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High-performance Liquid Chromatography

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Editorial

HPLC (high-performance liquid chromatography), originally 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 in manufacturing (e.g., during the manufacturing process of pharmaceutical and biological products), legal (e.g., detecting performance enhancement drugs in urine), research (e.g., separating the components of a complex biological sample or similar synthetic chemicals from one another), and medical applications.

Chromatography may be defined as an adsorption-based mass transfer method. Pumps transport a pressured liquid and a sample combination 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 comprised of solid particles (e.g., silica, polymers, etc.) with a size range of 2–50 m. 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 pressurized liquid that is generally a combination 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. These interactions are physical in nature, such as hydrophobic (dispersive), dipole–dipole, and ionic interactions, which are frequently combined.

HPLC differs from conventional ("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 tiny quantity of material, 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 method. A degasser, sampler, pumps, and detector are commonly included in an HPLC instrument's design. The sampler adds the sample mixture to the mobile phase stream, which then transports it to the column.

The pumps supply the desired mobile phase flow and composition via the column. Because the detector creates a signal proportional to the quantity of sample component emerging from the column, quantitative analysis of the sample components is possible. The HPLC apparatus is controlled and data is analysed by a digital microprocessor and user software. In an HPLC apparatus, some mechanical pumps may mix different solvents together 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 often used. The temperature at which the separation is accomplished may be adjusted using most HPLC equipment' column ovens.

The sample mixture to be separated and studied is put into the stream of mobile phase percolating through the column in a discrete tiny volume (usually microliters). Varying physical interactions with the adsorbent cause the components of the sample to travel along the column at different speeds (also called stationary phase). The chemical makeup of each component, as well as the nature of the stationary phase (column) and the composition of the mobile phase, all influence its velocity. The retention time of a certain analyte is the time it takes for it to elute (emerge from the column) [1-5].

Conflict of Interest

None.

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