A Review on High Performance Liquid Chromatography

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Abstract- *Today HPLC is widely applied for separations and purifications in a variety of areas including pharmaceuticals, biotechnology, environmental, polymer and food industries. It is accomplished by injection of a small amount of liquid sample into a moving stream of liquid (called the mobile phase) that passes through a column packed with particles of the stationary phase. The separation of a mixture into its components depends on different degrees of retention of each component in the column. HPLC is just one type of liquid chromatography, meaning the mobile phase is a liquid. Reversed-phase HPLC is the most common type of HPLC. The reversed-phase means the mobile phase is relatively polar, and the stationary phase is relatively non-polar. HPLC instrumentation includes a Solvent reservoir, pump, injector, column, detector, and integrator or acquisition and display system. The heart of the system is the column where separation occurs. The information that can be obtained using HPLC includes identification, quantification, and resolution of a compound. The major applications are in the area of Pharmaceuticals, food, research, manufacturing, forensics, and bio-monitoring of pollutants*

Keywords- Chromatography, Mobile phase, Stationary phase, Analytic

Aim- Review on High Performance Liquid chromatography.

Objective

1. The purpose high performance liquid chromatography (HPLC) analysis of any drugs is to confirm the identity of a drug and provide quantitative results and also to monitor the progress of the therapy of a disease.
2. Liquid chromatography (LC) separates molecules in a liquid mobile phase using a solid stationary phase.
3. Liquid chromatography can be used for analytical or preparative applications.

I. INTRODUCTION

High Performance Liquid chromatography can be used for analytical or preparative applications.- performance liquid chromatography or commonly known as HPLC, is an analytical technique used to separate, identify or quantify each component in a mixture.

The mixture is separated using the basic principle of column **chromatography** and then identified and quantified by spectroscopy.

In the 1960s, the column chromatography LC with its low-pressure suitable glass columns was further developed to the HPLC with its high-pressure adapted metal columns.

HPLC is thus basically a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres.

High Performance Liquid Chromatography (HPLC) is a form of column chromatography that pumps a sample mixture or analytic in a solvent (known as the mobile phase) at high pressure through a column with chromatographic packing material (stationary phase). The sample is carried by a moving carrier gas stream of helium or nitrogen. HPLC has the ability to separate, and identify compounds that are present in any sample that can be dissolved in a liquid in trace concentrations as low as parts per trillion. Because of this versatility, HPLC is used in a variety of industrial and scientific applications, such as pharmaceutical, environmental, forensics, and chemicals.

Sample retention time will vary depending on the interaction between the stationary phase, the molecules being analyzed, and the solvent, or solvents used. As the sample passes through the column it interacts between the two phases at different rate, primarily due to different polarities in the analytics. Analysts that have the least amount of interaction with the stationary phase or the most amount of interaction with the mobile phase will exit the column faster.

HPLC is an abbreviation for high-performance liquid chromatography. Chromatography refers to the measurement method, chromatogram refers to the measurement results, and chromatograph refers to the instrument. Chromatography

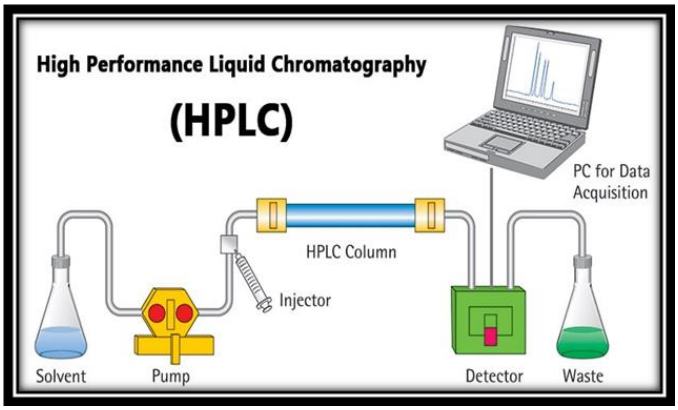
separates components in a particular substance and performs qualitative and quantitative analyses on those components. Qualitative analysis refers to "what kind of compound each component is", and quantitative analysis refers to "how much of each component is present.

HPLC is just one type of liquid chromatography, meaning the mobile phase is a liquid. Reversed-phase HPLC is the most common type of HPLC. HPLC is the most accurate analytical method widely used for the quantitative as well as qualitative analysis of drug products and used for determining drug product stability.

What is HPLC?

HPLC is a technique for separation, identification and quantification of components in a mixture. It is especially suitable for compounds which are not easily volatilized, thermally unstable and have high molecular weights.

Principle of high performance liquid chromatography



- The purification takes place in a separation column between a stationary and a mobile phase.
- The stationary phase is a granular material with very small porous particles in a separation column.
- The mobile phase, on the other hand, is a solvent or solvent mixture which is forced at high pressure through the separation column.
- Via a valve with a connected sample loop, i.e. a small tube or a capillary made of stainless steel, the sample is injected into the mobile phase flow from the pump to the separation column using a syringe.
- Subsequently, the individual components of the sample migrate through the column at different rates because they are retained to a varying degree by interactions with the stationary phase.
- After leaving the column, the individual substances are detected by a suitable detector and passed on as a signal to the HPLC software on the computer.
- At the end of this operation/run, a chromatogram in the HPLC software on the computer is obtained.
- The chromatogram allows the identification and quantification of the different substances.

