A Short review on High-performance liquid chromatography (HPLC)

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ABSTRACT :-
This paper describes a separation technique that can be used to analyze compounds from various samples in high performance liquid chromatography (HPLC). Depending on the nature, chemical structure and molecular weight of the analyte, a choice of HPLC type can be made. From this point of view, various types of HPLC have appeared, which make it possible to provide qualitative and quantitative information about individual parts of the studied sample. The purpose of this chapter is to explain the principles of HPLC and some methods as a tool for the qualitative and / or quantitative determination of the components present in a given sample that can be used as fingerprints in the identification process.

I.   INTRODUCTION:-

High-performance liquid chromatography (HPLC), formerly known as high-pressure liquid chromatography, is a technique in analytical chemistry used to separate, identify, and evaluate each component of a mixture. It relies on a pump to pass a pressurized liquid solvent containing the sample mixture through a column filled with solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates of different components and causing fractionation as they exit the column. HPLC is used for industry (for example, during the manufacturing process of pharmaceuticals and biological products), law (for example, the determination of performance-enhancing drugs in urine), research (for example, the separation of components from complex biological samples or similar synthetic chemicals). , and medicine (for example, the determination of vitamin D (blood serum level)) is the goal. Chromatography can be described as a mass transfer process involving adsorption. HPLC relies on a pump to move a pressurized liquid and sample mixture through a column filled with an adsorbent, which causes the sample components to separate. The active ingredient in milk, adsorbent, is a granular material usually made of solid particles (eg silica, polymer, etc.) with a size of 2-50 μm. The components of the sample mixture are separated from each other due to the different levels of their interaction with the adsorbent particles. The pressurized liquid is usually a mixture of solvents (eg water, acetonitrile, and/or methanol) and is called the "solid phase". Its composition and temperature play an important role in the separation process by influencing the interaction between the sample components and the adsorbent. These interactions are physical in nature, such as hydrophobic (dispersive), dipole-dipole, and ionic, and are often constitutive. HPLC differs from conventional ("low pressure") liquid chromatography in that it operates at higher operating pressures (50–350 bar), whereas conventional liquid chromatography relies on gravity to force the mobile phase through the column. A small amount separated in analytical HPLC has a column size of 2.1–4.6 mm and a length of 30–250 mm. Also, HPLC columns allow small particle sizes (2–50 μm) in the average particle size. This provides high resolving power (the ability to distinguish between compounds) in the separation of mixtures, making HPLC a popular chromatographic technique. (1)

II.   METHODOLOGIEY:-

High performance liquid chromatography, or high pressure liquid chromatography (HPLC), is a chromatographic technique used in analytical chemistry and biochemistry to separate mixtures of compounds for the identification, evaluation, or purification of individual components of the mixture. Reversed-phase HPLC or Ultra-High-Performance Liquid Chromatography (UHPLC) is a commonly used separation procedure. Provides dynamic retention of hydrophobic and organic functional compounds. The combination of all-target coupling and hydrophobic and van der Waals-type interactions between the stationary and mobile phases allows these compounds to be retained in the opposite phase. Mobile phase. There

are different columns and sorbents of different particle sizes and surfaces. The mixture flows through the column at various speeds and interacts with the sorbent, also known as the stationary phase.

1) chemical properties
2) the nature of the column and
3) mobile phase composition. The time during which a specific analyte is detected in milk is called the retention time. Retention time is measured under specific conditions and is considered a defining characteristic of a given analyte. Interrogative particles can be either hydrophobic or polar in nature. Commonly used mobile phases include water and miscible mixtures of organic solvents such as acetonitrile and methanol. A non-aqueous mobile phase can also be used.

The aqueous phase of the mobile phase may contain acids such as formic, phosphoric, or trifluoroacetic acid or hydrochloric acid to ensure separation of the sample components. The composition of the mobile phase is either constant or varied during the chromatographic analysis. The continuous approach is effective for separating sample fractions that are not very similar in proximity to the stationary phase. In different approaches, the composition of the mobile phase varies from lower to higher volumes. The elongating power of the mobile phase is reflected by the analytical retention times that produce fast elution with high noise (2,3).

