A Short Review of High-Performance Liquid Chromatography

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Article Info
Page Number: 410-414
Publication Issue:
Vol. 71 No. 2 (2022)

Article History
Article Received: *24 January 2022*
Revised: *26 February 2022*
Accepted: *18 March 2022*
Publication: *20 April 2022*

Abstract
High performance liquid chromatography (HPLC) is a critical qualitative and quantitative technique used to infer pharmaceutical and biological samples. It is the most versatile, safest, responsible, and fastest chromatographic technique for drug component quality control. This article was written with the intention of reviewing various aspects of HPLC, such as the principle, types, instrumentation, and application
Keywords**:** High performance liquid chromatography, instrumentation, elution, applications, mobile phase.

Introduction

High-performance liquid chromatography (HPLC) is a type of column chromatography that is used in biochemistry and analysis to separate, identify, and quantify active compounds. [1] HPLC primarily employs a column that contains packing material (stationary phase), a pump that moves the mobile phase(s) through the column, and a detector that displays the molecule retention times. The retention time depends on the interactions between the stationary phase, the molecules being analysed, and the solvent(s). [2] The sample to be analysed is added in small quantities to the mobile phase stream and is slowed down by specific chemical or physical interactions with the stationary phase. The amount of retardation depends on the nature and composition of the analyte.

Types Of HPLC

HPLC types are generally determined by the phase system used in the process. The following HPLC types are commonly used in analysis.

Normal phase chromatography

This method, also known as normal phase HPLC (NP-HPLC), separates analytes based on polarity. A polar stationary phase and a non-polar mobile phase are used in NP-HPLC. The polar stationary phase interacted with and retained the polar analyte. Adsorption strengths increase as analyte polarity increases, and the interaction between the polar analyte and the polar stationary phase prolongs elution time.

Reversed phase chromatography

Reversed phase HPLC (RP-HPLC or RPC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. RPC works on the basis of hydrophobic interactions caused

by repulsive forces between a polar eluent, a relatively non-polar analyte, and a non-polar stationary phase. The analyte's binding to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand in the aqueous eluent.

Size exclusion chromatography

Size exclusion chromatography (SEC), also known as gel permeation chromatography or gel filtration chromatography, separates particles based on their size. It is also useful for determining the tertiary and quaternary structures of proteins and amino acids. This technique is commonly used to determine the molecular weight of polysaccharides.

Ion exchange chromatography

Retention in ion- exchange chromatography is based on the attraction of solute ions to charged sites bound to the stationary phase. Ions with the same charge are excluded. This type of chromatography is widely used in water purification, Ligand-exchange chromatography, Ion-exchange chromatography of proteins, High-pH anion-exchange chromatography of carbohydrates and oligosaccharides, and so on.

Bio-affinity chromatography

Separation based on the specific reversible interaction of proteins with ligands. Ligands are covalently attached to solid support on a bio-affinity matrix, which retains proteins that interact with the column-bound ligands.

Proteins bound to a bioaffinity column can be eluted in two ways:

• Biospecific elution: inclusion of free ligand in elution buffer to compete with column bound ligand.

• Aspecific elution: a change in pH, salt, or other factors that weaken the interaction of the protein with the column-bound substrate.

Because of the specificity of the interaction, bioaffinity chromatography can achieve very high purification in a single step (10 - 1000-fold).

Parameters

Some parameters are used as a standard for a specific compound for accurate analysis. If the parameters change, the outcome may be greatly influenced. Internal diameter, particle size, pore size, and pump pressure are the most frequently used parameters. The parameters for different compounds can be changed depending on their nature and chemical properties.

Internal diameter

An HPLC column's internal diameter (ID), which also affects sensitivity, is a crucial factor in determining how much analyte can be placed onto the column. Larger columns are typically found in industrial settings, such as when a medicine product is being purified for future use. Low ID columns have reduced solvent use and increased sensitivity at the cost of loading capacity.

