Liquid chromatography

Liquid chromatography (LC) extracts molecules in a liquid mobile phase using a stationary phase i.e. a solid or an immiscible liquid. Liquid chromatography is used for analytical or preparative applications. In column liquid chromatography, the mobile phase along with the solutes to be separated passes through the column and components in the mixture interrelate to varying degrees with the stationary phase. Based on the physical state of stationary/mobile phase combinations the technique of chromatography is classified.

Liquid chromatography methods are named according to the mechanism involved:

1) Liquid-solid chromatography (adsorption chromatography): The separation apparatus in LSC is based on the struggle of the constituents of the mixture sample for the active sites on a solid stationary phase such as silica gel.

2) Liquid-liquid chromatography (partition chromatography): The stationary solid surface is coated with another liquid (stationary phase) which is immiscible in the solvent (mobile phase). Partitioning of the sample between two phases interrupts or retains some components more than the others to effect partitioning.

3) Ion exchange chromatography: Ion-exchange chromatography is based on the competition of distinct ionic compounds in the sample for the active sites on the ion-exchange resin (stationary phase).

4) Gel permeation chromatography (exclusion chromatography): Gel permeation chromatography is a mechanical sorting of molecules on the basis of size of the molecules in solution. Small molecules are capable of infusing into more pores and are, therefore, reserved longer than large molecules.

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Liquid chromatography

- Normal phase chromatography

In normal phase chromatography, stationary phase is polar in nature while the mobile phase is non-polar, meaning polar analyte interacts more with the stationary phase hence increasing its retention time inside the column.

- Reverse phase chromatography

In reversed phase chromatography, the stationary phase is non-polar whereas the mobile phase is polar in nature. If the analyte is polar, it will interrelate less with the stationary phase hence decreasing its retention time inside the column.

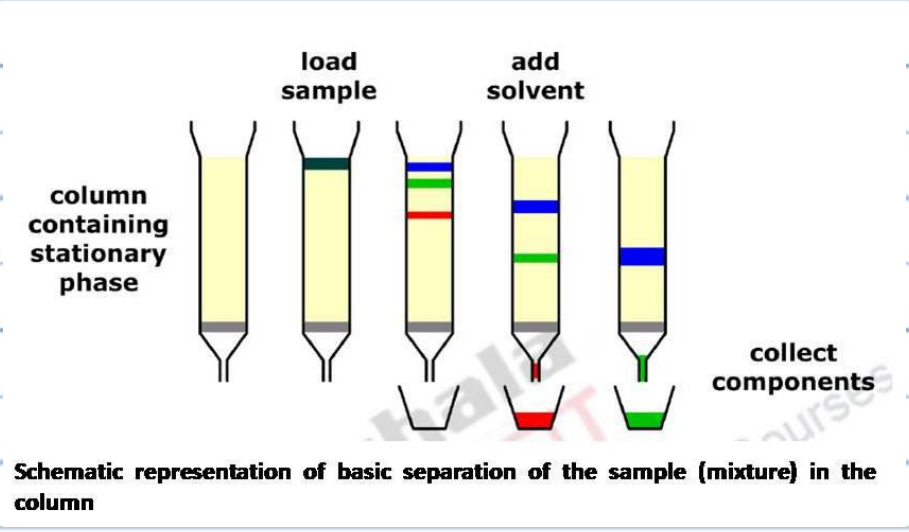
High Performance Liquid Chromatography (HPLC)

HPLC is basically an improvised variety of column chromatography. Instead of mobile phase being permitted to drip down through a column under the force of gravity, it is forced through a column under high pressures of up to 400 atmospheres, making it a much faster technique. For the column packing material, it permits the usage of much smaller particle size and that in turn helps in providing a larger surface area such that the solute can interact with the stationary phase. This promotes enhanced separation of the constituents of the mixture. Another major development over column chromatography is the use of highly sensitive detectors.

Principle of LC or HPLC

Components of interest present in the mobile phase are separated on the basis of their differing physicochemical interactions with the stationary and mobile phases. A fine adsorbent solid is chosen as the stationary phase wherein the solid is so chosen which will clamp on the outer surface of the liquid particles. The separating column is made by tightly filling the adsorbent solid generally silica into the glass tube. HPLC has high resolving power i.e. it can identify compounds with similar properties.