- **Instrumentation of HPLC consists as follows:**

  - Mobile Phase Reservoir.
  - Degasser.
  - Pump.
  - Sample Injector.
  - Column Heater.
  - Column (Stationary Phase).
  - Detector.
  - Data Analyzers
  - Recorder

Mobile Phase Reservoir

- Mobile phase contents are contained in a glass reservoir.
- The mobile phase, or solvent, in HPLC, is usually a mixture of polar and non-polar liquid components whose respective concentrations are varied depending on the composition of the sample.

Pump

- A pump aspirates the mobile phase from the solvent reservoir and forces it through the system's column and detector.
- Depending on several factors, including column dimensions, the particle size of the stationary phase, the flow rate and composition of the mobile phase, operating pressures of up to 42000 pea (about 6000 psi) can be generated.

Sample Injector

- The injector can be a single injection or an automated injection system.
- An injector for an HPLC system should provide an injection of the liquid sample within the range of 0.1-100 mol of volume with high reproducibility and under high pressure (up to 4000 psi).

Columns

- Columns are usually made of polished stainless steel, are between 50 and 300 mm long, and have an internal diameter between 2 and 5 mm.
- They are commonly filled with a stationary phase with a particle size of 3–10 µm.
- Columns with internal diameters of less than 2 mm are often called micro bore columns.
- Ideally, the temperature of the mobile phase and the column should be kept constant during an analysis.

Detector

- The HPLC detector, located at the end of the column, detects the analysts as they elute from the chromatographic column.
- Commonly used detectors are UV-spectroscopy, fluorescence, mass-spectrometric and electrochemical detectors.

Data analyzer

- Signals from the detector may be collected on chart recorders or electronic integrators that vary in complexity and their ability to process, store and reprocess chromatographic data.
- The computer integrates the detector's response to each component and places it into a chromatograph that is easy to read and interpret.

Recorder

- The change in lucent detected by a detector is in the form of an electronic signal, and thus it is still not visible to our eyes.
- In older days, the pen (paper)-chart recorder was popularly used. Nowadays, a computer-based data processor (integrator) is more common.
- There are various types of data processors; from a simple system consisting of the in-built printer and word processor while those with software that are specifically designed for an LC system which not only data acquisition but features like peak-fitting, baseline correction, automatic concentration calculation, molecular weight determination, etc.

Degasser

- The lucent used for LC analysis may contain gases such as oxygen that are non- visible to our eyes.
- When gas is present in the lucent, this is detected as noise and causes an unstable baseline.
- Degasser uses special polymer membrane tubing to remove gases.

Column Heater

- The LC separation is often largely influenced by the column temperature.
- In order to obtain repeatable results, it is important to keep consistent temperature conditions.
- Also for some analysis, such as sugar and organic acid, better resolutions can be obtained at elevated temperatures (50 to 80°C).
- Thus columns are generally kept inside the column oven (column heater)

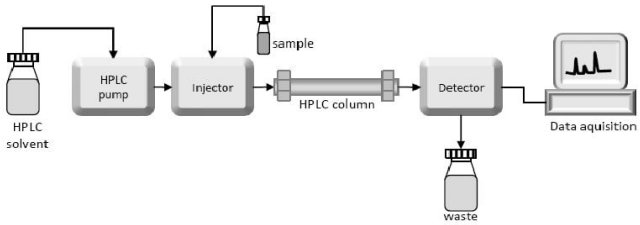


Diagram- instrumentation of HPLC

Different Types of HPLC:-

There are following variations of HPLC, contingent on the stage framework (stationary) in the process:

1. Normal Phase HPLC
2. Reverse Phase HPLC
3. Size-exclusion HPLC
4. Ion-Exchange HPLC

1. Normal Phase HPLC:

This strategy isolates analytic on the premise of extremity. NP-HPLC utilizes polar stationary stage and non-polar portable stage. Subsequently, the stationary stage is generally silica and normal versatile stages are hexane, ethylene chloride, chloroform, diethyl ether, and blends of these. Polar specimens are consequently held on the polar surface of the column pressing longer than less polar materials.

2. Reverse Phase HPLC:

The stationary stage is no polar (hydrophobic) in nature, while the versatile stage is a polar liquid, for example, blends of water and methanol or acetonitrile. It deals with the

rule of hydrophobic collaborations thus the more nonpolar the material is, the more it will be held.

3. Size-exclusion HPLC:

The column is loaded with material having definitely controlled pore sizes, and the particles are isolated by it's their atomic size. Bigger atoms are quickly washed through the column; littler atoms enter inside the permeable of the pressing particles and elute later.

