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A REVIEW ON CHROMATOGRAPHIC TECHNIQUES AND THEIR APPLICATION IN ANALYSIS OF PHARMACEUTICALS

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ABSTRACT:

Chromatography is a technique used for separating components of a mixture. This technique was first used by Tswet in 1890 for the separation of mixture of plant pigments the method used was paper chromatography. Now a day there are many different techniques of chromatography are used for the separation purpose. The separation, analysis and purification of different drugs is possible using chromatography, it requires very less quantity of sample. Chromatographic techniques such as HPLC, HPTLC can be used for analysis of samples including drugs, food, particles, plastics, pesticides, air and water and tissue extract. Chromatographic techniques are highly sensitive, it gives accurate and precise results. This review contains overview on different chromatographic techniques and their applications in analysis of pharmaceutical products.

Keywords: Chromatography, Adsorption Chromatography, Partition Chromatography, TLC, HPTLC, HPLC.

1. INTRODUCTION:

The term "chromatography" finds its roots in two Greek words, "chroma" and "graphein," both meaning "to write." This English word encompasses a method that involves applying a mixture, which requires separation, to a stationary phase (either solid or liquid). A pure solvent, typically water or gas, is then allowed to pass slowly over this stationary phase, separating the mixture's components based on their solubility in the solvent.

Chromatography is a separation process involving the combination of an analyte with a liquid or gaseous mobile phase, pumped through a stationary phase. Usually, there are two phases: a lipophilic (affinity for fats) phase and a hydrophilic (affinity for water) phase. The analyte's components interact differently with each phase, depending on their polarity. This interaction dictates how long they remain engaged with the stationary phase and how much they are slowed down. Consequently, the sample's constituents become separated. The time it takes for each constituent to elute from the stationary phase is known as the retention time (Rt). As the components' signals pass through the detector, they are recorded and plotted to form a chromatogram.

2. HISTORY OF CHROMATOGRAPHY:

1) The credit for the development of the first true chromatography is often attributed to the Russian-Italian botanist Mikhail Tswet.

2) Tswet created new column fractionation techniques in the 1890s for separating petroleum compounds based on his research using filter paper extraction.

3) Tswet used a liquid adsorption column containing calcium carbonate to separate plant pigments, such as xanthophylls, carotenes, and chlorophylls, which were known as yellow, orange, and green pigments at the time [1].

Chromatography has found applications in various branches of the biological and physical sciences. Remarkably, between 1937 and 1972, twelve Nobel Prizes were awarded for research in which chromatography played a major role [2].

3. TYPES OF CHROMATOGRAPHY:

3.1 Adsorption Chromatography

3.2 Partition Chromatography

3.3 Thin Layer Chromatography

3.4 High Performance Thin Layer Chromatography

3.5 Paper Chromatography

3.6 Column Chromatography

3.7 Ion Exchange Chromatography

3.8 High Performance Liquid Chromatography

3.9 Gel Permeation Chromatography

3.10 Gas Chromatography

3.1 Adsorption Chromatography:

Adsorption chromatography is among the earliest chromatographic techniques. It employs a mobile liquid or gaseous phase that has adhered to a stationary solid phase. The separation of different solutes is based on the equilibrium between these mobile and stationary phases. Here, more adsorbent compounds move more slowly, while fewer adsorbent compounds move faster in the stationary phase. Various forms of adsorption chromatography include column chromatography, gas-solid chromatography, thin-layer chromatography, and high-performance liquid chromatography (HPLC) [3].

Applications of Adsorption Chromatography:

1) Separation of amino acids

2) Isolation of antibiotics

3) Identification of carbohydrates

4) Distinction and identification of fatty acids and fats

5) Isolation and identification of peptides and proteins.

3.2 Partition Chromatography:

In partition chromatography, a thin liquid layer forms on the surface of a solid support. Equilibrium is established between the solute's mobile phase and the stationary liquid. Types of partition chromatography include high-performance liquid chromatography, paper chromatography, gas-liquid chromatography, thin-layer chromatography, and partition column chromatography [3].

