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A REVIEW ARTICLE ON HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY TECHNIQUE

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Abstract: The High-Performance Liquid Chromatography (HPLC) Technique is useful to distribute the component from the mixture. Chromatography is used in all research laboratories and pharmaceutical industries universally. This review article focuses on the principle, Techniques, and application of HPLC. The objective of this review article shown the different types of techniques involved in HPLC.

***Keywords*:** HPLC, Analysis, Detection, Identification, Quantization, Chromatography.

I. INTRODUCTION:

High-performance liquid chromatography or High-pressure liquid chromatography (HPLC) is the particular form of column chromatography mainly used in biochemistry and analysis to differentiate, identify, and quantify the active compounds. Mikhail Tsvet, a Russian botanist 1930 coined give the name chromatography from the Greek words Chroma means colour, and graphene means to write.
High-performance liquid chromatography (HPLC) is the more accurate analytical method for a long time used for the quantitative or also as a qualitative analysis of drug products. The principle is that a compound of the sample is inserted into the column of a porous material (stationary phase) and a liquid (mobile phase) is blown up at high pressure through the column. Qualitative analysis gives the knowledge about the identity of the sample it is informing about the presence or absence of certain components. A quantitative analysis gives powerful information as the comparative amount of one or more components.

II. PRINCIPLE:

The principle of High-performance liquid chromatography (HPLC) is the component of the sample is inserted into a column of porous material (stationary phase) and the liquid phase (mobile phase) is blown up at higher pressure through the column.

The principle of separation has followed the adsorption of solute in the stationary phase based on its affinity towards the stationary phase. HPLC is the main branch of column chromatography in their mobile phase was forced through the column at high speed.

III. CHROMATOGRAPHIC TECHNIQUES:

1. Normal Phase Chromatography:
2. Reverse-phase chromatography
3. Size Exclusion chromatography
4. Ion Exchange chromatography
5. Bio Affinity
6. Mode of separation

1) Normal phase chromatography:

Normal phase chromatography contains a stationary polar phase and a non-polar mobile phase. In there, the mixture of components to be separated & the analytes are relatively more polar to the polar stationary phase longer than those which are relatively less polar. In this method separation of analytes is based on their polarity.

2) Reverse-phase chromatography:

The Reversed-Phase HPLC (RP-HPLC or RPC) has a non-polar stationary phase and the aqueous, moderately polar mobile phase. In there, the polar solvents eluted first and nonpolar solvents stayed for a longer time. More amounts of drugs and pharmaceuticals are polar, but they are not staying for longer periods and get eluted faster.

3) Size Exclusion chromatography:

Size exclusion chromatography (SEC), also known as gel permeation chromatography or gel filtration chromatography in their mainly separates particles based on size using gels. The columns are packed with materials of controlled pore size, and the particles are separated according to their molecular size. The mechanism of separation is by stearic and diffusion effects. It is also useful for the determination of tertiary structure and quaternary structure of proteins and also useful for the determination of amino acids.

4) Ion Exchange Chromatography:

The principle of separation is ion-exchange chromatography which is the reversible exchange of functional groups. In the Ion-exchange chromatography, the retention depends based on attraction between solute ions and charged sites bound to the stationary phase. This technique is useful almost exclusively with ionic or ionisable samples.

5) Bio Affinity:

The Separation is based upon the specific reversible interaction of proteins with the ligands. It retains the proteins that have interacted with the column-bound ligands. The formation of these complexes

includes the participation of common molecular forces such as the Van der Waals interaction, electrostatic interaction, dipole-dipole interaction, hydrophobic interaction, and the hydrogen bond.

6) Mode Of Separation:

There are two modes of separation in HPLC, based on eluent composition these are:

I) **Isocratic:** Isocratic mode of separation involves the constant eluent composition; that means the equilibrium conditions in the column and the actual velocity of compounds moving through the column are constant.

II) **Gradient:** Gradient mode of separation involves the varying eluent composition. This technique significantly increases the separation power of a system mainly due to increasing the apparent efficiency (decrease of the peak width). The width of the peak depends on the rate of the composition of the eluent.

IV. INSTRUMENTATION :

HPLC equipment consists of nine basic components: mobile phase/solvent container, solvent delivery system, sampling device, chromatographic column, post-column device, detector, data acquisition and output system, and post-detector eluent, processing and pipe and fittings.

