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Use of HPLC in Biochemistry and Biotechnology

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High-performance liquid chromatography, abbreviated as HPLC, is a chromatographic technique of great versatility and analytic power used in many aspects of drug manufacturing and research. It separates or identifies mixtures of substances into their components based on their molecular structure and composition.

The other name for high-performance liquid chromatography is high-pressure liquid chromatography. High-performance liquid chromatography (HPLC) is the most widely used separation technique. It can be very sensitive, specific, and precise. It is a particular form of column chromatography used in biochemistry and analysis to separate, identify, and quantify the active compounds in a mixture. In HPLC, a column holds packing material (stationary phase), a pump moves the mobile phase(s) through the column, and a detector shows the retention times of the molecules. Retention time is variable and mainly depends on the interactions between the stationary phase, the molecules being analyzed, and the solvent(s) used.A small volume of sample to be analyzed is introduced to the mobile phase stream and is retarded by specific chemical or physical interactions with the stationary phase.The amount of retardation mainly depends on the nature of the analyte and the composition of both stationary and mobile phases.The most common solvents used in high-performance liquid chromatography (HPLC) are methanol and acetonitrile.

Brief history

Michael Tswett (1872-1920) is credited as the father of chromatography due to his demonstration of liquid chromatography. In 1903, he separated the green-leaf pigments into bands of colors. After that, in 1937-38, thin-layer chromatography (TLC) was used. The next significant advancement was the use of paper chromatography in the mid-1940s. Thin-layer chromatography (TLC) advanced slowly during the next few years, but Egon Stahl made significant development in 1956. Egon Stahl standardized the preparation of the sorbents used to make the plates. High-pressure liquid chromatography (HPLC) was later developed in the 1970s. The term high-performance liquid chromatography (HPLC) was introduced in the 1970s to distinguish the modern high-performance technique from classical low-pressure column chromatography, developed in the 1930s.

HPLC Principle

High-performance liquid chromatography (HPLC) involves the injection of a small volume of liquid sample into a tube packed with tiny particles (3 to 5 microns (µm) in diameter called the stationary phase) where individual components of the sample are moved down the packed tube with a liquid (mobile phase) forced through the column by high pressure delivered through a pump.

The column packing is used to separate the components from one another. It involves various chemical and/or physical interactions between their molecules and the packing particles. The separated components are then detected at the exit of the column by a detector that measures their amount. Output from this detector is called a "liquid chromatogram."

