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REVIEW ARTICLE ON HIGH- PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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INTRODUCTION

High-performance liquid chromatography (or High pressure liquid chromatography, HPLC) is a specific form of column chromatography generally used in biochemistry and analysis to separate, identify, and quantify the active compounds. HPLC mainly utilizes a column that holds packing material (stationary phase), a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the interactions between the stationary phase, the molecules being analyzed, and the solvent(s) used.

The sample to be analyzed is introduced in small volume to the stream of mobile phase and is retarded by specific chemical or physical interactions with the stationary phase.

A liquid sample is injected into a stream of solvent (mobile phase) flowing through a column packed with a separation medium (stationary phase). Sample components separate from one another by a process of differential migration as they flow through the column.

Chromatography is discovered by Russian–Italian botanist Mikhail Semyonovich Tswett at the commencement of the 20th century, it is a physicochemical process for partition of composite mixtures In his paper ‘On a new category of adsorption phenomena and its application to biochemical analysis’ presented on 21st March, 1903 in frequent meeting of the biology section of the Warsaw Society of Natural Sciences, Tswett gave a very detailed report of the newly discovered phenomena of adsorption-based separation of composite mixtures, which he later called ‘chromatography’. The word chromatography is a translation from Greek which means “color writing”.

Coincidentally, the Russian word “tswett” means color. He discussed in all his publications that he observed a colorful image of his first separation of plant pigments shown in Figure 1.1, so he has given that name for his new method. At that time of discovery, the chromatographic method was not appreciated among the scientists, additionally after almost 10 years when C. Dhere in Europe and L. S. Palmer.

The United States individually published the description of a similar separation process.

History of Development

Prior to HPLC scientists used standard liquid chromatographic techniques. Liquid chromatographic systems were largely inefficient due to the flow rate of solvents being dependent on gravity. Separations took many hours, and sometimes days to complete. Gas chromatography (GC) at the time was more powerful than liquid chromatography (LC), however, it was believed that gas phase separation and analysis of very polar high molecular weight biopolymers was impossible.

GC was ineffective for many biochemists because of the thermal instability of the solutes. As a result, alternative methods were hypothesized which would soon result in the development of HPLC.

Martin and Synge in 1941, it was predicted by Calvin Giddings, Josef Huber, and others in the 1960s that LC could be operated in the high-efficiency mode by reducing the packing-particle diameter substantially below the typical LC (and GC) level of 150 μm and using pressure to increase the mobile phase velocity. These predictions underwent extensive experimentation and refinement throughout the 60s into the 70s. Early developmental research began to improve LC particles, and the invention of Zipax, a superficially porous particle, was promising for HPLC technology.

The 1970s brought about many developments in hardware and instrumentation. Researchers began using pumps and injectors to make a rudimentary design of an HPLC system. Gas amplifier pumps were ideal because they operated at constant pressure and did not require leak-free seals or check valves for steady flow and good quantitation. Hardware milestones were made at Dupont IPD (Industrial Polymers Division) such as a low-dwell-volume gradient device being utilized as well as replacing the septum injector with a loop injection valve.

While instrumentational developments were important, the history of HPLC is primarily about the history and evolution of particle technology. After the introduction of porous layer particles, there has been a steady trend to reduced particle size to improve efficiency. However, by decreasing particle size, new problems arose. The practical disadvantages stem from the excessive pressure drop needed to force mobile fluid through the column and the difficulty of preparing a uniform packing of extremely fine materials. Every time particle size is reduced significantly, another round of instrument development usually must occur to handle the pressure.