Principle of HPLC :-

The principle of HPLC separation is based on the distribution of the analyte (sample) between the mobile phase (eluent) and the stationary phase (milk packing material). Depending on the chemical structure of the analyte, the molecule is left behind as it passes through the stationary phase. Specific intermolecular interactions between the sample molecules and the packing material determine the time 'in the column'. Therefore, different components of the sample are selected at different times. Thus, the separation of sample components is achieved. A detection unit (eg, UV detector) determines the analytes after they leave the column. The signal is converted and recorded by a data management system (computer software) and then displayed on a chromatogram. After passing through the detector section, the mobile phase may be subjected to additional detector section, particle collection section, or waste. In general, the HPLC system includes the following modules: solvent reservoir, pump, injection valve, column, detector unit, and data processing unit (Figure 1). The solvent (euent) is delivered by the pump at high pressure and at a constant rate in the system. Constant flow and pulses from the pump are important to keep detector signal drift and noise as low as possible. The analyte eluent (sample) is provided with an injection valve (3,4).
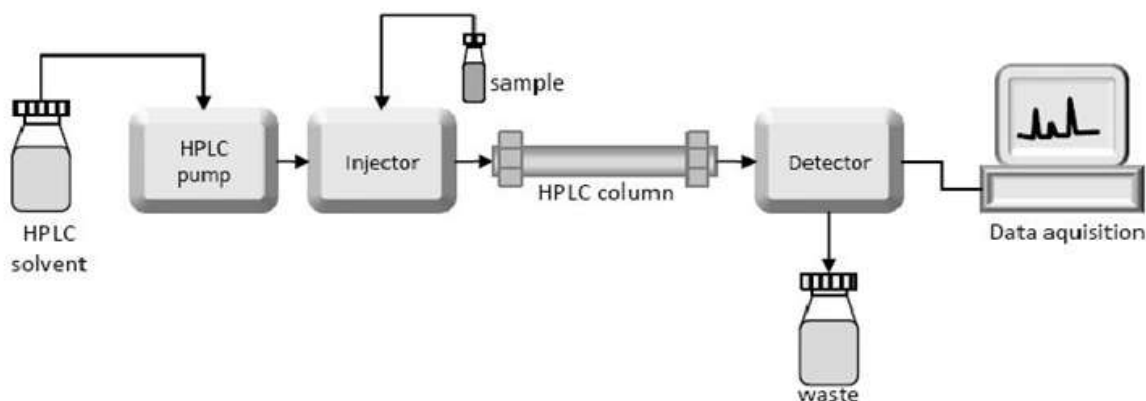

Principle of HPLC
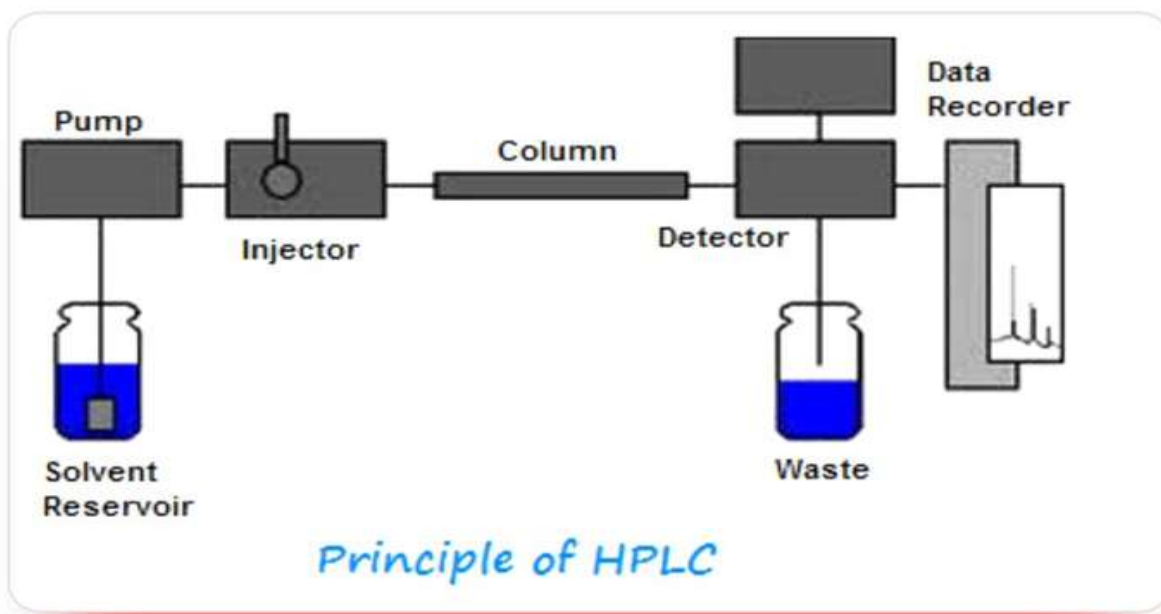
Instrumentation of High-Performance Liquid Chromatography:-

1. Pump
The development of HPLC led to the development of pump systems.

The pump is located upstream of the liquid chromatography system and draws the solvent from the reservoir into the system.

High pressure output is a "standard" requirement for the pump, which must also provide a constant pressure and flow rate that can be controlled and scaled in any situation.

Most pumps used in LC systems today create flow by moving a piston back and forth (reciprocating pump). This produces a "pulse" due to the movement of the piston.