Applications of Partition Chromatography:

1. Removal of detergents from protein solutions
2. Separation of mycotoxins, bile acids, and steroids
3. Elimination of insecticides, phenols, and pesticides
4. Determination of trace metal concentrations in water-based solutions.

3.3 Thin Layer Chromatography (TLC):

TLC involves separating a mixture into its components using a glass plate coated with a thin layer of an adsorbent, such as silica gel or alumina. The plate, referred to as a chromatographic plate, is used for this purpose. The sample to be separated is applied as a small spot 2 cm above one end of the plate. A liquid eluent is then added to a closed container containing the plate, causing the components of the mixture to rise up the plate and separate at different heights.

Components of Thin Layer Chromatography (TLC):

1) TLC plates: These plates come pre-manufactured with a fixed stationary phase consisting of a thin, uniform layer.

2) TLC chamber: This is where the development of the TLC plate takes place. It maintains a consistent atmosphere for proper spot formation, prevents solvent evaporation, and keeps the process free of dust.

3) Mobile phase: Composed of a solvent or solvent mixture, the mobile phase needs to be pure and free of particles. It should not react chemically with the sample's stationary phase and recommended solvents.

4) Filter paper: Moistened and placed in the chamber after the mobile phase is added, it allows the solvent to climb steadily during the stationary phase.

Instrumentation:

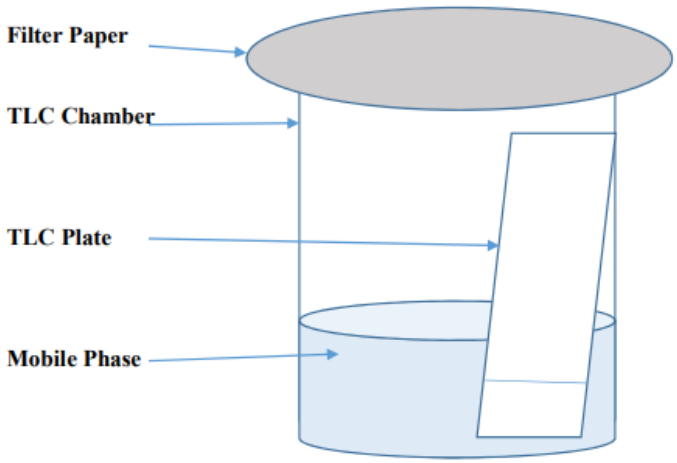

Figure: 1. Thin Layer Chromatography

It is not accurate to assess the amount of a compound solely by comparing the relative intensity of spots in chromatography. To quantify the movement of a compound within a chromatographic medium, we use a parameter called the "ratio to front" or Rf value, which is defined by the following equation:

Rf = (distance from the starting point to the solvent front) / (distance from the starting point to the centre of the separated spot)

It's important to note that numerous experimental factors can influence the Rf value, including the type of chromatography, the solvent system, and environmental conditions. [4].

Applications of Thin Layer Chromatography (TLC):

Monitoring the progress of reactions

1. Identifying components in a mixture
2. Assessing the purity of a substance
3. Analyzing fatty acids and ceramides
4. Detecting pesticides and insecticides in water and food supplies
5. Determining the dye content in fibers for legal purposes [1].
6. Identification of Drugs, Poisons and explosives: The identification of drugs, poisons, and explosives is a crucial aspect of various fields, including forensic science, law enforcement, toxicology, and national security. Several techniques and methods are employed to identify and analyze these substances [4].