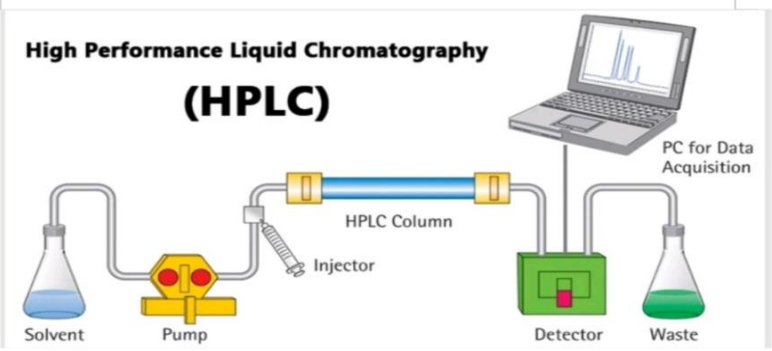
HPLC METHOD

HPLC, or high performance liquid chromatography is an amazing analytical technique for chemical compounds including biopolymers, small molecules, and polymers. In this method, a sample is first dissolved to make a solution. This solution is then injected into a "column" that contains resin that will interact with the sample. This will slow down the movement of the sample through the "column" and as the sample comes out the other side of the column, it is detected. This allows you to know both the time at which the sample comes out and the intensity of the sample that was detected. Here's an overview of this technique:

In a typical HPLC procedure you can decide the following variables:
Flow rate With fast flow peaks come out sooner but there's they're harder to resolve and tend to blend together. For more resolution, run slower. Pressure Affected by flow rate and solvent Solvent Buffer Determines signal intensity, how quickly the peaks come out, signal fidelity.

Column Type Determines the type of interaction with the sample Detection Parameters If using UV or FLD, you need to set the right excitation/emission wavelengths.

INSTRUMENTATION



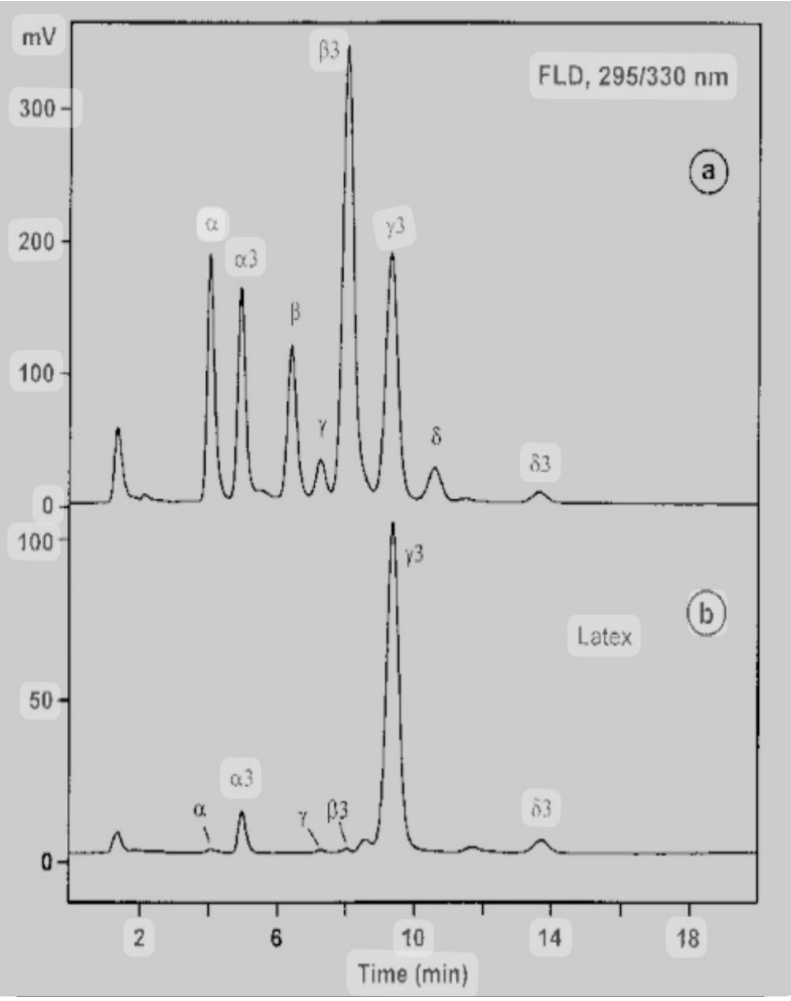
TYPES OF HPLC

1) Partition chromatography.
2) Normal–phase chromatography.
3) Displacement chromatography.
4) Reversed-phase chromatography (RPC)
5) Size-exclusion chromatography.
6) Ion-exchange chromatography.
7) Bioaffinity chromatography

1) Partition chromatography:- Partition Chromatography technique is defined as. The separation of components between two liquid phases viz original solvent and the film of solvent used in the column.

