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A Review on High Performance Liquid Chromatography (HPLC)

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Abstract— **The chromatography term is derived from the greek words namely chroma (colour) and graphein (to write). Chromatography is defined as a set of techniques which is used for the separation of constituents in a mixture. This technique involves 2 phases stationary phase and mobile phase. The separation of constituents is based on the difference between partition coefficients of the two phases. The chromatography is very popular technique and it is mostly used analytically. There are different types of chromatographic techniques namely Paper Chromatography, Thin Layer Chromatography (TLC), Gas Chromatography, Liquid Chromatography, Ion exchange Chromatography and lastly High Performance Liquid Chromatography (HPLC). This review mainly based on the HPLC technique its principle, types, instrumentation and applications.**

I INTRODUCTION

High Performance Liquid Chromatography which is also known as High Pressure Liquid Chromatography. It is a popular analytical technique used for the separation, identification and quantification of each constituent of mixture. HPLC is an advanced technique of column liquid chromatography. The solvent usually flows through column with the help of gravity but in HPLC technique the solvent will be forced under high pressure upto 400 atmospheres so that sample can be separated into different constituents with the help of difference in relative affinities [1-7].

In HPLC, pumps will be used to pass pressurized liquid solvent including the sample mixture which is allowed to enter into a column filled with solid adsorbent material. The interaction of each sample component will be varies and this causes difference in flow rates of each component and finally leads to separation of components of column.

Chromatography can be depicted as a mass exchange process including adsorption. HPLC depends on pumps to pass a pressurized fluid and an example blend through a section loaded with adsorbent, prompting the partition of the specimen segments. The dynamic segment of the section, the adsorbent, is regularly a granular material made of solid particles (e.g. silica, polymers, etc.) 2 µm to 50 µm in size. The segments of the example mixture/blend are isolated from each other because of their distinctive degrees of connection with the retentive particles. The pressurized fluid is commonly a blend of solvents (e.g. water, acetonitrile and/or methanol) and is known as 'mobile phase'. Its organization and temperature plays an important part in the partition procedure by affecting the connections occurring between sample segments and adsorbent [8-15].

HPLC is recognized from traditional ("low weight") liquid chromatography because operational pressures are fundamentally higher (50 bar to 350 bar), while normal liquid chromatography regularly depends on the power of gravity to pass the portable stage through the segment. Because of the small sample amount isolated in scientific HPLC, column section measurements are 2.1 mm to 4.6 mm distance across, and 30 mm to 250 mm length. Additionally, HPLC segments are made with smaller sorbent particles (2 µm to 50 µm in normal molecule size). This gives HPLC high determining or resolving power (the capacity to recognize components) while isolating mixtures, which makes it a prominent chromatographic method.

II HISTORY

Liquid chromatographic systems were to an inefficient because of the flow rate of solvents being reliant on gravity. Separations took numerous hours, and some of the time days to finish. Gas chromatography (GC) at the time was more effective than liquid chromatography (LC), in any case, it was trusted that gas stage partition and investigation of extremely polar high atomic weight biopolymers was impossible. GC was ineffectual for some organic chemists due to the thermal instability of the solutes. Accordingly, alternative techniques were hypothesized which would soon bring about the advancement of HPLC.

Taking after on the original work of Martin and Synge in 1941, it was anticipated by Cal Giddings, Josef Huber, and others in the 1960s that LC could be worked in the high-proficiency mode by decreasing the pressing molecule measurement generously beneath the run of the mill LC (and GC) level of 150 µm and utilizing pressure to expand the versatile stage velocity. These expectations experienced broad experimentation and refinement all through the 60s into the 70s. Early developmental exploration started to enhance LC particles, and the innovation of Zipax, an externally permeable molecule, was promising for HPLC technology. The 1970s achieved numerous advancements in equipment and instrumentation. Specialists started utilizing pumps and injectors

to make a simple configuration of a HPLC system. Gas amplifier pumps were perfect since they worked at consistent pressure and did not require release free seals or check valves for steady flow and great quantitation. While instrumentational advancements were important, the historical backdrop of HPLC is principally about the history and development of molecule technology. After the presentation of permeable layer particles, there has been a steady pattern to reduced molecule size to enhance efficiency. However, by decreasing molecule size new issues arrived. The disadvantage from the unnecessary pressure drop is expected to drive versatile liquid through the segment and the trouble of setting up a uniform pressing of to a great degree fine materials. Every time molecule size is diminished altogether, another round of instrument advancement normally should occur to handle the pressure.