4. Ion-Exchange HPLC:

The stationary stage has an ironically charged surface of inverse charge to the example particles. This strategy is utilized only with ionic specimens. The more grounded the charge on the example, the more grounded it will be pulled in to the ionic surface and along these lines, the more it will take to elute. The portable stage is a fluid cradle, where both pH and ionic quality are utilized to control elution time.

Separation Techniques

Isocratic versus Gradient Elution

Elution techniques are methods of pumping mobile phase through a column. In the isocratic method, the composition of the mobile phase remains constant, whereas in the gradient method the composition changes during the separation process. The isocratic method is the simplest technique and should be the first choice when developing a separation. Fluent gradients are usually generated by combining the pressurized flows from two pumps and changing their individual flow rates with an electronic controller or data system while maintaining the overall flow rate constant.

Derivatization

Derivatization of samples involves a chemical reaction that alters the molecular structure of the analytic of interest to improve detection. In HPLC, derivatization of a drug is usually unnecessary to achieve satisfactory chromatography. Derivatization is used to enhance the sensitivity and selectivity of detection when available detectors are not satisfactory for the underivatized compounds.

Advantage and disadvantages of HPLC

Advantagages

1. Separations are fast and efficient (high-resolution power)
2. Continuous monitoring of the column effluent
3. It can be applied to the separation and analysis of very complex mixtures
4. Accurate quantitative measurements.
5. Repetitive and reproducible analysis using the same column.
6. Adsorption, partition, ion exchange, and exclusion column separations are excellently made
7. HPLC is more versatile than GLC in some respects because it has the advantage of not being restricted to volatile and thermally stable
8. Solute and the choice of mobile and stationary phases is much wider in HPLC.
9. Aqueous and non-aqueous samples can be analyzed with little or no sample pre-treatment.
10. A variety of solvents and column packing are available, providing a high degree of selectivity for specific analyses.
11. It provides a means for the determination of multiple components in a single analysis and etc.

Disadvantages

1. Column performance is very sensitive, which depends on the method of Packing.
2. further, no universal and sensitive detection system is available
3. Very costive, have low sensitivity for certain compounds, and some cannot be detected as they are irreversibly adsorbed.

Application of High Performance Liquid Chromatography

The applications of HPLC encompass a broad range of fields and include testing the quality of products you use every day. HPLC helps keep consumers safe through:

- Impurity analysis in medicine
- Characterizing the quality of ingredients in foods and drinks
- Monitoring the levels of pollutants in water and soil.

Pharma & Biopharma

Pharma

The pharmaceutical industry uses HPLC for research and development, manufacturing quality control, and impurity and degradation analysis to ensure our medications are free of unintended or harmful ingredients.

- **Biopharma**

Biopharma companies use HPLC to characterize and identify molecular targets, screen drug targets, and produce medicine from peptide mapping and sequencing, analyzing antibodies, and purifying the biological actives.

- **Proteomics & metabolomics**

Researchers studying complex proteomics and metabolomics biological samples use nano-, capillary-, and micro-flow liquid chromatography hyphenated with high-resolution accurate-mass or triple quadruple mass spectrometry.

- **Clinical research**

Clinical labs utilize HPLC to analyze complex biological fluids like blood, plasma, and serum, aiding the development of targeted precision medicines.

- **Food & beverage**

HPLC analysis helps ensure foods and beverages are unadulterated and free of harmful toxins and carcinogens by detecting residual pesticides and verifying the purity and authenticity of ingredients.

Environmental

Environmental companies use HPLC to identify, monitor, and quantify metal-free organic-based pollutants in water and soil.

Forensics & toxicology

Forensic and toxicology labs depend on HPLC analysis to screen, quantify, and confirm drugs associated with criminal investigations and drug screenings.

Analytical method validation

Method validation is documented evidence which provides a high degree of assurance for a specific method that the process used to confirm the analytical process is suitable for its intended use.Method Valid

1. Linearity
2. Accuracy
3. Precision
4. Specificity
5. Range

1. Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

2. Accuracy

Accuracy is measured by spiking the sample matrix of interest with a known concentration of analyte standard and analyzing the sample using the "method being validated.

3. Precision

Precision of a method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings. Precision is measured by injecting a series of standards or analyzing series of samples from multiple samplings from a homogeneous lot.

4. Specificity

Specificity is the ability to assess unequivocally the analyzed in the presence of components which may be expected to be present. Typically these might include impurities, degredents, matrix, etc.

5. Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyzed in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

II. CONCLUSION

High Performance Liquid Chromatography is one of the most widely used analytical techniques. Using this technique, it is possible to produce very pure compounds. HPLC is useful both at the laboratory as well as clinical level and provides accurate, precise results with increased specificity. In this paper, the authors have tried to conclude that HPLC is a reproducible and versatile chromatographic method for analyzing drug products, having a wide range of applications in both qualitative and quantitative estimation of various biological and drug molecules. Along with this, a number of patents and research have also proved the applicability of the HPLC technique in the healthcare sector in various areas, which paves the path for many successful prospects of this analysis technique.

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