3.4 High-Performance Thin Layer Chromatography:

In the ever-evolving realm of chromatographic techniques, HPTLC emerges as a versatile tool for the identification of constituents, the detection and quantification of impurities, and the quantitative determination of active substances. Utilizing modern apparatus like video scanners, densitometers, and advanced chromatographic chambers, along with more efficient elution techniques, high-resolution sorbents with selected

particle sizes, and chemically modified surfaces, HPTLC stands out as a crucial alternative method to HPLC or gas chromatography. The enhanced accuracy, reproducibility, and capacity for result documentation place HPTLC at the forefront, making it the most suitable TLC technique for cGMP compliance. The extensive use of TLC in pharmaceutical analysis is evidenced by the multitude of articles published in this field. HPTLC remains one of the most adaptable, dependable, and cost-effective separation techniques, ideally suited for the analysis of botanicals and herbal drugs. Employed with standardized procedures, it consistently delivers reproducible results, a vital element in the routine identification of complex fingerprints in plant extracts and pharmaceutical products. It has established itself as the method of choice for handling complex analytical tasks related to herbal drugs and botanicals [5].

Application of HPTLC:

1)Numerous pharmacological formulations and dosage forms are examined for purity and effectiveness using HPTLC. For the purpose of simultaneously determining ofloxacin and ornidazole in solid dose form, Puranik et al. devised and validated two straightforward, quick, and accurate chromatographic techniques (HPLC and HPTLC). Ofloxacin and ornidazole were found to have an estimated percentage of label claims of 100.23 and 99.61%, respectively, with mean percent recoveries of 100.47 and 99.32%. It was discovered that these two techniques were easy to use, swift, selective, accurate, and exact. They could also be effectively used to determine the purity of tablets and combinations made in a lab [5].

2) For the examination of celecoxib, etoricoxib, and valdecoxib in pharmaceutical preparations, a reasonably quick, easy, and accurate approach has been developed. The method can be easily applied for the selective examination of medicines, according to Małgorzata Starek et al., and repeatable outcomes are achieved free from interference from other compounds [5].

3) For the purpose of quantifying omeprazole in capsule dose form, a novel HPTLC approach has been devised and validated. The approach verified its accuracy, precision, linearity, specificity, and robustness in compliance with the guidelines set forth by the International Conference on Harmonization. In addition to bulk drug analysis of omeprazole, the suggested method can be utilized for quality control and stability testing of various dosage forms, including tablets and capsules [5].

4) We have developed and confirmed a simple HPTLC method to measure etoricoxib and thiocolchicoside in a combination tablet. The trial employed Nucoxia-MR tablets as the pharmacological dose form. In compliance with the criteria of the International Conference on Harmonization, the approach was validated in terms of linearity, accuracy, precision, and robustness. Drugs in pharmaceutical formulations have been effectively analyzed using this technology [5].

5) The process of identifying a molecule involves comparing the retention times of both the sample and the standard.

6) Verifying the purity of a compound involves assessing the number of peaks present. If more peaks are observed, it indicates the presence of contaminants in the perceived region of the acquired peaks, suggesting that the chemical is not pure [6].

7) When compared to a reference standard or reference material, the presence of additional peaks indicates the existence of contaminants. It is also possible to calculate the percentage of contaminants using peak regions [6].

8) Quantitative analysis includes various methods for determining the quantity of a component, such as multicomponent analysis, drug isolation, and identification [6].

9) The separation and identification of a combination of components, whether of natural or artificial origin [6].

3.5 Paper Chromatography:

Developed by Richard Laurence Millington Synge and Archer John Porter Martin, paper chromatography was a significant breakthrough. It successfully solved the problem of distinguishing highly similar amino acids. This technique typically employs purified paper sheets, which are known to have absorbed water. The choice of paper varies based on the specific research at hand. Different liquids, such as paraffin oil and silicone, are also used. Varieties of chromatographic sheets by Whatman are commonly employed [1].

Principle of Paper Chromatography:

Separation is primarily governed by partitioning rather than adsorption. Substances partition between a mobile phase and a stationary phase. Water occupies the stationary phase within the cellulose layers of the filter paper, while the mobile phase comprises organic solvents and buffers. The developing solution carries the sample up to the stationary phase. The sample components separate based on their solubility in the mobile phase and their affinity for the stationary phase [1].

