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A SHORT REVIEW ON: HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT: High liquid chromatography (HPLC) performance is an important quality and quantitative method used to measure pharmaceutical and environmental samples. It is the most flexible, safest, most reliable, and fastest chromatographic method for quality control of drug components**.** High-performance liquid chromatography (HPLC; formerly referred to as high-pressure liquid chromatography) is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture.

KEYWORDS: High performance liquid chromatography, instrumentation, elution, applications, mobile phase

INTRODUCTION:

High-performance liquid chromatography (HPLC; formerly referred to as high-pressure liquid chromatography) is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture.[1,2] It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column.[3]

- The mixture is separated using the basic principle of column chromatography and then identified and quantified by spectroscopy.
- In the 1960s the column chromatography LC with its low-pressure suitable glass columns was further developed to the HPLC with its high-pressure adapted metal columns.
- HPLC is thus basically a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. The purification takes place in a separation column between a stationary and a mobile phase.
- The stationary phase is a granular material with very small porous particles in a separation column.

- The mobile phase, on the other hand, is a solvent or solvent mixture which is forced at high pressure through the separation column.
- Via a valve with a connected sample loop, i.e. a small tube or a capillary made of stainless steel, the sample is injected into the mobile phase flow from the pump to the separation column using a syringe.
- Subsequently, the individual components of the sample migrate through the column at different rates because they are retained to a varying degree by interactions with the stationary phase.
- After leaving the column, the individual substances are detected by a suitable detector and passed on as a signal to the HPLC software on the computer.
- At the end of this operation/run, a chromatogram in the HPLC software on the computer is obtained.
- The chromatogram allows the identification and quantification of the different substances.

Principle

- The purification takes place in a separation column between a stationary and a mobile phase. The stationary phase is a granular material with very small porous particles in a separation column. The mobile phase on the other hand is a solvent or solvent mixture which is forced at high pressure through the separation column. Via a valve with a connected sample loop,i.e. a small tube or a capillary made of stainless steel, the sample is injected into the mobile phase flow from the pump to the separation column using a syringe. Subsequently the individual components of the sample migrate through the column at different rates because they are retained to a varying degree by interactions with the stationary phase. After leaving the column the individual substances are detected by a suitable detector and passed on as a signal to the HPLC software on the computer. At the end of this operation a chromatogram in the HPLC software on the computer is obtained, which allows the identification and quantification of the different substances.[4,5,6]

Types

There are following variants of HPLC, depending upon the phase system (stationary) in the process :

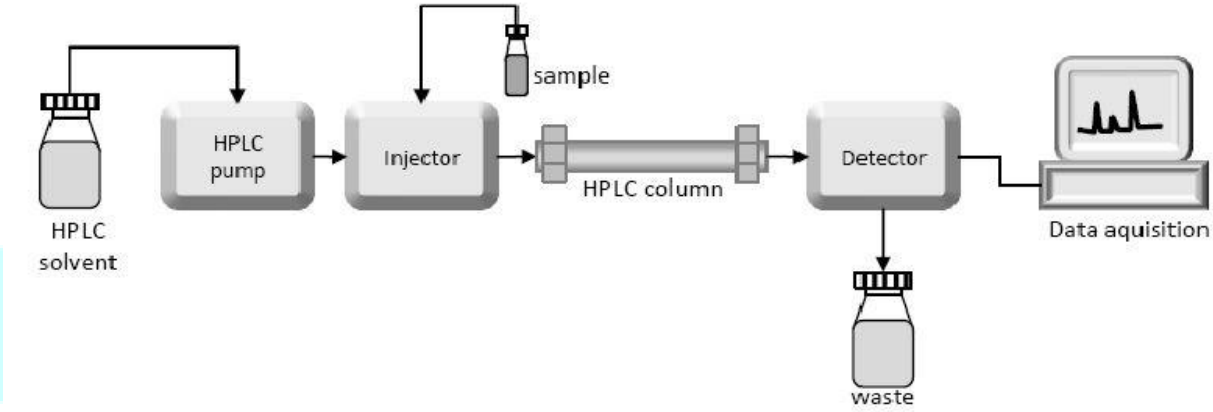
Normal Phase HPLC: This method separates analytes on the basis of polarity. NP-HPLC uses polar stationary phase and non-polar mobile phase. Therefore, the stationary phase is usually silica and typical mobile phases are hexane, methylene chloride, chloroform, diethyl ether, and mixtures of these. Polar samples are thus retained on the polar surface of the column packing longer than less polar materials.[2,7]

- **ii. Reverse Phase HPLC**
- The stationary phase is nonpolar (hydrophobic) in nature, while the mobile phase is a polar liquid, such as mixtures of water and methanol or acetonitrile. It works on the principle of hydrophobic interactions hence the more nonpolar the material is, the longer it will be retained.
- **iii. Size-exclusion HPLC:** The column is filled with material having precisely controlled pore sizes, and the particles are separated according to its their molecular size. Larger molecules are rapidly

washed through the column; smaller molecules penetrate inside the porous of the packing particles and elute later.

