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A Brief Note on High-Performance Liquid Chromatography (HPLC)

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Brief Report

High-performance liquid chromatography (HPLC) 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 convey 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 variable flow rates and separation of the components as they flow out of the column.

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 move 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 offers HPLC better resolving power (the capacity to discriminate between substances), making it a preferred chromatographic method.

In column chromatography, a solvent drips through an adsorbent-loaded column under gravity. HPLC is a far more advanced type of column chromatography. Under high pressures of up to 400 atmospheres, a pump pushes a solvent through a column. A granular substance composed of solid particles such as silica or polymers is used as the column packing material, adsorbent, or stationary phase. In comparison to column chromatography, the method is significantly quicker because to the pressure. This enables the column packing material to be made up of considerably smaller particles. The surface area of the smaller particles allows for more interactions between the stationary phase and the molecules moving past it. As a result, the components of the mixture are separated considerably better.

Types of HPLC

HPLC are the two most popular types Normal-phase and Reversed-phase

Normal-phase HPLC

A non-polar solvent, such as hexane, is used to fill the column with small silica particles. A typical column has a length of 150 to 250 mm and an interior diameter of 4.6 mm or less. Non-polar chemicals in the combination will move through the column faster than polar compounds, since polar molecules cling to the polar silica for longer than non-polar compounds.

Reversed-phase HPLC

The column sizes are same. The column is packed with non-polar silica particles that have been modified. Attaching lengthy hydrocarbon chains (8–18 C atoms) to its surface accomplishes this. A polar solvent, such as a combination of water and an alcohol such as methanol, is employed. Because of the high attraction between the polar solvent and the polar molecules in the mixture, polar compounds in the mixture will move through the column more quickly.

On their passage through the column, non-polar molecules are slowed. Through van der Waals dispersion forces and hydrophobic interactions, they generate different degrees of affinity with the hydrocarbon groups. Reversal phase HPLC is the most popular type of HPLC.

Uses of column chromatography

- Active compounds are isolated via column chromatography.
- It's extremely beneficial for separating chemicals from mixtures.
- Column chromatography is used to estimate drug concentrations from drug formulations; it is also used to eliminate contaminants.
- Aids in metabolite separation from biological fluids.

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