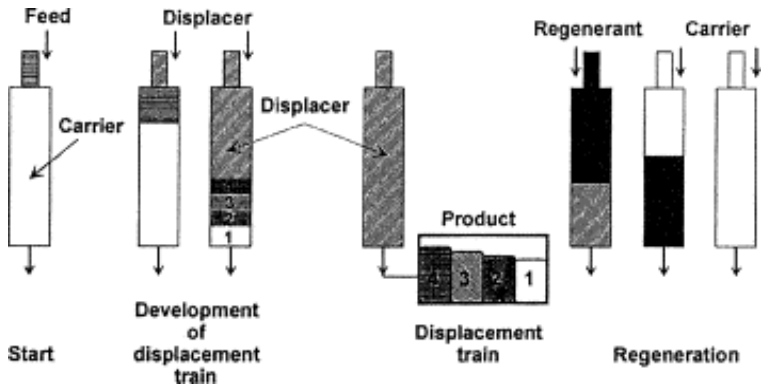
In partition chromatography, the separation of the components from the sample takes place through the process of partition the components between two phases, where both the phases are present in liquid form. In this procedure, the immiscible solid surface that is covered with the liquid surface on the stationary phase is in the mobile phase. The stationary phase immobilizes the liquid surface which ultimately changes into a stationary phase. The components are separated just after the mobile phase shifts from the stationary phase. The separation is because of the differences in partition coefficients.

2) Normal–phase chromatography:- Normal-phase liquid chromatography (NPLC) is a technique that uses columns packed with polar stationaryphases combined with nonpolar or moderately-polar mobile phases to separate the components of mixtures. In normal-phase chromatography, the stationary phase is polar and the mobile phase is nonpolar. In reversed phase the opposite is true; the stationary phase is nonpolar and the mobile phase is polar. Typical stationary phases for normal-phase chromatography are silica or organic moieties with cyano and amino functional groups.

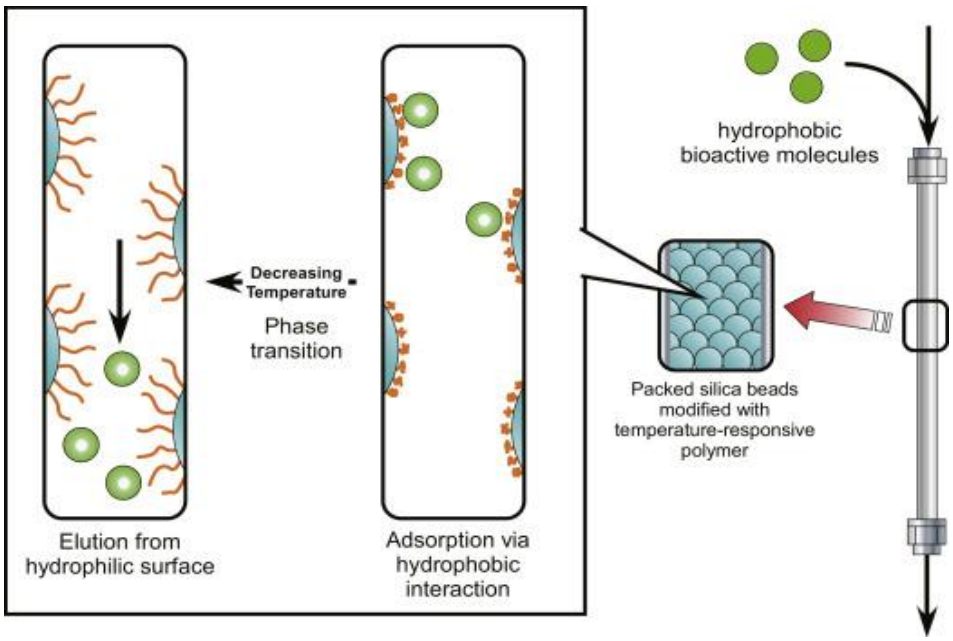


3) Displacement chromatography:- In displacement chromatography, the sample is introduced onto the column, and then displaced by a constant infusion of a displacer solution.