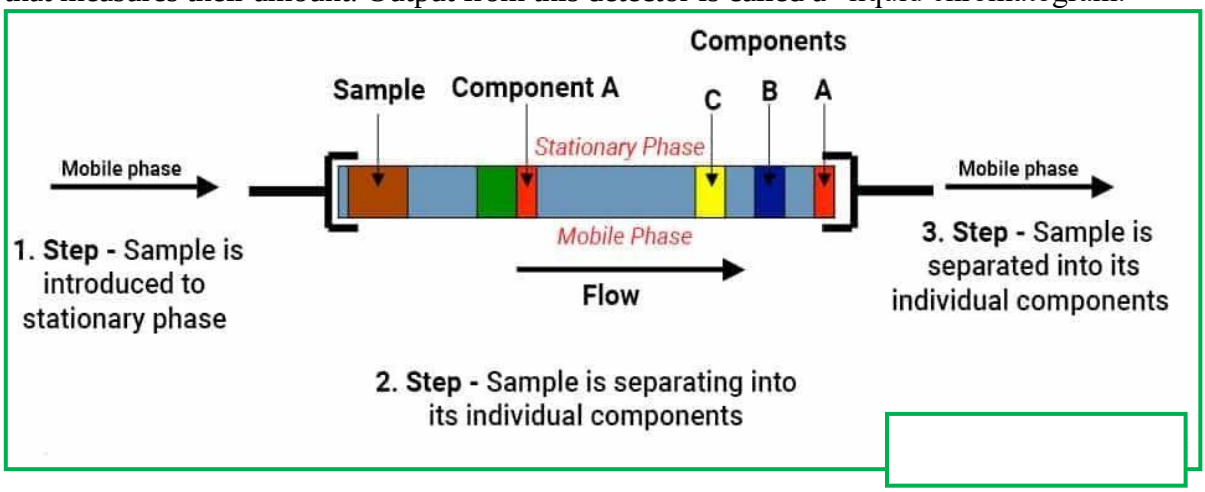


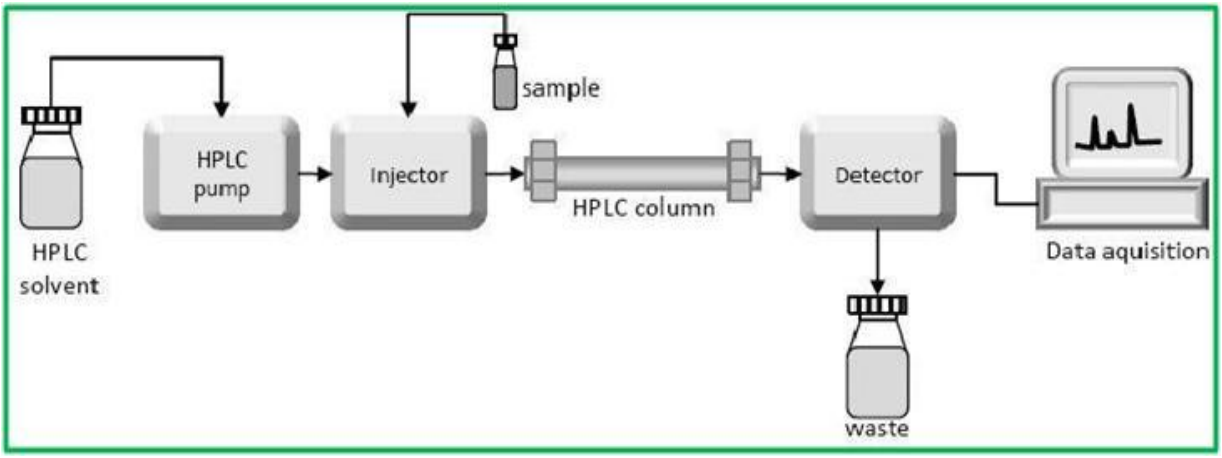
Figure: 1 Describes the basic principle of HPLC.
Step 1. The sample is introduced (mobile phase).
Step 2. The sample is separated into its components (stationary phase). Step 3. The sample is separated into its components (mobile phase).

Advantages over low-pressure column liquid chromatography

There are many advantages of High-performance liquid chromatography (HPLC) over traditional low-pressure column liquid chromatography.

- Greater sensitivity (various detectors can be employed)
- Improved resolution
- Speed
- Easy sample recovery (less fluent volume to remove)
- A wide variety of stationary phases

Instrumentation of HPLC



1. Solvent Reservoir

- Mobile phase contents are contained in a glass reservoir.
- The mobile phase, or solvent, in HPLC, is usually a mixture of polar and non-polar liquid components whose respective concentrations are varied depending on the composition of the sample.

2. Pump

- A pump aspirates the mobile phase from the solvent reservoir and forces it through the system's column and detecter.
- Depending on several factors, including column dimensions, the particle size of the stationary phase, the flow rate and composition of the mobile phase, operating pressures of up to 42000 kPa (about 6000 psi) can be generated.

3. Sample Injector

- The injector can be a single injection or an automated injection system.
- An injector for an HPLC system should provide an injection of the liquid sample within the range of 0.1-100 mL of volume with high reproducibility and under high pressure (up to 4000 psi).

4. Columns

- Columns are usually made of polished stainless steel, are between 50 and 300 mm long, and have an internal diameter between 2 and 5 mm.
- They are commonly filled with a stationary phase with a particle size of 3–10 µm.
- Columns with internal diameters of less than 2 mm are often called microbore columns.
- Ideally, the temperature of the mobile phase and the column should be kept constant during an analysis.

5. Detector

- The HPLC detector, located at the end of the column, detects the analytes as they elute from the chromatographic column.
- Commonly used detectors are UV-spectroscopy, fluorescence, mass-spectrometric and electrochemical detectors.

6. Data Collection Devices

- Signals from the detector may be collected on chart recorders or electronic integrators that vary in complexity and their ability to process, store and reprocess chromatographic data.
- The computer integrates the detector's response to each component and places it into a chromatograph that is easy to read and interpret.

Applications of HPLC

The information that HPLC can obtain includes resolution, identification, and quantification of a compound. It also aids in chemical separation and purification. The other applications of HPLC include

1. Pharmaceutical Applications: control drug stability.
2. Environmental Applications: Detection of phenolic compounds in drinking water.
3. Applications in Forensics: Quantification of drugs in biological samples.
4. Food and Flavor: Measurement of Quality of soft drinks and water.
5. Applications in Clinical Tests: Urine analysis, antibiotics analysis in blood.

Limitations

The limitation of using high-performance liquid chromatography (HPLC) is the following.

1. HPLC is much more costly requires a large number of expensive organics.
2. HPLC may have low sensitivity for certain compounds, and some cannot even be detected as they are irreversibly adsorbed.

3. Complexity
4. Volatile substances are much better to be separated by gas chromatography.

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