III OPERATION

The sample blend to be isolated and dissected is presented, in a discrete little volume (commonly microliters), into the stream of mobile phase permeating through the column. The segments of the sample travel through the segment at various speeds, which are a component of particular physical connections with the adsorbent (likewise called stationary stage). The velocity of every component relies on upon its compound nature, composition of mobile phase. The time at which a particular analyte elutes (rises up out of the column) is called its retention time. The retention time measured under specific conditions is a distinguishing normal for a given analyte.

Various sorts of columns are available, loaded with adsorbents varying in molecule size, and in the nature of their surface ("surface science"). The utilization of small molecule size packing materials requires the utilization of higher operational pressure ("backpressure") and regularly enhances chromatographic resolution (i.e. the degree of division between sequential analytes rising up out of the column). Sorbent particles might be hydrophobic or polar in nature. Basic mobile phases utilized incorporate any miscible mixture of water with different natural solvents (the most widely recognized are acetonitrile and methanol).Some HPLC systems use without water mobile phases. The aqueous segment of the mobile phase may contain acids, (for example, formic, phosphoric or trifluoroacetic corrosive) or salts to help with the seperation of the sample components. The composition of the mobile phase might be kept constant ("isocratic elution mode") or changed ("inclination elution mode") during the chromatographic examination. Isocratic elution is normally successful in the partition of sample components that are not altogether different in their proclivity for the stationary stage. In gradient elution the organization of the mobile phase is fluctuated ordinarily from low to high eluting quality. The eluting quality of the mobile phase is reflected by analyte maintenance times with high eluting quality delivering quick elution.

The selected structure of the mobile phase (additionally called eluent) relies on upon the force of connections between different example parts ("analytes") and stationary stage (e.g. hydrophobic connections in turned around stage HPLC). Dependent upon their partiality for the stationary and mobile stages analytes partition between the two.During the detachment procedure occurring in the sample. This procedure is like what happensamid a liquid–liquid extraction however is continuous, not step-wise. In this case, utilizing a water/acetonitrile angle, more hydrophobic parts will elute (fall off the column) late, once the mobile stage gets more packed in acetonitrile (i.e. in a versatile period of higher eluting quality)

IV INSTRUMENTATION

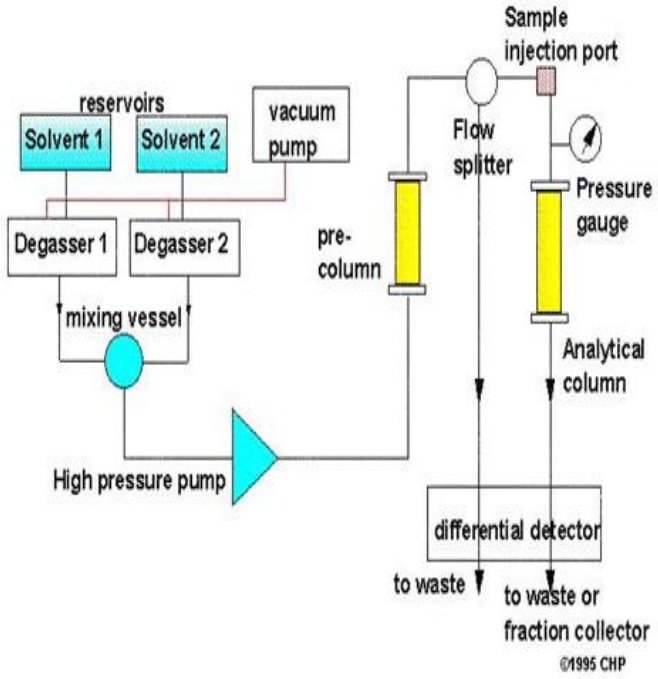

Figure 1 Schematic representation of HPLC

The HPLC instrumentation involves pump, injector, column, detector, integrator and display system. In the column the separation occurs. The parts include:

Solvent Reservoir: The contents of mobile phase are present in glass container. In HPLC the mobile phase or solvent is a mixture of polar and non-polar liquid components. Depending on the composition of sample, the polar and non-polar solvents will be varied.

Pump: The pump suctions the mobile phase from solvent reservoir and forces it to column and then passes todetector. 40000 KPa is the operating pressure of the pump. This operating pressure depends on column dimensions, particle size, flow rate and composition of mobile phase.

Sample Injector: The injector can be a solitary infusion or a computerized infusion framework. An injector for a HPLC framework should give infusion of the fluid specimen inside the scope of 0.1 mL to 100 mL of volume with high reproducibility and under high pressure (up to 4000 psi).