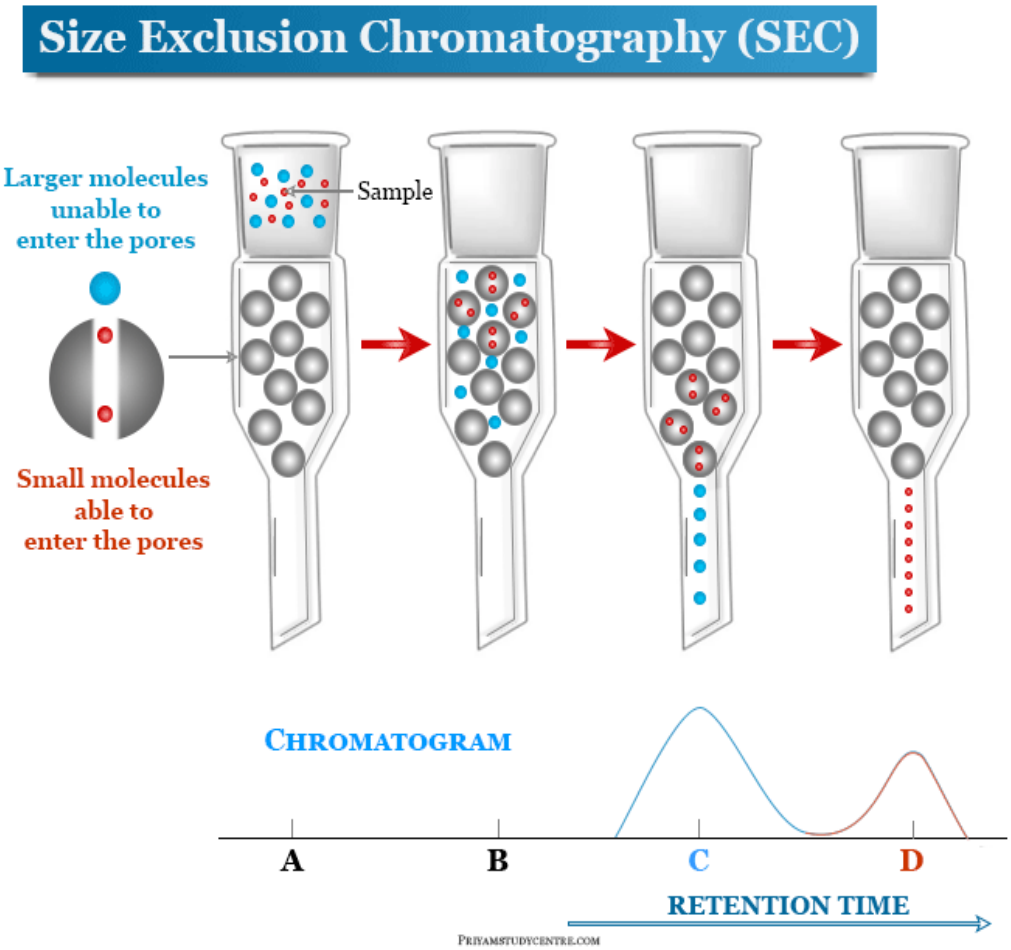
Displacement chromatography is a chromatography technique in which a sample is placed onto the head of the column[n 1] and is then displaced by a solute that is more strongly sorbed than the components of the original mixture. The result is that the components are resolved into consecutive "rectangular" zones of highly concentrated pure substances rather than solvent-separated "peaks.



4) Reversed-phase chromatography (RPC):-Reversed-phase chromatography (RPC) is a particular form of bonded-phase chromatography in which the mobile phase is more polar than the stationary phase. Reversed-phase chromatography is a technique using hydrophobic molecules covalently bonded to the stationary phase particles in order to create a hydrophobic stationary phase, which has a stronger affinity for hydrophobic or less polar compounds.