Steps in Paper Chromatography:

1) Select a sturdy support.
2) Choose the mobile phase.
3) Set up a saturated chamber.
4) Prepare and load a sample.
5) Construct chromatograms.
6) Age the chromatograms.
7) Detection [1].

Applications of Paper Chromatography:

1) Counting the components in a sample using the appropriate mobile phase
2) An economical method that requires minimal setup and a small sample quantity
3) Effectively separating free amino acids in human serum
4) Evaluating reaction kinetics makes it a valuable tool in synthetic chemistry [7].

3.6 Column Chromatography:

Column chromatography is a method employed for the separation of a mixture's components using a column filled with a suitable adsorbent material, typically packed within a glass tube. The mixture is introduced at the top and washed with an appropriate eluent, which gradually descends down the column. The components are separated based on the degree to which they adhere to the column wall. The most strongly adsorbed component remains at the top, while others are displaced to different heights within the column.

Principle Column Chromatography:

When the mixture that needs to be separated and the mobile phase are injected from the top of the column, the various components of the mixture move at different speeds. Components with lower affinity and adsorption to the stationary phase move more quickly than those with higher affinity and adsorption. The elements that move more slowly are separated from the elements that move more swiftly [8].

Application Column Chromatography:

1. Analytical Uses
2. Separation of Diastereomers
3. Separation of Tautomeric Mixtures
4. Separation of Geometrical Isomers
5. Separation of Racemates [9].

3.7 Ion-Exchange Chromatography:

This method capitalizes on the ionic charges found in the twenty common amino acids, which are the building blocks of proteins. Some of these amino acids have positively or negatively charged side groups, also known as "R" groups. The net balance of positive and negative charges helps classify the type of protein. For instance, a protein with more positive than negative charges are called a "basic protein," while a protein with a net charge greater than zero is considered acidic. If a protein primarily carries ionic charges, it can bind to a support with an opposite charge. Ion-exchange chromatography is used to separate chemicals based on their charges, simplifying the identification of different chemical families (acidic, basic, and neutral). This technique is frequently used for protein purification [10].

Applications:

1. Separation of similar ions due to differing exchange reactions
2. Softening hard water by removing divalent ions contributes to its hardness.
3. Purification of organic compounds by eliminating ions
4. Biochemical separations are useful in extracting biochemical substances from biological fluids or identifying specific drugs and metabolites [11].

3.8 High-Performance Liquid Chromatography (HPLC):

HPLC, standing for high-performance liquid chromatography, is a widely used method for pharmaceutical analysis due to its simplicity and versatility. It is a comprehensive process that involves the use of specific equipment known as an HPLC system. HPLC serves various purposes:

1) Recognizing specific components or groups of compounds in a sample, e.g., determining the presence and quantity of diazepam in a blood sample or caffeine in a plant extract.

2) Identifying unknown components in a sample requires the use of detection aids like mass spectrometers. Collecting eluent fractions with the peak of interest for off-line characterization is often essential [12].

Components of an HPLC System:

1. Solvent reservoirs
2. Degasser
3. Low-pressure gradient pumps (LPG)
4. High-pressure pump
5. Pre-column, saturation column or guard column
6. Sampling port
7. The main column
8. Detector
9. Data presentation and analysis method [12].

Figure of HPLC:

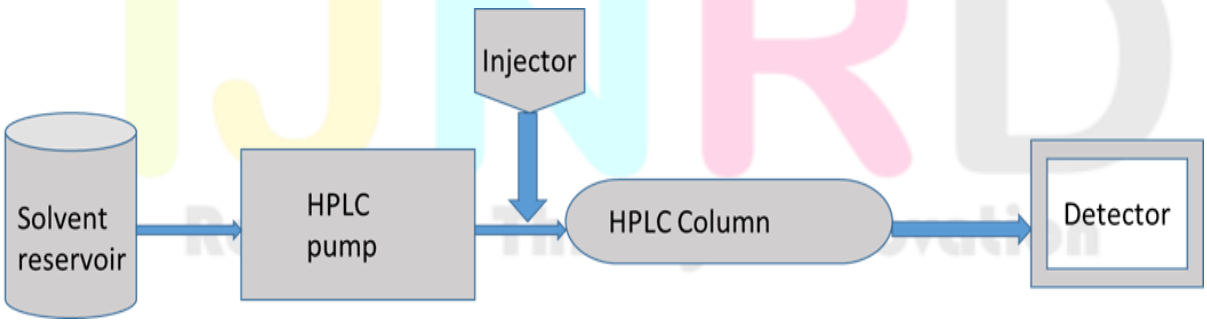

Figure: 2. High Performance Liquid Chromatography (HPLC)

Applications of HPLC:

1. Analyzing dissolving tablets in medicinal dosage forms
2. Regulating medication stability and estimating shelf life
3. Identifying and quantifying the elements in use

4. Monitoring the quality of pharmaceuticals [13].

3.9 Gel Permeation Chromatography (GPC):

Also known as Size Exclusion Chromatography (SEC) or Molecular Sieve Chromatography (MSC), GPC utilizes a porous polymeric matrix composed of spongy particles with pores filled by the mobile phase (gel). The gels, which are polymers, consist of cross-linked, long polymeric chains that form open, three-dimensional networks. The size of the pores depends on the degree of cross-linking and is essential for separation. Larger molecules are excluded from entering the pores, while smaller molecules can partially or fully penetrate them based on their size. The mobile phase used is either water or diluted alcohol, depending on the molecular weights of the solutes. Molecules segregate within and outside of the pores, depending on their size [2].

Applications of GPC:

1. Measuring the weight in grams of peptides, proteins, and polysaccharides
2. Desalinating colloids like albumin are produced with (NH4)2SO4.
3. Separating poly- and monosaccharide mixtures
4. Extracting proteins and peptides from amino acids.
5. Separating soluble RNA and muco-polysaccharides
6. Differentiating between hemoglobin and myoglobin
7. Separating alkaloids and purifying enzymes [2].

3.10 GAS CHROMATOGRAPHY:

A glass or metal column in a temperature-controlled oven, an injector (sample entry point), and a detector with an electronically interfaced recorder or recording device constitute the components of gas-liquid chromatography. The fundamental parts of GLC systems have not changed significantly since the technology's inception [6].

Application of Gas Chromatography:

1)Determination of Fatty Acids by Gas Chromatography: Gas chromatography is a widely used analytical technique in food science, biochemistry, and lipid chemistry, among other domains, for the assessment of fatty acid concentrations. Based on their chemical characteristics, such as volatility and affinity for the stationary phase, individual fatty acids in a mixture can be separated and quantified using gas chromatography (GC) [14].

2) Improve the Effective Analysis of Chemical Density and Precision: To improve the effective analysis of chemical density and precision, you can follow best practices in laboratory procedures, instrumentation, and data analysis [14].

3) Determination of Pahs by Gas Chromatography: Multiple fused aromatic rings are found in a class of chemical molecules known as polycyclic aromatic hydrocarbons, or PAHs. Because of their mutagenic and carcinogenic qualities, PAHs pose a threat to human health and the environment. The presence and concentration of PAHs in a variety of materials, such as food, biological tissues, and environmental samples, can be ascertained using gas chromatography (GC), a widely used analytical method [14].

4) Application of Gas Chromatography in Petrochemical Field: Hydrocarbons and other substances found in crude oil, petroleum products, and other petrochemical processes can be analyzed using gas chromatography (GC), a frequently used analytical technique in the petrochemical business [14].

4 Migration Parameters:

The locations of migrating spots on the chromatography are represented by symbols such as RF, RI, RM, and RC. These parameters are both qualitative and quantitative and help in characterizing substances:

RF (Retention Factor) is the solute's movement concerning the solvent front, given by the formula:

RF = (Distance Traveled by Solute) / (Distance Traveled by Solvent) [7].

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