Injector The injector is placed next to the pump. The simplest method is to use a syringe and the sample is introduced into the eluent stream. The most commonly used injection method is based on the sampling cycle. The use of the autosampler system (automatic-injector) is widely used, so that injections are repeated at certain times.

2. Milk
• Separation is done in columns.
• End columns are often made of stainless steel instead of glass columns.
• The commonly used packing material is silica or polymer gel as opposed to calcium carbonate.
The eluent used for LC varies from acidic to basic solvents.
• Most column housings are made of stainless steel because stainless steel is resistant to many different solvents.

3. Detector
• Separation of the analytes is done inside the column, and the detector is used to observe the resulting separation.
• In the absence of analyte, the eluent composition is constant. The presence of analyte changes the eluent composition. What the detector does is measure this difference.
• This difference is monitored as a type of electronic signal. There are several types of detectors.

4. Dictaphone
• The change of electrons detected by the detector is in the form of an electronic signal, which is still not visible to our eyes.

• In the past, writing pen (paper) was widely used. Today, computer-based data processors (integrators) are more common.
• There are different types of data processors; From a simple system consisting of an internal printer and word processor, it is capable of not only data acquisition, but also advanced, baseline correction, automatic concentration calculation, molecular weight determination, and much more. Such as software designed specifically for LC systems. .

5. Degasser
• The eluent used for LC analysis may contain invisible gases such as oxygen.
• If gas is present in the eluent, it is detected as noise and creates an unstable base.
• Degasser uses a special polymer membrane tube to separate the gas.
• Many pores on the surface of the polymer tube allow air to pass through, preventing liquid from passing through the holes.
• LC separation is often affected by column air.
• It is important to maintain consistent temperature conditions to obtain reproducible results.
• Better solutions can also be obtained at higher temperatures (50-80°C) for some analytes such as sugars and organic acids.

• So the column is often placed in a column oven (column heater). (5,6,7,8)

III. APPLICATION:-

1. Chemical separations: -This is based on the fact that certain compounds have different migration rates given a given column and mobile phase.

2. Purification: Purification is defined as the process of removing or removing the target compound from a mixture of compounds or impurities. Each compound shows a characteristic peak under certain chromatographic conditions

3. Determination Usually screening of compounds is done by HPLC (9).

TYPES OF HPLC :-

Depending on the substrate used, which is the stationary phase used, HPLC is divided into the following types

A. Normal Phase HPLC- This method is based on the polarity of separation. The stationary phase is polar, especially silica, and the non-polar phase is hexane, chloroform, and diethyl ether. The polar sample is placed in the column

B. Reverse Phase HPLC- Reversed to normal phase HPLC. The mobile phase is polar and the stationary phase

C. Non-polar or hydrophobic. The more non-polar nature it has, the more it will remain.

D. Quantitative HPLC- Milk will be combined with precisely controlled substrate molecules. Components are separated based on differences in molecular size.

E. Exchange HPLC- The stationary phase has an ionically charged surface opposite to the charge of the sample. The mobile phase used is an aqueous buffer to control pH and ionic strength (10,11).

Procedure :-

1. Set up a mobile phone
2. Create a component solution
3. Extraction of 7 standard solutions
4. Check the initial settings of the HPLC system.
5. With the manual injection injector handle, slowly inject 100 µL of the solution through the septum port while collecting the sample and data. Check that the data acquisition program is set to collect data for 300 s, which allows enough time for all 3 peaks to elongate through the detector.
6. Diet soda sample
7. Calculation From the concentration of the component solution, calculate the concentration of all the components in the standard based on solution (12).

ADVANTAGES :-

1. Simple, fast, reproducible.
2. Hypersensitivity.
3. High performance.
4. Fast operation and therefore save time.
5. It has high resolution and resolution capabilities.
6. Accuracy and precision.
7. The stationary phase is chemically impermeable.
8. Various stationary phases.
9. The mobile phase is not chemically internal.
10. Less need for mobile phase in the growth chamber.
11. early recovery of detached components.
12. Easy to view disconnected components.
13. Good reproducibility and reproducibility.
14. Analytical methods are important for product inspection and product quality control.
15. It is important for qualitative and quantitative analysis. Used for analytical and training purposes (13)

IV. CONCLUSION:-

From this review, it can be concluded that HPLC is a versatile and reproducible chromatographic technique for drug evaluation. It has a wide application in various fields in terms of quantitative and qualitative evaluation of active molecules. HPLC is the main analytical technique used. It has several advantages. Ultrapure compounds can be produced using HPLC. It can also be used in laboratory and clinical science. Accuracy, precision and specificity can be improved with the use of HPLC. The only drawback of HPLC is Its high cost

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