A COMPLETE REVIEW ON HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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***Abstract*: High-performance liquid chromatography (HPLC) is a qualitative and quantitative technique which is employed for the evaluation of the medicine and biological samples. HPLC is that the safe, reliable, more versatile, and fast chromatographic technique for quality control of pharmaceutical ingredients. This article was created to explore various aspects of HPLC, including principles, types, equipment, and applications.**

***Keywords*: Instrumentation, applications, high-performance liquid chromatography, elution, mobile phase.**

INTRODUCTION

High-performance liquid chromatography is generally used in biochemistry and analysis to separate, identify, and quantify the active compounds. HPLC is a form of column chromatography. HPLC mainly includes packing material (stationary phase), a pump that moves the mobile phase(s) within the column, and a detector that displays the retention time of the molecule. This depends on the interaction between the retention time of the stationary phase, the molecule to be analyzed, and the solvent used. The sample is introduced into the mobile phase flow in a small amount and is delayed due to some chemical or physical interaction with the stationary phase. Properties of the composition containing the analyte are fixed, and the mobile phase depends on its retention. The time it takes for a solute to elute a particular analytical object in a column is called the retention time. In HPLC common solvents include a combination of water-miscible organic liquid, or (most commonly used are methanol and acetonitrile). The component is divided into two phases, one is fixed (stationary), and the other moves in the defined direction and is called a gradient elution. The gradient elution off to allow separation of components in a sample having a significant affinity for different surfaces. The choice of solvent, additive and gradient depends upon the nature of the fixed (stationary) phase and the drug or bimolecular sample to be analyzed.

1. PRINCIPLE:

This process involves analyzing the interaction of the compound in the sample substance (moving with the mobile phase) through a fixed surface (stationary phase). The compound binds to a specific area of the stationary phase according to specific physical and chemical properties. These bound molecules are then eluted with an appropriate buffer and collected over time.

2. TYPES OF HPLC

The types of HPLC usually depend on the phase system to be used in the process. The following types of HPLC are used in the analysis:

1) Normal phase chromatography: This method, also known as normal phase HPLC (NP-HPLC), separates objects to be analyzed according to the polarity of the sample. NP-HPLC uses a polar fix (stationary) phase and a non-polar (moving) mobile phase. Polar analysts interact and are retained by the polar stationary phase. As the adsorption capacity of the image to be analyzed increases with increased polarity, the polarity of the interaction between the analyte and the polarity of the stationary phase increases wash off time (elution time).

2) Reversed phase chromatography: RPC is operating on the principle of hydrophobic interactions resulting from repulsive forces between polar eluent, relatively non-polar objects of analysis, and non-polar stationary phases. No polar segments surrounding the analysis target molecules bind to the stationary phase is proportional to the area of the contact surface of the analysis object, and the aqueous washed off upon ligand binding liquid.

3) Size exclusion chromatography: Size Exclusion Chromatography (SEC), also known as gel permeation chromatography or gel filtration chromatography, separates particles primarily based on size. It also helps determine the tertiary and quaternary structure of proteins and amino acids.

4) Ion exchange chromatography: In ion-exchange chromatography, retention is based on the attractive force between the solute ion and the potential point on the stationary phase. Ions of the same charge are not included. This type of chromatography is widely used in water purification, ligand exchange chromatography, protein ion-exchange chromatography, high pH anion exchange chromatography of carbohydrates and oligosaccharides, etc.

5) Bio-affinity chromatography: Separation based on specific reversible interactions between proteins and ligands. The ligand covalently binds to solid support within the bioaffinity matrix and interacts with the ligand bound to the column to retain the protein. Bound protein affinity bio-columns can be eluted in two ways:

1) Biospecific elution: Include the free ligand in the elution buffer that competes with the ligand bound to the column.

2) Aspecific elution: Changes in pH, salt, etc. This weakens the interaction between the protein and the substrate attached to the column.
Due to the specificity of the interaction, bio-affinity chromatography can lead to very high one-step purification.

3. PARAMETERS

Several parameters are used as standards for a particular compound to analyze the compound accurately. Changes to the parameters can have a significant impact on the results. The most commonly used parameter is the inner diameter, particle size, pore size and the pump pressure. You can change the parameters of various compounds according to their properties and chemical properties.

1) Internal diameter: HPLC column internal diameter (ID) is an important aspect, which determines the amount of analysis that can be loaded onto the column material, but also affects the sensitivity. Larger columns are commonly used in industrials, such as for purifying the drugs for later use. DI columns increase sensitivity and consume less solvent at the expense of load capacity.

2) Particle size: Most conventional HPLC by the fixed connection to the small spherical silicone rubber particles (very small beads) to the outside. For smaller particles, the surface area usually increases, but to improve the separation, the pressure required for the optimal linear velocity, the particle size 2 increases with the reciprocal of the square.

