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An Overview of High-Performance Liquid Chromatography (HPLC)

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Introduction

HPLC (high-Performance Liquid Chromatography), formerly known as high-pressure liquid chromatography, is an analytical chemistry technique for separating, identifying, and quantifying each component in a mixture. Pumps are used to move a pressured liquid solvent containing the sample combination through a solid adsorbent material-filled column. Each component in the sample interacts with the adsorbent material in a slightly different way, resulting in varying flow rates and separation of the components as they flow out of the column. HPLC has been used for manufacturing, legal (e.g., identifying performance enhancement drugs in urine), research (e.g., separating the components of a complicated biological sample, or of comparable synthetic substances from each other), and medicinal (e.g., detecting vitamin D levels in blood serum) applications [1].

Description

Chromatography can be defined as an adsorption-based mass transfer method. Pumps transport a pressured liquid and a sample mixture through a column loaded with adsorbent, allowing the sample components to be separated. The adsorbent, or active component of the column, is commonly a granular substance consisting of 2–50 m solid particles (e.g., silica, polymers, etc.). Because of their varying degrees of contact with the adsorbent particles, the components of the sample mixture are separated from one another. A "mobile phase" is a pressurised liquid that is typically a mixture of solvents (e.g., water, acetonitrile, and/or methanol). Its composition and temperature have a big impact on the separation process because they influence how sample components interact with the adsorbent. Physical contacts include hydrophobic (dispersive), dipole–dipole, and ionic interactions, which are frequently combined [2].

HPLC differs from traditional ("low pressure") liquid chromatography in that the operating pressures are much greater (50–350 bar), whereas regular liquid chromatography depends on gravity to transfer the mobile phase through the column. Because analytical HPLC only separates a small amount of sample, typical column diameters are 2.1–4.6 mm diameter and 30–250 mm length. Smaller adsorbent particles (2–50 m in average particle size) are also used in HPLC columns. When separating mixtures, this affords HPLC greater resolving power (the capacity to differentiate between chemicals), making it a preferred chromatographic technique.

The schematic of an HPLC instrument typically includes a degasser, sampler, pumps, and a detector. The sampler brings the sample mixture into the mobile phase stream which carries it into the column. The pumps deliver the desired flow and composition of the mobile phase through the column. The detector generates a signal proportional to the amount of sample component emerging from the column, hence allowing for quantitative analysis of the sample components. A digital microprocessor and user software control the HPLC instrument and provide data analysis. In some HPLC instruments, mechanical pumps can mix various solvents in time-varying ratios, resulting in a composition gradient in the mobile phase. UV/Vis, photodiode array (PDA), and mass spectrometry-based detectors are all commonly used. The temperature at which the separation is accomplished can be adjusted using most HPLC equipment' column ovens [3].

History and development

Prior to HPLC, scientists relied on traditional liquid chromatographic methods. Because the flow rate of solvents is determined by gravity, liquid chromatographic devices are inefficient. Separations could take several hours or even days to accomplish. Although Gas Chromatography (GC) was more potent than liquid chromatography (LC) at the time, gas phase separation and analysis of extremely polar high molecular weight biopolymers was thought to be unachievable. Because of the heat instability of the solutes, GC was useless for many biochemists. As a result, various approaches were proposed, eventually leading to the invention of HPLC.

Cal Giddings, Josef Huber, and others predicted in the 1960s that LC could be operated in the high-efficiency mode by reducing the packing-particle diameter substantially below the typical LC (and GC) level of 150 m and using pressure to increase the mobile phase velocity, building on the seminal work of Martin and Synge in 1941. Throughout the 1960s and 1970s, these predictions were tested and refined extensively. Early research into improving LC particles began, and the discovery of Zipax, a superficially porous particle, looked promising for HPLC technology.

Many advancements in hardware and instrumentation occurred throughout the 1970s. Researchers began by constructing a crude HPLC system utilising pumps and injectors. Gas amplifier pumps were excellent since they worked at constant pressure and didn't require leak-free seals or check valves for consistent flow and accurate quantification. At Dupont IPD (Industrial Polymers Division), hardware milestones were achieved, such as the use of a low-dwell-volume gradient device and the replacement of the septum injector with a loop injection valve.

