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CHROMATOGRAPHIC ANALYSIS - A VITAL TOOL FOR SYNTHETIC CHEMISTRY

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ABSTRACT
Chromatography is the separation and identification technique. Structurally or chemically similar components of homogeneous mixture can be separated using this technique. Separation is based upon component's relative ability to adsorb and/or partition between mobile phase and the stationary phase. Depending upon separation principle, geometry of method, mode of chromatography, the technique is classified in various types. Chromatography is very commonly used technique in synthetic chemistry for identifying compounds, determining their purity and following the progress of a reaction by studying the components present; and in separating reaction intermediates. The TLC based chemical screening approach has been developed for the investigation of metabolites from the microbial culture. In the field of synthetic chemistry and drug 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. The current paper attempts to analyze the importance of chromatography in synthetic chemistry.
KEYWORDS- *TLC, HPTLC, Paper Chromatography, Synthetic Chemistry, Drug Discovery.*

1. INTRODUCTION-

There are very few, if any, methods for chemical analysis that are exclusive for a single chemical species. Generally analytical methods are selective for a few species or a class of species. Consequently, the separation of the analyte from potential interference is quite often a rate limiting step in the research. **(1)**
The term ***"chromatography"*** is derived from greek, ***chroma*** meaning "***color"*** and ***graphein*** meaning "***to write***". The chromatographic technique is now widely used for the separation, identification, and determination of the chemical component in complex mixture. it is a separation process applicable to essentially molecular mixture and relies on distribution of mixture between an essentially to dimensional or thin phase and one or more bulk phase which are brought into contact in a differentials counter current manner. Chromatography is a non-destructive procedure for resolving a multi-component mixture of trace, minor, or major constituents into its individual's fraction. Chromatography may be defined as a method of separating a mixture of component into individual component through equilibrium distribution

between two phases the technique of chromatography is based on the differences in the rate at which the component of a mixture move through a porous medium called stationary phase, under the influence of some solvent or gas called moving phases. Chromatography is one of the widely used physiochemical method of separation of inorganic and organic substances related in their composition and properties. in general, chromatography is an effective method of separating element by their non-uniform distribution between a mobile and stationary phase. The mobile phase can be a gas (gas chromatography) or liquid (liquid chromatography), while the stationary phase can be liquid or a solid. the chromatography method of analysis are classified by different features, namely by the state of aggregation of the phase used, by the nature of sorbate- sorbent interaction (the separation mechanism), by the techniques used. [(2)]

2. CLASSIFICATION OF CHROMATOGRAPHY

Chromatography is the separation and identification technique. Structurally or chemically similar components of homogeneous mixture can be separated using this technique. Separation is based upon component's relative ability to adsorb and/or partition between mobile phase and the stationary phase. Depending upon separation principle, geometry of method, mode of chromatography, the technique is classified in various types.[(3)] Chromatographic techniques can be classified into five types based on the type of equilibrium process. These are

- **Adsorption chromatography**-
  The stationary phase is a solid on which the sample component is adsorbed. The mobile phase may be liquid or gas. The components distribute between the two phases through a combination of sorption and desorption processes. [(4)] Column chromatography is a typical example of adsorption chromatography in which the solid stationary phase is a packed in a column, and mobile phase is allowed to flow through the solid.*(L. R. Snyder and M. A. Stadalius;1983)*
- **Partition chromatography**-
  The stationary phase is a liquid supported on an inert solid. And mobile phase is liquid or gas. Paper chromatography is a type of partition chromatography in which the stationary phase is a layer of water adsorbed on a sheet of paper. [(5)] This favors retention of polar compounds and elution of nonpolar compounds and is called normal-phase chromatography. If a stationary phase is used along with a polar mobile phase, then nonpolar solutes are retained favoring elution of polar solutes. This is called re-versed-phase chromatography. [(6)]
- **Ion exchange chromatography**-
  This technique uses as an ion exchange resins the stationary phase.ion exchange resin is a polymeric matrix with the surface of which ionic functional group, such as carboxylic acid or quaternary amines, have been chemically bonded. The mechanism of separation is based on ion exchange equilibrium.[(7)] As the mobile phase passes over this surface, ionic solutes are retained by forming electrostatic chemical bonds with the functional groups. the mobile phase used in this type are always liquid.
- **Size Exclusion chromatography**-
  In this technique, the stationary phase is a polymeric substance containing numerous pores of molecular dimensions.[(8)] The mobile phase containing analyses as solvated molecules are separated according to their size by their ability to penetrate a sieve like structure (the stationary phase).larger molecules that will not fit into the pore remain in the mobile phase and not retained.[(9)]
- **Affinity chromatography**-
  This technique utilize highly specific interaction between one kind of solute molecules and second molecules covently attached (immobilized) to the stationary phase.the immobilized molecules can be an antibody to a particular proteins.[(10)] When a crude mixture containing a large number of protein is passed through the column, only the protein that react with the antibody is

bound to the column. After washing all the other solutes off the column, the desired proteins is disloadsed from the antibody by changing the P^H or ionic strength.[11]

3. TYPES OF CHROMATOGRAPHY USED IN SYNTHETIC CHEMISTRY

(a) Thin Layer Chromatography (TLC)

The technique of thin layer chromatography closely resembles those of column and paper Chromatography. In thin layer chromatography, partition, however, occur on a layer of finely divided adsorbent, which is supported on a glass plate.[12] This chromatography using thin layers of an adsorbent held on a glass plate or other supporting medium is known as thin layer chromatography.