Columns: The heart of the system is the column. In order to achieve high efficiency of separation, the column material (micro-particles, 5-10 µm size) packed in such a way that highest numbers of theoretical plates are possible. Silica (SiO2, H2O) is the most widely used substance for the manufacture of packing materials. It consists of a network of siloxane linkages (Si-O-Si) in a rigid three dimensional structure containing inter connecting pores. Thus a wide range of commercial products is available with surface areas ranging from 100 to 800 m2/g. and particle sizes from 3 to 50 µm.

The silanol groups on the surface of silica give it a polar character, which is exploited in adsorption chromatography using non-polar organic eluents. Silica can be drastically altered by reaction with organochlorosilanes or organoalkoxysilanes giving Si-O-Si-R linkages with the surface. The attachment of hydrocarbon change to silica produces a non-polar surface suitable for reversed phase chromatography where mixtures of water and organic solvents are used as eluents. The most popular material is octadecyl-silica (ODS-Silica), which contains C18 chains, but materials with C2, C6, C8 and C22 chains are also available. During manufacture, such materials may be reacted with a small mono functional silane (e.g. trimethylchlorosilane) to reduce further the number of silanol groups remaining on the surface (end-capping). There is a vast range of materials which have intermediate surface polarities arising from the bonding to silica of other organic compounds which contain groups such as phenyl, nitro, amino and hydroxyl. Strong ion exchangers are also available in which sulphonic acid groups or quaternary ammonium groups are bonded to silica. The useful pH range for columns is 2 to 8, since siloxane linkages are cleaved below pH-2 while at pH values above eight silica may dissolve.

In HPLC, generally two types of columns are used, normal phase columns and reversed phase columns. Using normal phase chromatography, particularly of non-polar and moderately polar drugs can make excellent separation. It was originally believed that separation of compounds in mixture takes place slowly by differential adsorption on a stationary silica phase.While normal phase seems the passage of a relatively non-polar mobile phase over a polar stationary phase, reversed phase chromatography is carried out using a polar mobile phase such as methanol, Acetonitrile, water, buffers etc., over a non-polar stationary phase. Ranges of stationary phases (C18, C8, - NH2, -CN, -phenyl etc.) are available and very selective separations can be achieved. The pH of the mobile phase can be adjusted to suppress the ionization of the drug and thereby increase the retention on the column. For highly ionized drugs ion-pair chromatography is used.

Detector: Solute property detectors respond to a physical property of the solute, which is not exhibited by the pure mobile phase. These detectors measure a property, which is specific to the sample, either with or without the removal of the mobile phase prior to the detection. Solute property detectors which do not require the removal of the mobile phase before detection include spectrophotometric (UV or UV-Vis) detector, fluorescence detectors, polarographic, electro-chemical and radio activity detectors, whilst the moving wire flame ionization detector and electron capture detector both require removal of the mobile phase before detection. UV-Vis and fluorescent detectors are suitable for gradient elution, because many solvents used in HPLC do not absorb to any significant extent

Data Collection Devices or Integrator: Signals from the detector might be gathered on graph recorders or electronic integrators that fluctuate in many-sided quality and in their capacity to process, store and reprocess chromatographic information. The PC coordinates the reaction of the indicator to every part and places it into a chromatograph that is anything but difficult to interpret.

V TYPES OF HPLC

The types of high performance liquid chromatography are often classified by separation mechanism or by the type of stationary phase

- **Partition or liquid-liquid chromatography**.
- **Adsorption or liquid-solid chromatography.**
- **Ion exchange or ion chromatography.**
- **Size exclusion chromatography.**
- **Affinity chromatography.**
- **Chiral chromatography.**

VI APPLICATIONS OF HPLC

The HPLC has several applications in the fields of pharmacy, forensic, environment and clinical. It also helps in the separation and purification of compound .

Pharmaceutical Applications: The pharmaceutical applications include controlling of drug stability, dissolution studies and quality control.

Environmental Applications: Structure elucidation and Monitoring of unknown pollutants and detecting components of drinking water.

Forensic Applications: Analysis of textile dyes, quantification of drugs and steroids in biological samples.

Food and Flavour Applications: Sugar analysis in fruit juices, detecting polycyclic compounds in vegetables, analysis of preservatives.

Clinical Applications: Detecting endogeneous neuropeptides, analysis of biological samples like blood and urine.

VII CONCLUSION

HPLC is an assertive analytical technique with sophisticated technologies that have been extensively practiced from decades. It is having several advantages. With the use of HPLC one can produce extremely pure compounds. It can be used in both laboratory and clinical science. With the use of HPLC the accuracy, precision and specificity can be increased. The practice of HPLC is bygone limited to analyzers, but is now widely performed by students, chemists, biologists, production workers, and other novices in academia, research, and quality control laboratories

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