- **iv. Ion-Exchange HPLC:** The stationary phase has an ionically charged surface of opposite charge to the sample ions. This technique is used almost exclusively with ionic or ionizable samples. The stronger the charge on the sample, the stronger it will be attracted to the ionic surface and thus, the longer it will take to elute. The mobile phase is an aqueous buffer, where both pH and ionic strength are used to control elution time.[7,8]

Instrumentation



a. Solvent Reservoir : Mobile stage substance are contained in a glass reservoir. The versatile stage, or dissolvable, in HPLC is typically a blend of polar and non-polar liquid segments whose particular fixations are changed relying upon the arrangement of the specimen.

b. Pump : A pump suctions the versatile stage from the dissolvable reservoir and drives it through the framework's column and detector. Contingent upon various components including column measurements, molecule size of the stationary stage, the stream rate and sythesis of the versatile stage, working weights of up to 42000 kPa (around 6000 psi) can be created.

c. Sample Injector : The injector can be a solitary infusion or a mechanized infusion framework. An injector for a HPLC framework ought to give infusion of the liquid specimen inside the scope of 0.1–100 mL of volume with high reproducibility and under high weight (up to 4000 psi).

d. Columns: Columns are generally made of cleaned stainless steel, are in the vicinity of 50 and 300 mm long and have an inside distance across of in the vicinity of 2 and 5 mm. They are normally loaded with a stationary stage with a molecule size of 3–10 μm. Columns with interior distances across of under 2 mm are regularly alluded to as microbore HPLC columns. In a perfect world the temperature of the portable stage and the column ought to be kept steady amid an examination.

e. Detector : The HPLC indicator, situated toward the finish of the column distinguish the analytes as they elute from the chromatographic column. Regularly utilized finders are UVspectroscopy, fluorescence, mass-spectrometric and electrochemical indicators.

f. Data Collection Devices : Signals from the indicator might be gathered on outline recorders or electronic integrators that differ in many-sided quality and in their capacity to process, store and reprocess chromatographic information. The PC coordinates the reaction of the identifier to every part and places it into a chromatograph that is anything but difficult to peruse and decipher.[8,9,10]

g. Degasser

The eluent used for LC analysis may contain gases such as oxygen that are non-visible to our eyes. When gas is present in the eluent, this is detected as a noise and causes unstable baseline.Generally used method includes sparging (bubbling of inert gas), use of aspirator, distillation system, and/or heating and stirring. However, the method is not convenient and also when the solvent is left for a certain time period (e.g., during the long analysis), gas will dissolve back gradually. Degasser uses special polymer membrane tubing to remove gases. The numerous very small pores on the surface of the polymer tube allow the air to go through while preventing any liquid to go through the pore. By placing this tubing under low pressure container, it created pressure differences inside and outside the tubing (higher inside the tubing). This difference let the dissolved gas to move through the pores and remove the gas. Compared to classical batch type degassing, the degasser can be used on-line, it is more convenient and efficient. Many of new HPLC unit system contain a degasser.[10, 11]

h. Column Heater

The LC separation is often largely influenced by the column temperature. In order to obtain repeatable results, it is important to keep the consistent temperature conditions. Also for some analysis, such as sugar and organic acid, better resolutions can be obtained at elevated temperature (50 to 80°C). It is also important to keep stable temperature to obtain repeatable results even it is analyzed at around room temperature. There are possibilities that small different of temperature causes different separation results. Thus columns are generally kept inside the column oven (column heater).

Applications

The HPLC has developed into a universally applicable method so that it finds its use in almost all areas of chemistry, biochemistry, and pharmacy.[8, 9, 12]

- Analysis of drugs
- Analysis of synthetic polymers
- Analysis of pollutants in environmental analytics
- Determination of drugs in biological matrices
- Isolation of valuable products
- Product purity and quality control of industrial products and fine chemicals
- Separation and purification of biopolymers such as enzymes or nucleic acids.[13]
- Water purification
- Pre-concentration of trace components
- Ligand-exchange chromatography
- Ion-exchange chromatography of proteins
- High-pH anion-exchange chromatography of carbohydrates and oligosaccharides

Limitations

1. **Cost:** Despite its advantages, HPLC can be costly, requiring large quantities of expensive organics. [14]
2. **Complexity**
3. HPLC does have **low sensitivity** for certain compounds, and some cannot be detected as they are irreversibly adsorbed.
4. Volatile substances are better separated by gas chromatography.

CONCLUSION

It can be concluded from all reviews that HPLC is a flexible, repetitive chromatographic method for measuring drug products. It has a wide range of uses in various fields with a limited and limited range of active molecules such as all areas of chemical, biochemistry, and pharmacy.

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