



A Review on High Performance Liquid Chromatography

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ABSTRACT :

HPLC method development and validation are critical in the discovery, development, and manufacturing of pharmaceutical drugs, as well as in various studies related to humans and animals. Validation of HPLC methods, in accordance with ICH Guidelines, encompasses key performance characteristics such as accuracy, precision, specificity, linearity, range, limits of detection and quantification, robustness, and system suitability testing. As a leading separation technique in pharmaceutical and biomedical analysis, HPLC offers highly efficient separations with exceptional detection sensitivity. Its numerous advantages, including speed, specificity, accuracy, precision, and ease of automation, make it an indispensable tool in analytical procedures designed to evaluate specific characteristics of drug substances or drug products.

Introduction :

HPLC is a widely used technique for separating complex mixtures, known for its rapid process due to the use of high-resolution and high-speed columns. Developed in the early 1970s, the technique was initially introduced by Kirttand and Habber.

The term "chromatography" was coined by Russian botanist Mikhail Tsvet in the 1930s. Derived from the Greek words chroma (meaning color) and graphein (meaning to write), it reflects the technique's origins in separating colored compounds.

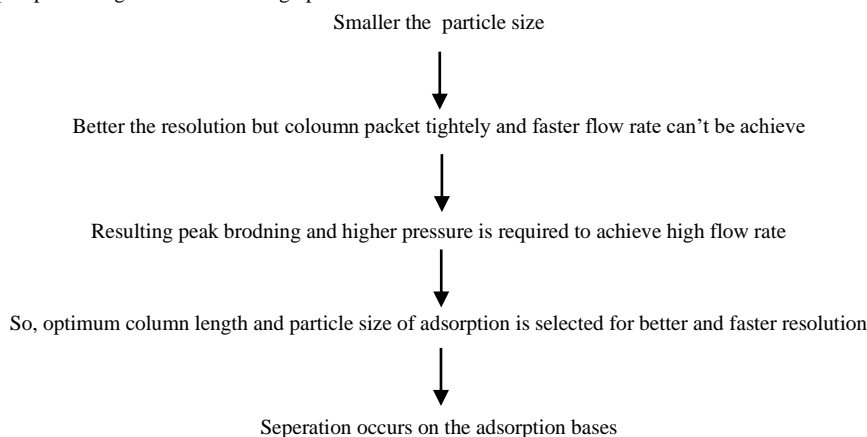
The principle of HPLC involves introducing the sample into a column filled with a porous material (stationary phase). A liquid (mobile phase) is then passed through the column under high pressure, facilitating the separation of compounds within the sample.

- **Definition**

The technique involves the system pumping the mobile phase through a packed column.

- **Principle**

High-performance liquid chromatography (HPLC) involves introducing a sample into a column filled with porous material (stationary phase), while a liquid (mobile phase) is pumped through the column at high pressure.



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Chromatographic Techniques

1. **Normal Phase Chromatography:**

In normal phase chromatography, the stationary phase is polar, while the mobile phase is non-polar. Components in the mixture interact differently with the polar stationary phase, with more polar analytes having stronger interactions and, therefore, staying on the stationary phase longer than less polar analytes. This method separates analytes based on their polarity.

2. Reverse-Phase Chromatography:

Reversed-phase HPLC (RP-HPLC or RPC) employs a non-polar stationary phase and a moderately polar, aqueous mobile phase. In this method, polar compounds elute first, while non-polar compounds interact more strongly with the stationary phase and remain longer. Since many drugs and pharmaceuticals are polar, they typically elute quickly and do not stay in the column for extended periods.

3. Size Exclusion Chromatography (SEC):

Also known as gel permeation chromatography or gel filtration chromatography, SEC separates particles based on their size using gel-filled columns. These columns are packed with materials of controlled pore sizes, allowing separation based on molecular size through steric and diffusion effects. This technique is particularly useful for studying the tertiary and quaternary structures of proteins, as well as for determining amino acids.

4. Ion Exchange Chromatography:

Ion exchange chromatography is based on the principle of reversible exchange of ions between the sample and the functional groups on the stationary phase. This technique facilitates the separation of components based on their ionic charge.

5. Bioaffinity Chromatography:

In bioaffinity chromatography, separation occurs based on the specific, reversible interaction between proteins and ligands. Proteins that bind to the ligands on the column are retained, while unbound components are eluted. The formation of these complexes involves various molecular forces, including Van der Waals interactions, electrostatic interactions, dipole-dipole interactions, hydrophobic interactions, and hydrogen bonding.