Particle size

The internal diameter (ID) of an HPLC column, which also affects sensitivity, has a significant impact on how much analyte can be placed onto it. Larger columns are typically seen in industrial settings, such as when a pharmaceutical product is being purified for future use. Low ID columns feature increased sensitivity and lessened solvent use at the expense of

loading capacity.

Pore size

To give a larger surface area, many stationary phases are porous. Bigger holes have superior kinetics, particularly for larger analytes, while smaller pores offer more surface area. The size of the pores determines how well analyte molecules can interact with the interior surface of the particle and penetrate into it. Given that the outer particle surface and its inner surface are around 1:1000, this is particularly significant. Specifically on the inner particle surface does the surface molecular interaction take place.

Pump pressure

Pumps come in a variety of pressure capacities, but the ability to produce a steady flow rate is the key performance indicator. In order to operate at significantly greater pressures, modern HPLC systems have been enhanced, allowing them to use columns with particles as small as 2 micrometres.

Instrumentation

***Injection of the sample*:** There are septum injectors on hand for injecting sample solution. When the mobile phase is flowing or halted, a sample injection can be made. Reproducible outcomes can be obtained by using a brand-new, high-tech rotary valve and loop injector.

The detector: The passage of a substance through the column can be determined in a number of ways. Typically, UV spectroscopy is used to detect the particular chemicals. Numerous organic substances absorb UV light of different wavelengths. The amount of a certain substance that is now travelling through the beam will determine how much light is absorbed.

Interpreting the output from the detector:

Each peak in the output, which is recorded as a series of spikes, represents a component of th e mixture that passed through the detector and absorbed UV light.

The amount of substance that passes through the detector is proportional to the area under the peak, and the computer connected to the display can automatically calculate this area.

Applications

A compound's identification, quantity, and resolution can all be learned through HPLC. The term "preparative HPLC" describes the separation and purification of chemicals. The goal of analytical HPLC, in contrast, is to learn more about the sample substance.

Chemical Separations: The extent or degree of separation is mostly influenced by the choice of stationary phase and mobile phase because various chemicals migrate at different rates depending on the column and mobile phase used.

Purification: Purification is the process of removing the desired component from a mixture of unwanted substances. Under specific chromatographic circumstances, each component displayed a distinctive peak. In order to collect or extract the pure target component without introducing any contaminants, the migration of the compounds and contaminants through the column needs to differ sufficiently from one another.

Identification: In general, HPLC is used for compound assay. The assay's settings should allow for a clear peak of the known sample to be seen on the chromatograph. At the detection

levels where the test will be run, the identifying peak should have a respectable retention period and be clearly distinguished from unrelated peaks.***Other applications of HPLC*:** Other applications of HPLC includes

Pharmaceutical applications [5-8]

- A study of how pharmaceutical dose forms dissolve in tablets.
- Determining the pharmaceutical items' shelf lives
- Labeling of dosage forms' active components
- Control of pharmaceutical quality

Environmental applications [9-12]

- Phenolic compound detection in drinking water
- diphenhydramine detection in sedimented samples; and pollutant bio-monitoring

Forensics *[13-15]*

- Measuring the drug's effect in biological samples.
- Finding anabolic steroids in urine, perspiration, serum, and hair
- Investigative study of textile dyes.
- Blood tests to check for cocaine and its metabolites

Clinical [16-19]

- Measuring the ions in human urine Antibiotics in blood plasma are examined.
- Calculating blood plasma levels of bilirubin and bilivirdin in cases of hepatic diseases.
- Finding endogenous neuropeptides in the brain's extracellular fluids

Food and Flavor [20]

- Monitoring the quality of water and soft drinks;
- Beer analysis.
- A study of sugar in fruit juices.
- Vegetable polycyclic compound analysis.
- Military high explosive traces on agricultural crops

Conclusion

The analysis as a whole suggests that HPLC is a flexible, repeatable chromatographic method for the quantification of pharmacological products. In terms of quantitative and qualitative estimation of active compounds, it has numerous applications in numerous domains.

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