. The definition, principle, advantage, and disadvantage of chromatographic techniques are the main topics of the review. Chromatographic methods enhance instrumentation and chemistry productivity by providing additional details because enhanced robustness, dependability, and productivity, sensitivity, speed, and resolution. The amount of time There is a significant reduction in the need to refine new methods.
- 3.Chromatographic techniques are essential for guaranteeing the effectiveness, security, and caliber of phytochemical analyses of herbal products. goods. Methods such as TLC, HPLC, GC, and HPTLC provide accurate, sensitive, and efficient methods for separating, identifying, and quantifying bioactive compounds.

REFERENCES

- [1]. Rajput JM, Nandre DS, Pawar BG. A comprehensive review on advanced chromatographic techniques and spectroscopic techniques in pharmaceutical analysis. Int. J. Pharm. Res. Appl. 2022;7:53-62.
- [2]. Coskun O. Separation techniques: chromatography. Northern clinics of Istanbul. 2016 Nov 11;3(2):156.
- [3]. Marston A. Role of advances in chromatographic techniques in phytochemistry. Phytochemistry. 2007 Nov 1;68(22-24): 2786-98.
- [4]. Kasture AV. Pharmaceutical Analysis Vol.-Ii. Pragati Books Pvt. Ltd.; 2008 Sep 7
- [5]. Hostettmann K, Hostettmann M, Marston A. Preparative chromatography techniques. Applications in Natural Product Isolation. 1986.
- [6]. Vagare RD, Mane SR, Bais SK. Review on phytochemical analysis of finished product by chromatographic techniques. Int. J. Pharm. Herbal. Technol. 2025;3:2583-8962.
- [7]. de Souza JA, da Silva WA, Bezerra IC, Ferreira MR, Soares LA. Chemical profiles by thin-layer chromatography and high-performance liquid chromatography of plant species from Northeast Brazil. Pharmacognosy Magazine. 2018;14(56).



- [8]. Hongbao M. Wikipedia: The free encyclopedia. Nature and Science. 2006;4(2):79-91.
- [9]. Sonawane GS, Lonare RK, Kumbhar AD, Bagal RR, Kulkarni K. A REVIEW ON THIN LAYER CHROMATOGRAPHY: PRINCIPLES, APPLICATIONS AND ADVANCES.
10. Vagare RD, Mane SR, Bais SK. Review on phytochemical analysis of finished product by chromatographic techniques. Int. J. Pharm. Herbal. Technol. 2025;3:2583-8962.
- [10]. Rajput JM, Nandre DS, Pawar BG. A comprehensive review on advanced chromatographic techniques and spectroscopic techniques in pharmaceutical analysis. Int. J. Pharm. Res. Appl. 2022;7:53-62.
- [11]. Luxminarayan L, Sharma N, Viswas A, Khinchi MP. A review on chromatography techniques. Asian Journal of Pharmaceutical Research and Development. 2017 Mar 1:1-08.
- [12]. Zweig G, Sherma J. Paper chromatography—past, present and future. Journal of Chromatographic Science. 1973 Jun 1;11(6):279-83.
- [13]. Supriya K. A review on experimental designs in hplc method development and validation. Journal of Innovations in Applied Pharmaceutical Science (JIAPS). 2025 Feb 14:7-11.
- [14]. Shastak Y, Pelletier W, Kuntz A. Insights into analytical precision: understanding the factors influencing accurate vitamin A determination in various samples. Analytica. 2024 Feb 2;5(1):54-73.
- [15]. Dongarwar AS. PHARMACEUTICAL ANALYSIS: ADVANCED CHROMATOGRAPHIC AND SPECTROSCOPIC METHODS.
- [16]. Priyadarshini R, Raj G, Shewade D. Chromatography—The essence of bioanalysis. Eur. J. Biomed. Pharm. Sci. 2016;3(1):366-77.
- [17]. Brännhammar C, Främme M. Identification and Categorisation of Toxic Chemical Gases in Shipping Containers.
- [18]. Rigaud J, Escribano-Bailon MT, Prieur C, Souquet JM, Cheynier V. Normal-phase high-performance liquid chromatographic separation of procyanidins from cacao beans and grape seeds. Journal of Chromatography A. 1993 Nov 19;654(2):255-60.
- [19]. Iram S, Nuthana Y, Shireesha M, Teja KS, Chary SS. A new analytical novel RP-HPLC method development and validation for the quantitative determination of Dasatinib in pure form and marketed pharmaceutical dosage form.
- [20]. Karlsson E, Rydén L, Brewer JO. Ion exchange chromatography. Wiley-VCH; 1998.
- [21]. Yang H, Viera C, Fischer J, Etzel MR. Purification of a large protein using ion-exchange membranes. Industrial & engineering chemistry research. 2002 Mar 20;41(6):1597-602.

