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Commentary

Note on High-Performance Liquid Chromatography (HPLC)

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DESCRIPTION

Prior to High-Performance Liquid Chromatography (HPLC), scientists used standard liquid chromatographic techniques. Liquid chromatographic systems are largely inefficient because the flow rate of fluids depends on gravity. Partitions took many hours, sometimes days, to complete. At that time gas chromatography (GC) was more powerful than liquid chromatography (LC), however, analysis of gas phase separation and very polar high molecular weight biopolymers was impossible. GC is ineffective for most biochemists due to the thermal instability of the solutions. As a result, alternative methods are envisioned, which will soon lead to the development of HPLC.

High-Performance Liquid Chromatography (HPLC), formerly referred to as High-Pressure Liquid Chromatography, is a technology in analytical chemistry used to isolate, identify, and compute each component. It relies on pumps to send a pressurized liquid containing the sample mixture through a column filled with solid absorbent material. Each part of the sample interacts in a slightly different manner with the adsorbent material, resulting in different flow rates for the different components and causing the components to separate as they flow out of the column.

Chromatography can be described as a mass transfer process with adsorption. HPLC relies on pumps to deliver a pressurized fluid and sample mixture through a column filled with adsorbent, which leads to separation of sample components. The active component of the column, adsorbent, is a granular material usually made up of solid particles (e.g., silica, polymers, etc.) 2-50 m in size. The components of the sample mixture are separated from each other due to different levels of interaction with the adsorbent cells. The pressurized fluid is usually a mixture of solvents (eg, water, acetonitrile and / or methanol) and is referred to as the "mobile phase". Its composition and temperature play a major role in the separation process by influencing the interactions that take place between the sample components and the adsorbent. These interactions are physical in nature, most often a combination of hydrophobic (diffuser), dipole-dipole and ionic.

HPLC is distinguished from conventional ("low pressure") liquid chromatography because the operating pressures are significantly higher (50-350 bar), although general liquid chromatography usually relies on the force of gravity to cross the mobile phase through the column. Due to the small sample separated in the analytical HPLC, the typical column dimensions are 2.1-4.6 mm in diameter and 30-250 mm in length. Also HPLC columns are made with small absorbent particles (average particle size 2-50 m). This gives HPLC superior resolving power (the ability to distinguish between compounds) when separating compounds, making it a well-known chromatographic technique.

The schematic of an HPLC device usually consists of a degasser, a model, pumps and a detector. The sample is brought into the mobile phase stream which carries the mixture into the column. The pumps provide the desired flow and composition of the mobile phase through the column. The sample generated from the detector column produces a signal proportional to the sum, thus allowing quantitative analysis of the sample components. Digital microprocessor and user software control the HPLC device and provide data analysis. Some models of mechanical pumps in the HPLC device can combine multiple solvents in changing ratios over time, producing composition gradient on the mobile stage. Various detectors based on UV / V is, photodiode array (PDA) or mass spectrometry is commonly used. Most HPLC devices also have a column oven, which allows you to adjust the temperature at which the separation is performed.

HPLC has many applications in both laboratory and clinical science. This is a common technology used in the development of pharmaceuticals because it is a reliable way to obtain and ensure product purity. Although HPLC is able to produce high quality (pure) products, it is not always the primary method used in the production of bulk drug materials. According to European Pharmacopoeia, HPLC is used in only 15.5% of syntheses. However, it plays a role in 44% of synthesis in the United States pharmacopoeia. This may be due to differences in monetary and time constraints, as large-scale HPLC is an expensive technology.

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