While equipment advancements were crucial, the history of HPLC is mostly on particle technology improvement. There has been a continual trend toward smaller particle sizes since the advent of porous layer particles to improve efficiency. However, reducing particle size caused new issues. The enormous pressure drop required to drive mobile fluid through the column, as well as the difficulty of making a uniform packing of extremely thin materials, are the practical drawbacks. [When particle size is lowered sufficiently, another round of instrument development is frequently required to accommodate the increased pressure [3,4].

Types

Partition chromatography: One of the first types of chromatography established by chemists was partition chromatography. Paper chromatography, thin layer chromatography, gas phase, and liquid–liquid separation applications have all used the partition coefficient principle. Archer John Porter Martin and Richard Laurence Millington Synge were awarded the Nobel Prize in

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Chemistry in 1952 for developing the technique that was utilised to separate amino acids. As with paper chromatography, partition chromatography uses a retained solvent on the surface or inside the grains or fibres of an "inert" solid supporting matrix; or takes advantage of coulombic and/or hydrogen donor interactions with the stationary phase. The eluent and a liquid stationary phase separate the analyte molecules. This technology, like Hydrophilic Interaction Chromatography (HILIC; a sub-technique within HPLC), separates analytes based on polarity differences. A bonded polar stationary phase and a mobile phase comprised mostly of acetonitrile with water as the strong component are most commonly used in HILIC. Historically, partition HPLC has been performed on unbonded silica or alumina supports. Each successfully separates analytes based on relative polar differences. The advantage of HILIC bound phases is that they can separate acidic, basic, and neutral solutes in one chromatographic run [3].

Normal–phase chromatography: One of the first types of HPLC created by chemists was normal–phase chromatography. This method, also known as normal-phase HPLC (NP-HPLC), separates analytes based on their affinity for a polar stationary surface such as silica, and thus on the ability of the analyte to engage in polar interactions (such as hydrogen-bonding or dipole-dipole type interactions) with the sorbent surface. NP-HPLC is a technique that uses a non-polar, non-aqueous mobile phase (such as Chloroform) to separate analytes that are easily soluble in non-polar solvents.

The analyte binds to the polar stationary phase and is held by it. Adsorption strengths rise as the polarity of the analyte increases. The strength of the interaction is determined not only by the functional groups in the analyte molecule's structure, but also by steric variables. This method uses the influence of steric hindrance on interaction strength to resolve (separate) structural isomers [5].

Displacement chromatography: The fundamental premise of displacement chromatography is that a molecule having a high affinity for the chromatography matrix (the displacer) would efficiently compete for binding sites, displacing any molecules with lower affinities. Displacement and elution chromatography are two different types of chromatography. Substances often emerge from a column as thin, Gaussian peaks in elution mode. To ensure maximal purification, a wide separation of peaks is desired, preferably to baseline. Many factors influence how quickly any component of a combination moves down the column in elution mode. However, there must be significant differences in some interaction between the biomolecules and the chromatography matrix for two compounds to flow at different speeds and therefore be resolved [4].

Size-Exclusion Chromatography

SEC, also known as gel permeation chromatography or gel filtration chromatography, separates particles based on their molecular size (really, their Stokes radius). Because it is a low-resolution chromatography, it is frequently used for the purification's final "polishing" stage. It can also be used to figure out the tertiary and quaternary structures of purified proteins. SEC is primarily used to examine big compounds like proteins and polymers. SEC works by trapping smaller molecules within a particle's pores. The larger molecules just pass through the pores because they are too big to fit inside. Larger molecules flow through the column faster than smaller molecules, hence the smaller the molecule, the faster it moves through the column [2].

Ion-exchange chromatography: The attraction between solute ions and charged sites attached to the stationary phase determines retention in Ion-exchange Chromatography (IC). Solute ions with the same charge as the charged sites on the column are prevented from binding, but solute ions with the opposite charge are kept on the column. By altering the solvent conditions, solute ions that have accumulated on the column can be eluted (e.g., increasing the ion effect of the solvent system by increasing the salt concentration of the solution, increasing the column temperature, changing the pH of the solvent, etc.) [5].

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.

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