Modes of Separation :

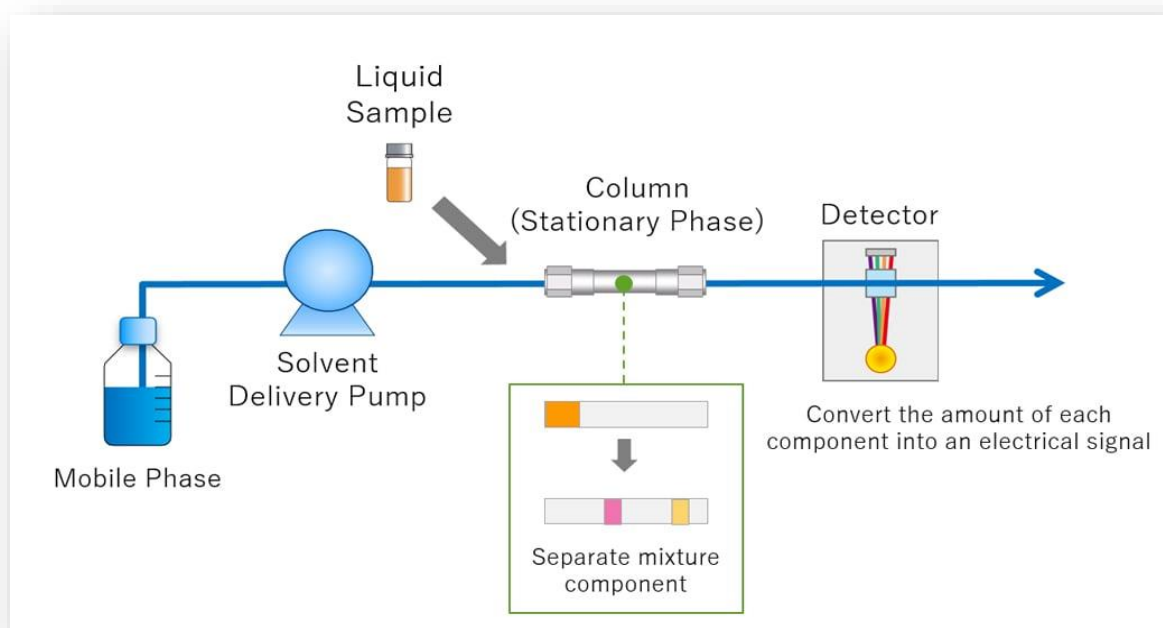
In HPLC, there are two modes of separation based on the composition of the eluent:

- Isocratic:** In the isocratic mode, the eluent composition remains constant throughout the separation process. This results in stable equilibrium conditions within the column, and the velocity of compounds moving through the column remains unchanged.
- Gradient:** In gradient mode, the eluent composition varies during the separation. This approach enhances the separation power of the system by improving its efficiency, primarily by reducing peak width. The width of the peaks depends on the rate at which the eluent composition changes.

Instrumentation of HPLC

HPLC equipment consists of nine basic components:

1. Mobile Phase/Solvent Container
2. Solvent Delivery System
3. Sampling Device
4. Chromatographic Column
5. Post-Column Device
6. Detector
7. Data Acquisition and Output System
8. Post-Detector Eluent
9. Processing, Pipes, and Fittings



1. Mobile Phase:

The solvent used to separate the components in a liquid sample during HPLC analysis is known as the mobile phase.

2. Pump:

The pump delivers the mobile phase through the system at a controlled flow rate.

3. Syringe:

HPLC syringes are specifically designed for manual or automatic injection into high-pressure ports. They typically feature a blunt, electro-polished needle to minimize wear on the injection port seals. These syringes ensure precise sample introduction into the system.

4. Injection Port

The injection port of an HPLC system includes a valve, sample loop, and needle port, which facilitate the controlled introduction of the sample into the column for separation.

5. Column:

The column in HPLC is responsible for separating the components of the sample. Typical column lengths range from 5 to 30 cm, with diameters ranging from 2 to 50 mm. The particle size of the column is typically 1–2 μm . The column is made of spherical, uniform-sized, and porous material to ensure effective separation.

6. Column Oven:

The column oven ensures precise temperature control, with an accuracy of better than $\pm 0.1^\circ\text{C}$. The typical dimensions of the oven are 30 cm in length and 22 cm in diameter, which maintain the column at a stable temperature for optimal separation.

7. Detector:

The detector measures the compounds that exit the column. Common types include UV detectors, refractive index detectors, and conductivity detectors, each tailored for detecting different types of compounds.

8. Display:

The display shows the particle size of the mobile phase and helps determine the value of the range for the analysis. It provides a visual representation of the chromatographic data, aiding in the analysis of the separation process.

Types of Chromatography Based on Modes:

- **Normal Phase:**
 - Stationary Phase (S.P.): Polar (e.g., silica gel)
 - Mobile Phase (M.P.): Non-polar
- **Reversed-Phase:**
 - Stationary Phase (S.P.): Non-polar (e.g., ODS C18, C8, C4)
 - Mobile Phase (M.P.): Polar

Types of Chromatography Based on Modes:

- 1) Adsorption Chromatography
- 2) Ion Exchange Chromatography
- 3) Ion Pair Chromatography
- 4) Size Exclusion or Gel Permeation Chromatography
- 5) Affinity Chromatography
- 6) Chiral Phase Chromatography

Applications:

- Stability studies
- Bioassays and their complementation's
- Dosage design
- Cosmetic industry applications
- Isolation of naturally occurring pharmaceutical compounds

Conclusion

In conclusion, HPLC is a versatile and reproducible technique used for both qualitative and quantitative analysis of pharmaceutical and biological samples. At the end of the analysis, a chromatogram is generated using HPLC software, which helps in identifying and quantifying the various compounds present. HPLC is highly efficient due to its short run time per sample, among other factors.

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