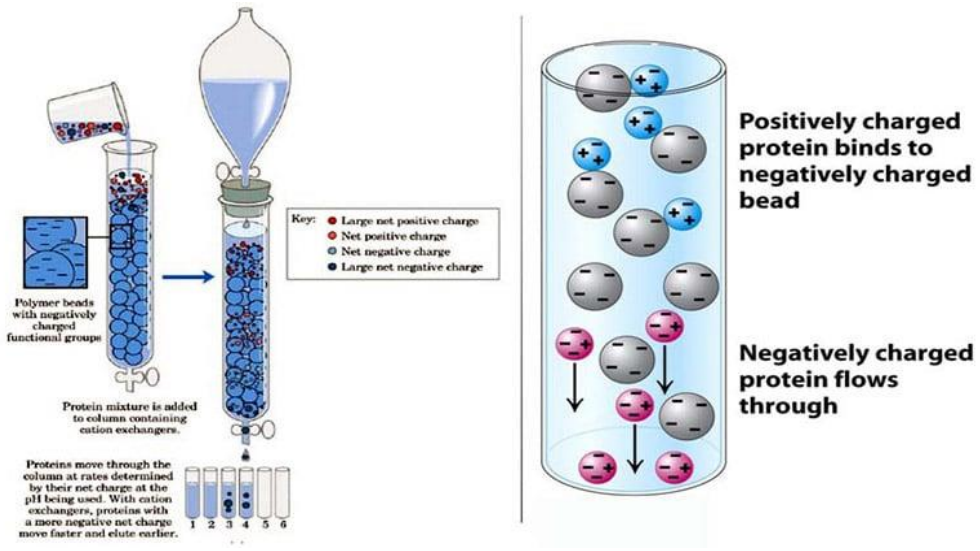
5) Size-exclusion chromatography:-Size-exclusion chromatography (SEC), also known as molecular sieve chromatography, is a chromatographic method in which molecules in solution are separated by their size, and in some cases molecular weight. Size exclusion chromatography (SEC) separates molecules based on their size by filtration through a gel. The gel consists of spherical beads containing pores of a specific size distribution. Separation occurs when molecules of different sizes are included or excluded from the pores within the matrix.