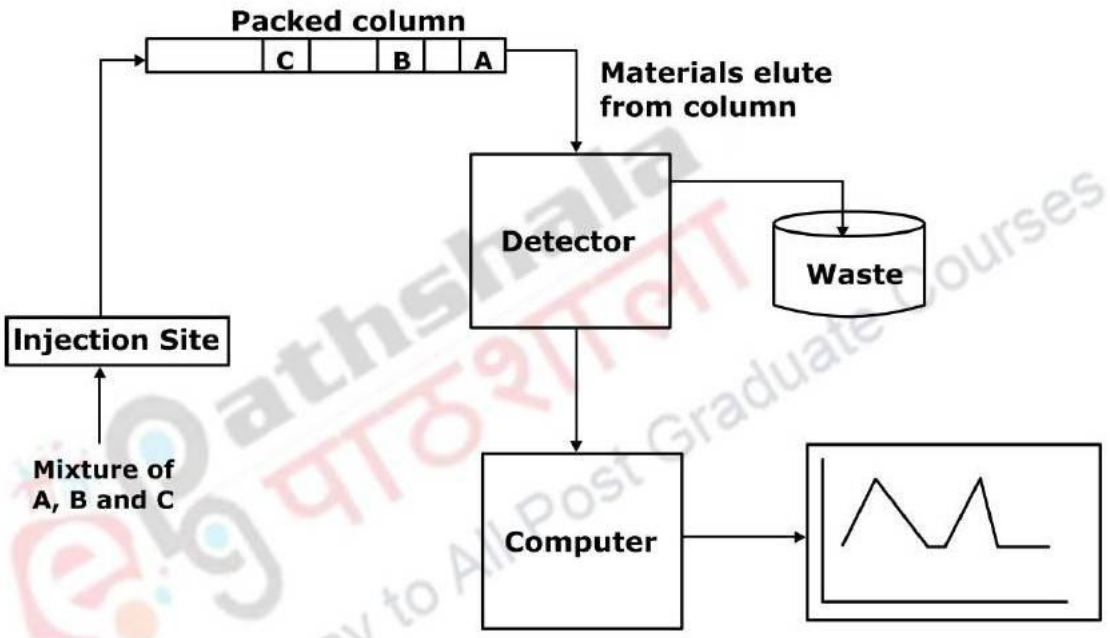
Liquid chromatography



Schematic representation of basic separation of the sample (mixture) in the column

Instrumentation and Working

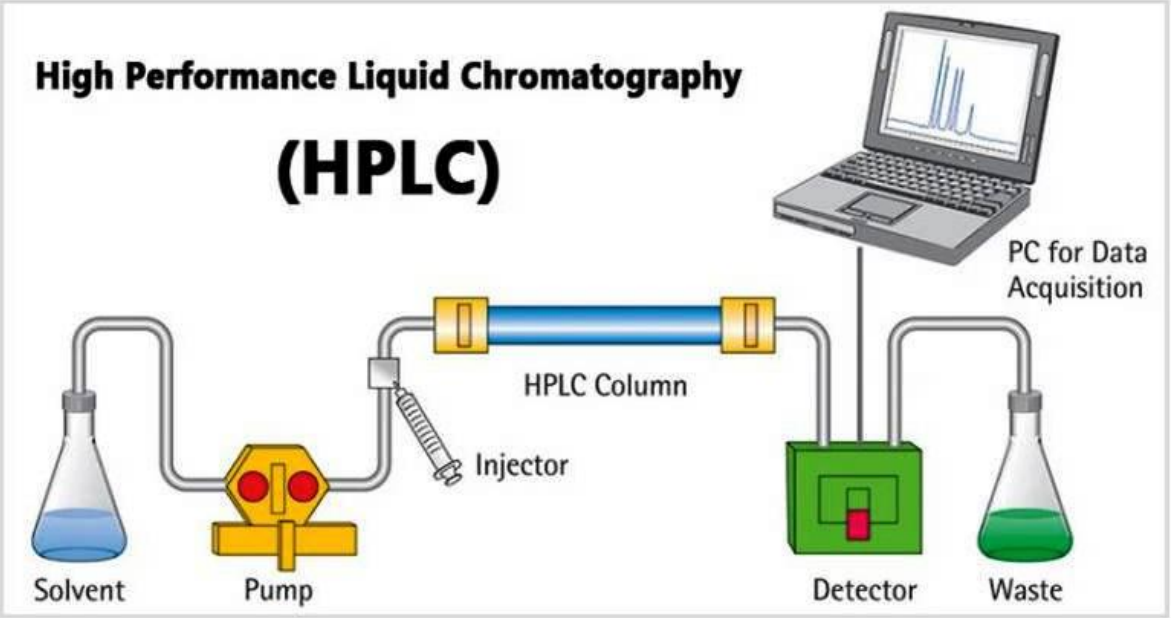
The mixture is injected at the injection port. The mobile phase carries it forward through the packed column where it separates into its components. The detector detects the separated analytes and the recorder, usually a computer records this information.



Instrumentation of Liquid Chromatography

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

The schematic representation in figure below shows HPLC instrumentation. It consists of a solvent reservoir, injector, pump, column, detector and an integrator or acquisition and display system. The pump is considered as the heart of this chromatography system.



The sample is injected through a port in the high pressure liquid carrier stream between the pump and the column. The separation takes place in the column which varies from 3-30 cm in length and 3 mm in diameter. The signal is then amplified and recorded as a detector response v/s retention time. The graph thus obtained is called **chromatogram**. The effluent may be discarded, recycled or saved for any further research or studies in a fraction collector which is synchronized with the detector.

Retention time:

The total time taken by the particular compound to travel to the detector, through the column is called as retention time. This time is initiated at the moment the sample is injected till the point at which the display shows a maximum peak height for the compound being run. Different compounds show diverse retention times.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Column Efficiency and Selectivity:

The column selectivity depends upon the partition coefficient, K, where K is given by the relation

$$K = \frac{\text{Concentration of solute in stationary phase}}{\text{Concentration of solute in mobile phase}}$$

Applications of LC and HPLC

Therapeutic Drug Monitoring and Toxicology

Measurements of Steroid Hormones

Analysis of drugs, analysis of explosives, ink analysis in case of Questioned Documents, Dyes in illicit drugs, fibers, lipstick smears, foods, and comparison of soil samples, etc

HPLC can analyze very small samples and quantify trace amounts of impurities in the drug samples.

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