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Chromatographic Technique

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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 text 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 ceu

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 of the 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. D 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 the General Introduction typical HPLCcolumn 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 mi” [10].of chromatographic bed shape [12,13], (III) Techniques by physical state of mobile phase

Various types of chromatography

Chromatography can be classified by various way 1.on the basis of interaction of solute to the stationary phase 2. On the basis chromatography bed shaped.3. Technique on 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 shaped

- Column chromatography
- Planar chromatography
- Paper chromatography
- Thin layer chromatography
- Displacement chromatography

Tecinques by physical state of Mobile phase

- Gas chromatography
- Liquid chromatography
- Affinity chromatography

Technique of chromatography

HPLC: High performance liquid chromatography

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

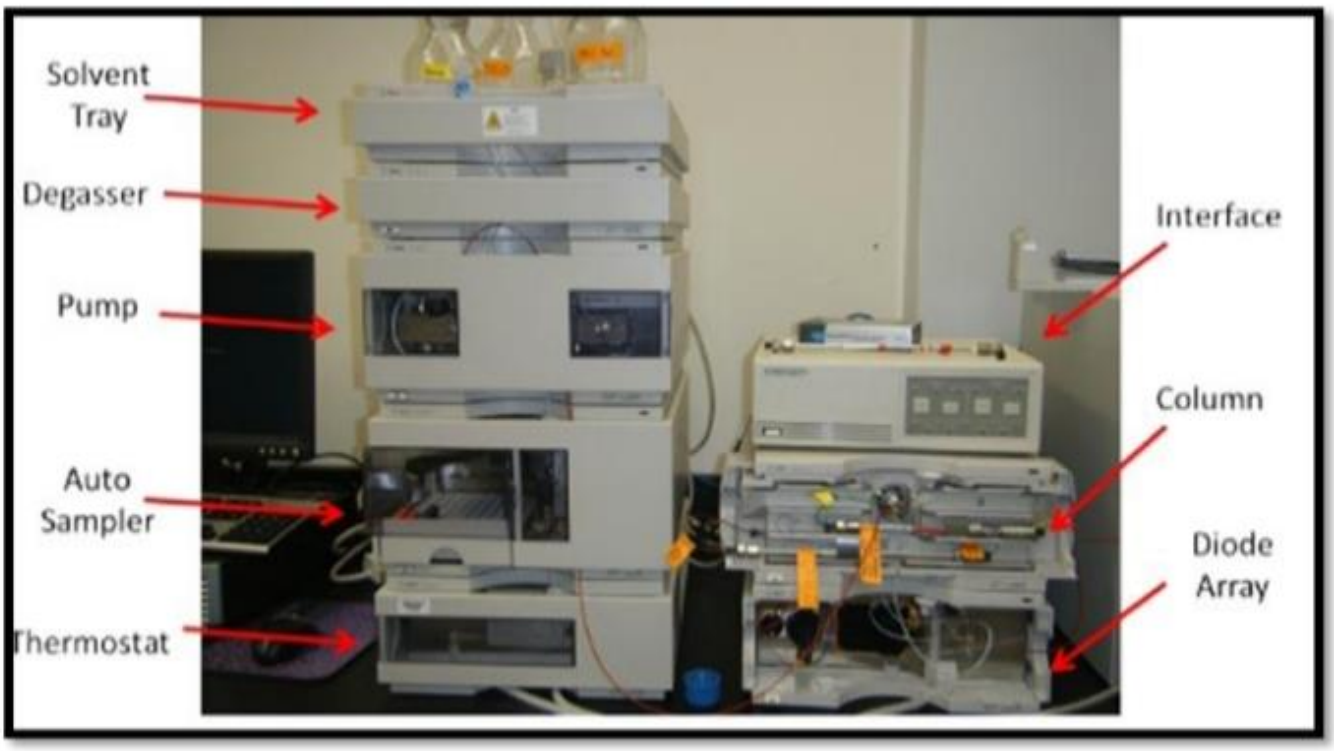

Fig.1 Labeled Agilent 1100 Series HPLC at UAF.

the individual components of the mixture

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-

Gas chromatography

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 reproducible, and rapid. Be controlled,

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 subjectedto 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 compon solvent can be removed by evaporation and the pure components can be fixed .

Application of 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.

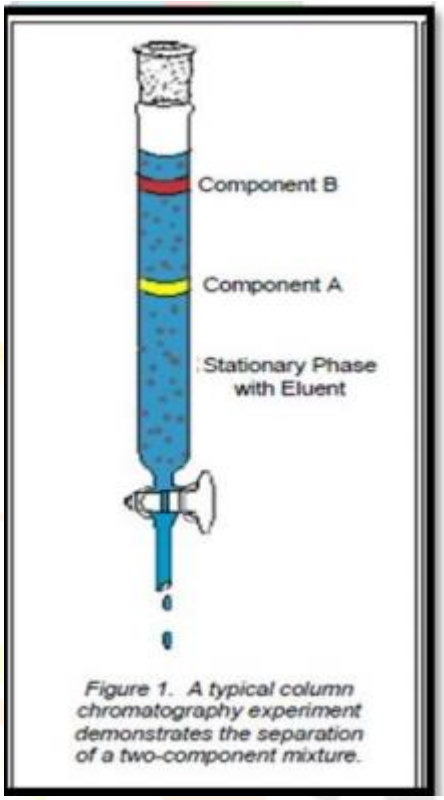

Figure 1. A typical column chromatography experiment demonstrates the separation of a two-component mixture.

• 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 secondaryglycoside in digitalis leaf. And

• Separation of diastereomers.

• Separation of inorganic ions copper,cobalt, nickel,etc. like and

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 adeal 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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