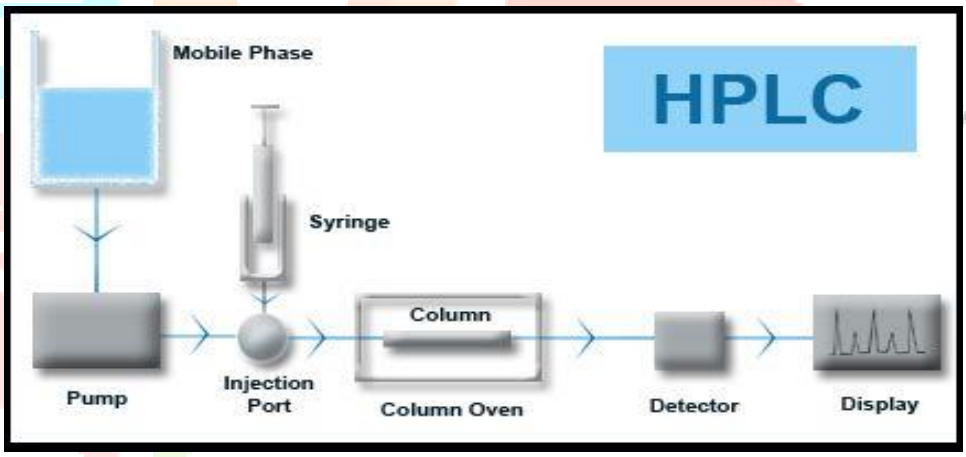

Fig.1: HPLC Instrumentation Overview

1) Mobile phase/solvent reservoir:-

The reservoir holds the solvent, this solvent is referred to as the mobile phase because it moves. Usually, there are a minimum of two reservoirs in the system, in there, each reservoir holds up to 1000 cc of solvent and it is usually fitted with a gas diffuser through which helium can be bubbled. In there the mixture is dissolved in a fluid solvent (gas or liquid) known as the mobile phase, which carries through a system (a column, a capillary tube, a plate, or a sheet) which material known as the stationary phase is fixed.

2) Solvent delivery system:-

In a gradient solvent delivery system, the volume (time) will be required for the gradient to pass through the HPLC system. This is the main volume of the fluid path, there as the solvent flows, from

the point where the A and B solvents first-come to each other until the mixture enters the column. There are two common types of HPLC pumps: the first one reciprocating pumps and the second one a syringe pumps. Reciprocating pumps are the most common pump used and they have a single piston or a dual piston.

3) Sample introduction system:-

The liquid sample is inserted into a stream of solvent (mobile phase) flowing through the column packed with a separation medium (stationary phase). The Sample components are separated from one another by the process of differential migration as they flow through the column. And the injector will be placed next to the pump. The simplest method is to use a syringe, and a sample is introduced to the flow of eluent. Since the precision of LC measurement is more amount of affected by the reproducibility of sample injection, the design of the injector is an important factor. The most widely used injection method is based on sampling loops. A use of autosampler (auto-injector) system is also used far away for allowing repeated injections in a set scheduled timing.

4) Column:-

The Columns are the main component in HPLC because the column is responsible for the separation of sample components. A sample passes through the column with the mobile phase and it separates the components when it comes out from the column. A separation is performed inside the column; that's why it can be said that the column is the heart of an LC system. Recent columns are prepared from stainless steel housing, instead of glass columns used in Tswett's experiment. Packing material is generally used as silica or polymer gels compared to calcium carbonate used by Tswett.

5) Post-column apparatus:-

The Post-column derivatization also called post-column reaction, renders visible certain compounds that are normally invisible. Normally you use the reaction that produces the strong colour or makes a fluorescent product. You can increase the sensitivity of detection by different orders of magnitude in favourable cases. The Most reagents are selective for a particular class of substances, so the analytes of these classes are more easily seen against a complex background. So, the post-column derivatization is used to increase sensitivity and selectivity in HPLC analysis.

6) Detector:-

The chromatography detector is a device used in gas chromatography (GC) or liquid chromatography (LC) to analyse components of a mixture being eluted off the chromatography column. Normally there are two types of detectors these are the first one destructive and the second one non-destructive. The universal detector is defined as the one which 'can respond to every component in the column effluent except the mobile phase'2. In a contrast, selective detectors are defined as 'detectors which respond to a related group of sample components in the column effluent.

i. UV, VIS, and PDA Detectors
ii. Refractive-Index Detector
iii. Evaporative Light Scattering Detector
iv. Multi-Angle Light Scattering Detector
v. Mass Spectrometer
vi. Conductivity Detector
vii. Fluorescence Detector

7) Data collection & output system:-

The Sample is carried by a moving gas stream of Helium or Nitrogen. High-Performance Liquid Chromatography (HPLC) is a form of column chromatography that pumps a sample mixture or analyte in a solvent (known as the mobile phase) at high pressure through the column with chromatographic packing material (stationary phase).

An output or results of an HPLC run is usually viewed as a chromatogram This is a horizontal series of peaks representing compounds eluted from the column with different Rf values.

8) Post detector eluent processing:-

The eluent moves the analytes through the chromatograph. In liquid chromatography, the eluent is a liquid solvent; in gas chromatography, it's a carrier gas. Gradient elution on HPLC refers to a method of changing the composition of the mobile phase while performing chromatography. The High Performance of Liquid Chromatography relies on the pumps to pass a pressurized liquid and the sample mixture through a column filled with the adsorbent, leading to the separation of the sample components. Phases in a reverse chromatography are probably acetonitrile and methanol. Solutes having a wide range of capacity factors are to be separated.

9) Connective tubing & fittings:-

High-performance liquid chromatography" (HPLC) equipment is finding the water pollution, toxic chemicals, and airborne impurities. The Longer lengths of small diameter stainless steel capillary tubing are used to plumb many high-precision systems to increase the sampling efficiencies.

HPLC columns are mainly packed with pellicular or porous particles. The Pellicular particles are made up of polymer or glass beads. The diameter of the beads ranges from 30 to 40 µm. They are surrounded by a thin uniform layer of silica, alumina, or other types of ion-exchange resins.

V. APPLICATION:

• The Pharmaceutical applications

• The Environmental applications

• Forensics

• Clinical

• Food and flavour

•To survey food and drug products

• To identify the confiscated narcotics

VI. CONCLUSION:

It is concluded that HPLC is a multipurpose & repeatable qualitative or quantitative technique used for the evaluation of pharmaceutical and biological samples.

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