3) Pore size: Many stationary phases are porous, providing a larger surface area. The smaller the pore size, the larger the surface area, the larger the pore size, and the faster the reaction speed, especially when the object to be analyzed is larger. The size of the pore determines the ability of the analyte molecule to penetrate the particle and interact with its inner surface. This is important because the ratio of the outer to the inner surface of the particle is approximately 1: 1000. Surface molecular interactions occur primarily on the inner surface of the particle.

4) Pump pressure: Pump pressure capabilities vary, but their performance through their production of raw capacity is consistent and the reproducible flow rate is to be measured.

4. INSTRUMENTATION

1) Pumps: The pump is used to pass the mobile phase through the chromatographic column at a high-pressure controlled flow rate. In addition, HPLC pump maximum pressure used was 6000 PSI, a flow rate of 0.1 ~ 10 ml/min, producing green 0.5 ± % of the flow rate control and reproducibility of the flow, the composition is an anti-output pulse cannot. It should be easy to switch from one mobile phase to another, and the pump should be easy to disassemble and repair.

2) Injection of the sample: Septum injector is available; which is used to inject sample solution. Sample can be injected while flowing or stopped mobile phase. Use the new advanced rotary valve and loop sampler to obtain reproducible results.

3) Column: There are two kinds of columns, "analytical column is 25-100 cm, the internal diameter of 2-6 mm" and "preparative column is 25-100 cm, 6 mm or greater." The main advantages of these columns are minimum solvent speed and less consumption. These columns must be possible to withstand high pressure and also has a temperature of the control system. Film/pellicle and porous particles, HPLC is used for 2 basic types of packaging.

4) The detector: There are several methods to detect whether the substance has passed the chromatographic column. It is usually equipped with an ultraviolet spectrometer to detect specific compounds. An organic compound absorbs the UV rays of various wavelengths. The amount of light absorbed depends on the amount of specific compound passing through the beam at that time.

5) Interpreting the output from the detector: As a series of peaks in the output recorded, each one representing the first UV light absorption through which the compound in the mixture passes through the detector. The area below the peak is proportional to the amount of passing through the detector, which can be calculated automatically with the help of a computer linked to the display.

5. ADVANTAGES OF HPLC:

- It provides a specific, sensitive and accurate method for analyzing various complex samples.
- Easy sample preparation and input.
- There is analysis speed.
- HPLC analysis is specific, accurate and precise.
- It is superior to gas chromatography in analyzing many polarities, ions, metabolites, and heat-labile and non-volatile substances.

6. APPLICATION

The information obtained by HPLC includes the identification, quantification and separation of compounds. Preparative HPLC refers to the process of separating and purifying compounds. This is different from analytical HPLC, which aims to obtain information about sample compounds.

1) Chemical Separations: This is based on certain compounds show different mobility depending on the chromatographic column and specific mobile phase. The degree or degree of separation largely depends on the choice between the stationary phase and the mobile phase.

2) Purification: Purification is defined as the compound or dirt separating or extracting the title compound in a mixture of dyed material processes. Each of the compounds exhibited Laid chromatographic conditions under certain intrinsic peaks. Passing through the column and the compounds of dirt transfer pollutants must be sufficiently different to be able to collect or extract pure compounds of interest, without producing raw other unwanted compounds.

3) Identification generally assay of compounds are carried using HPLC: The parameters of this determination should be such that a clean peak is observed from a known sample on the chromatograph. The identified peaks should have a reasonable retention time and be well separated from irrelevant peaks at the detection level of the detection.

4) Other applications of HPLC:

• Studies that shows tablet dissolution of pharmaceutical dosages form.
• Determination of shelf-life of pharmaceutical products
• Identification of active pharmaceutical ingredients of the dosage forms
• Pharmaceutical quality control of dosage forms

5) Environmental applications

• Phenolic compounds Detection in drinking water
• Diphenhydramine identification in sedimented samples
• Biological monitoring of pollutant

6) Forensic Medicine

• Identification of the anabolic steroids in serum, urine, sweat, and hair
• Forensic analysis of the textile dyes.
• Cocaine and metabolites determination in blood

7) Clinical

• Quantification of ions in human urine
• Analysis of antibiotics in blood plasma.
• Estimation of bilirubin and biliverdin in blood plasma in case of liver disorders.
• Detection of the extracellular fluid of the brain of the endogenous neural peptides.

8) Food and Flavor

• Ensure the quality of soft drinks and drinking water.
• Beer analysis.
• Analysis of sugar in fruit juice.
• Analysis of polycyclic compounds in plants/vegetables.
• Analysis of traces of high military explosives in crops.

CONCLUSION

Through a comprehensive complete review, we can conclude, HPLC is a chromatographic technique for the assessment of a drug's generic and reproducible. From the perspective of quantitative and qualitative estimation of active molecules, it has a wide range of applications in various fields in terms of quantitative and qualitative estimation of active molecules.

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