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A Short Review on: Definition, Principle and Applications of High Performance Liquid Chromatography



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ABSTRACT

The term HPLC was referred to as High-Pressure Liquid chromatography due to the involvement of high pressure. High-performance liquid chromatography is one of the most widely in the identification and quantifies the potency of drug substances. The flexibility of this technique numerous applications has been adopted for routine in pharmaceutical industries, research and development center quality control laboratories, food testing and educational institution. This review focus on the definition, principle, and applications of High-performance liquid chromatography.



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INTRODUCTION:

The analytical technique of High-Performance Liquid Chromatography (HPLC) is used extensively throughout the pharmaceutical industry. It is used to provide information on the composition of drug-related samples. ^[1] It can separate, identify, and quantify the compounds that are present in any sample that can dissolve in liquid. HPLC is the most accurate analytical method widely used for the quantitative as well as qualitative analysis of drug products and used for the determination of drug product stability. ^[2] High pressure should apply to have an eluent flow through the column due to the physical properties of HPLC. ^[3] HPLC is recognized from traditional liquid chromatography because operational pressures are fundamentally higher (50bar to 350 bar). The small sample amount of isolated in scientific HPLC, column section measurements are 2.1nm to 4.6nm distance across, and 30 nm to 250nm in length. Additionally, the HPLC segment is made with the smaller sorbent particles (2 μ m to 50 μ m in a normal molecule size). This gives HPLC determine high resolving power (the capacity is recognized components) while isolating mixtures which makes its prominent chromatographic method. ^[4]

DEFINITION:

High-performance liquid chromatography (HPLC) is a chromatographic technique used to split a mixture of compounds in the fields of analytical chemistry, biochemistry, and industrial. The main purposes of using HPLC are identifying, quantifying, and purifying the mixture of the individual compounds. ^[5]

PRINCIPLE:

- HPLC principle is the solution of the sample injected into a column of porous material (stationary phase) and the liquid phase (mobile phase) is pumped at higher pressure through the column.
- The principle is separation followed is the adsorption of solute on the stationary phase based on affinity towards the stationary phase. ^[6]
- Depending on the nature of the stationary phase, the separation process can be four different modes.
 - i. Adsorption chromatography, where the separation is based upon repeated adsorption-desorption steps;

- ii. Partition chromatography where the separation is based on the partition between the mobile and the stationary phase;
- iii. Ion – exchange chromatography where the separation phase is made up of the anionic surface of opposite charge to that of the sample; and
- iv. Size exclusion chromatography where the sample is separated according to its molecular size through a column filled with a material having precisely controlled pore size.^[7]

The principle of HPLC is based on the Van Deemter equation which relates the efficiency of the chromatographic column to the particle size of the column, molecular diffusion, and thickness of the stationary phase.

The Van Deemter Equation is given is:

$$H \text{ or HETP} = A + \frac{B}{v} + Cv$$

Where,

A=represents eddy's diffusion

B=represents molecular diffusion

C=represents rate of mass factor

v= represents flow rate^[8]

Type of HPLC:

I. Normal phase chromatography:

In a normal phase chromatography, the mobile phase is a non- polar, and the stationary phase is polar. An increase in polarity of solute molecules increases the adsorption capacity leading to an increased elution time. The chemically modified silica (cyanopropyl, aminopropyl, and diol) is used as a stationary phase in this chromatography. A polar compound in the mixture is passed through the column will stick a longer and the non-polar pass more quickly through the column.

II. Reversed-phase HPLC:

The non-polar stationary phase and polar moderately polar mobile phase. RP-HPLC is based on the principle of hydrophobic interaction. The non-polar stationary phase is retained longer time if the period compared to more polar hence the most polar compounds are eluted first. [9]

III. Size exclusion chromatography:

Size exclusion chromatography is also called gel permeation chromatography or gel filtration chromatography mainly separate particles basis of size. These techniques widely use the determination of the molecular weight of polysaccharides. It is also useful to the determination of tertiary and quaternary structure.

IV. Ion exchange chromatography:

In ion-exchange chromatography, retention is based on the attraction between solute ion and charged sites bound to the stationary phase. Ions of the same charge are excluded. This chromatographic technique is widely used for purifying water, ligand exchange chromatography, ion-exchange chromatography of proteins.

V. Bio-affinity Chromatography:

Separation is based on a specific reservoir interaction of the protein with ligands. Ligands are covalently attached to solid support on a bio-affinity matrix; retain proteins with interaction to the column bound ligands. Protein-bound to bio-affinity column can be two ways:

○ Biospecific elution:

Inclusion of free ligand in elution buffer which competes with column bound ligand.

○ A specific elution:

Change in pH, salt, etc. which weak interaction protein with column bound substrate.

Because of the specificity of the interaction, bio-affinity chromatography can result in very high purification in a single step (10-1000 folds).^[10]

Advantages of HPLC:

The advantages of HPLC are:

- Good repeatability.
- High Sensitivity (ppm-ppb).
- Continuous monitoring of the column effluent.
- It can be applied to the separation and analysis of a very complex mixture.
- No need to vaporize the sample like GC.^[11]
- High efficiency.
- High accuracy.
- High-resolution speed.
- Versatile and extremely precise when it comes to identifying and quantifying chemical components.^[12]

Disadvantages of HPLC:

- HPLC can be an expensive method; it required a large number of expensive. organic needs a power supply, and regular maintenance is required.
- It can be applied to troubleshoot the problem or develop a new method.^[13]
- High cost.
- Complex to operate.^[14]

Applications of HPLC:

High Performances Liquid chromatography system and their components we now introduced to typical applications.

HPLC has contributed to the analytical solution in diverse filed such as pharmaceuticals, foods, life science, environment, forensics, etc. ^[15]

PHARMACEUTICAL APPLICATIONS:

1. Tablet dissolution study of the pharmaceutical dosage form.
2. To control drug stability, shelf-life determination.

3. Identification of active ingredient.

4. Pharmaceutical quality control. ^[16]

Food applications:

1. HPLC is widely using the food analysis of particular product research and quality control.

2. It is suited for testing of the labile compound for a complex matrix.

3. Analysis of natural compounds (sugar, fats, protein, amino acids) food additives and contaminants are determined.

4. Multiresidues testing of contaminants and pesticides. ^[16]

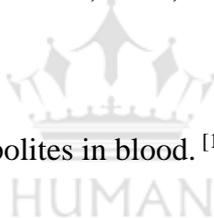
Forensics applications:

1. Quantification of the drug in a biological sample.

2. Identification of anabolic steroids in serum, urine, sweat, and hair.

3. Forensics analysis of textile dyes.

4. Determination of cocaine and metabolites in blood. ^[17]



Clinical Applications:

1. Clinical research and routine clinical analysis.

2. Measuring the glycated hemoglobin, it means monitoring long term plasma glucose control in diabetic patients.

3. It is also widely used to identify the cause of poisoning.

4. Identifying the drug metabolism that may be important in drug toxicity. ^[18]

CONCLUSION:

It can conclude the entire review of the HPLC is the versatile, reproducible chromatographic technique for the estimation of the drug product and the drug substances. HPLC is the best separation technique for the quantitative trace analysis of toxic chemicals, impurities, manufacturing of high purity products, medicinal uses, and research purposes.

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