Feature and applicability of TLC-

All chromatography principles functioning in solid liquid, liquid system are also applicable to thin layer chromatography. adsorption chromatography has widely been used, but ion exchange, partition and reversed phased partition can also be applied in thin layer technique. [13]The choice of the chromatographic principle is determined by the chemical nature of the compounds to be resolved and by the desired pattern of fractionation.

(b) Paper chromatography

This is probably the simplest, type of chromatography, where a drop of a test mixture is placed on a piece of chromatography paper and allowed to dry. The mixture separates as the solvent front advances past the mixture.[14] Separation is the most efficient if the atmosphere is saturated in the solvent vapor. Paper chromatography works by the partition of solutes between water in the paper fibers (stationary phase) and the solvent (mobile phase).[15]

(c) Ion exchange Chromatography

Here stationary phase is ion exchange resin and ions of the opposite charge are electrostatically bound to the surface of the resin (insoluble, high molecular mass solid). When the mobile phase (always a liquid) is passed through the resin, the electrostatically bound ions are released as other ions are bonded preferentially.[16] This technique involves the exchange equilibria between ions in solution and ions of like sign on the resin surface.

(d) Column chromatography

Each compound in a mixture will have a particular solubility in the solvent and a particular tendency to be absorbed by the solid adsorbent.[17] Mostly no two compounds behave exactly alike in these respects. This principle is utilized in column chromatography.

(e) HPTLC Analysis

Standardization manufacturing procedures and suitable analytical tools are required to establish the necessary frame work for quality control in herbals. Among those tools separation techniques include high performance liquid chromatography (HPLC), High Performance Thin Layer Chromatography (HPTLC) and capillary electrophoresis are most widely used methods to establish reference fingerprints of herbals, against which raw material as well as finished products can be assayed.[18] HPTLC also known under the name of planner chromatography is a modern powerful analytical technique with better resolution, performance and reproduct ability superior to classics TLC.[19] Based on the use of HPTLC plates with small particle size and precise instruments for each steps of the chromatographic p[procedure (Sample application, Chromatograph Development and Chromatograph Evaluation).[20] HPTLC provides the means for demanding quantitative determination. Instruments can be easily validated and are fully compliant with GMP. For the analysis of herbals, HPTLC offers a number of advantages. The technique is especially suitable for comparison of samples based on fingerprints. Finger print analysis by HPTLC is one of the most powerful tools to link the botanical identity to the chemical constituent profile of the plant.[21] From constituent profile, a number of marker compounds can be chosen, which might further describe the quality of herb ort the herbal preparation. HPTLC can also be employed for quantitative determination of such marker compounds.

4. APPLICATIONS OF CHRMATOGRAPY IN SNTHETIC CHEMISTRY

Thin-layer chromatography (TLC) is a very commonly used technique in synthetic chemistry for identifying compounds, determining their purity and following the progress of a reaction by studying the components present; and in separating reaction intermediates. The TLC based chemical screening approach has been developed for the investigation of metabolites from the microbial culture. The large numbers of products created by the combinatorial chemistry are then identified by fast LC-MS methods.[22] Almost half of the drugs in use are chiral. Molecules which are revealed from combinatorial chemistry may be mixture of two enantiomers. It is well known that the pharmacological effect is restricted in most of the cases to one of the enantiomers. Only about 25% of drugs are administered as pure enantiomers. There can be qualitative and quantitative differences in the activity of the enantiomers. The pharmacologically inactive enantiomers can show unwanted side effects; in some cases antagonistic and even toxic effects are observed. The enantiomers can differ in absorption, distribution, protein binding and affinity to the receptor. Furthermore, the metabolic pathways can differ. Therefore, the separation of racemic mixtures of intermediate or final products is often required. For enantiomers' separation on analytical scale A great variety of methods based on chiral chromatographic techniques such as HPLC, GC, SFC, TLC have been developed where chiral reagent is added in mobile phase or chiral stationary phase is used.[23] Affinity based chiral separations and the use of affinity chromatography for the study of drug or hormone interactions with binding proteins. Some areas of possible future developments are then considered, such as tandem affinity methods and the use of synthetic dyes, immobilized metal ions, molecular imprints, as affinity ligands for clinical analytes. Ion exchange chromatography has been applied to a variety of organic and biochemical systems, drugs, their metabolites serum, food preservative, vitamin mixtures, and pharmaceutical preparations. Ion exchange is probably the most frequently used chromatographic technique for the separation and purification of proteins, polypeptides, nucleic acids, polynucleotides and other charged biomolecules. [24] The objective of the cleaning validation is to verify the effectiveness of the cleaning procedure for removal of product residues, degradation products, preservatives, excipients, and/or cleaning agents as well as the control of potential microbial contaminants.[25] In addition one need to ensure there is no risk associated with cross-contamination of active ingredients. The analytical method involved in here should be sensitive, specific, fast and accurate to establish the assurance that the equipments used in manufacturing are free of the above unwanted impurity, presence of which may alter the safety and efficacy of the drug product.[26] HPLC, UPLC techniques have established their role in pharmaceutical cleaning validation. IPQC testing are integral part during manufacturing process, which give assurance that any process in manufacturing is running as per the laid standards and will produce the products with predetermined specifications. HPLC, GC, UPLC studies are commonly utilized to check drug release, dissolution testing, content uniformity etc.[27] Identification of leachable is utmost important in pharmaceutical manufacturing. Chromatographic techniques have justified its role in detection of leachable. [28]

5. CONCLUSION

In the field of synthetic chemistry and drug 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.[29] 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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