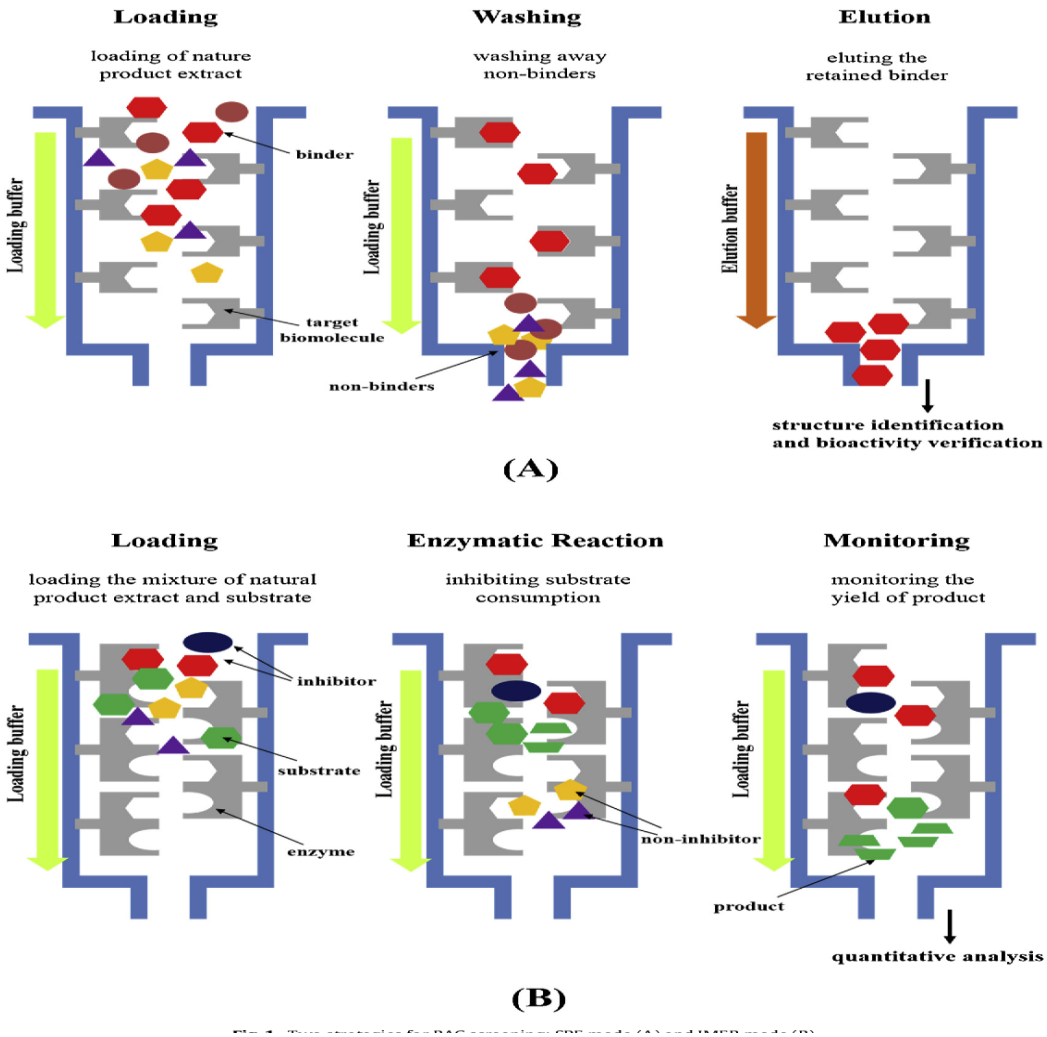
6) Ion-exchange chromatography:- Ion exchange chromatography is a technique for separating compounds based on their net charge. The ion exchangers basically contain charged groups covalently linked to the surface of an insoluble matrix.

The charged groups of the matrix can be positively or negatively charged.

When suspended in an aqueous solution, the charged groups of the matrix will be surrounded by ions of the opposite charge.



7) Bioaffinity chromatography:- Affinity chromatography is a separation method based on a specific binding interaction between an immobilized ligand and its binding partner. Examples include antibody/antigen, enzyme/substrate, and enzyme/inhibitor interactions.



This section provides an overview of affinity chromatography of proteins, general considerations for affinity purification of proteins, and provides some commonly used affinity chromatography methods.

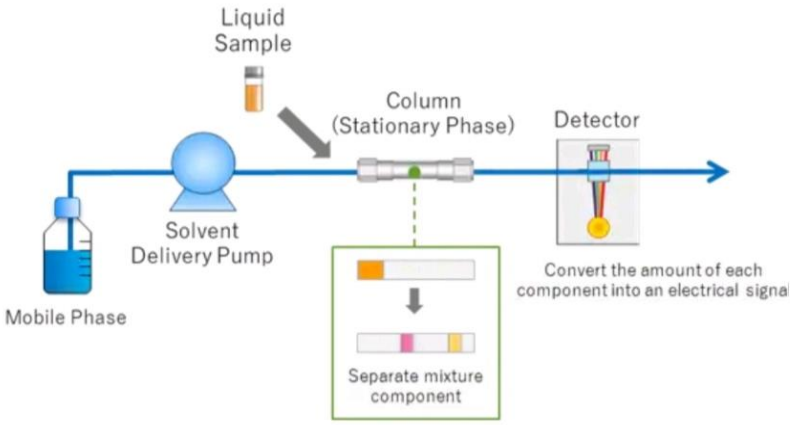
LATEST ADVANCEMENT IN HPLC

HPLC qualifies as a mature technology, especially if we focus on legacy instruments, which were designed for packed columns with 4 mm or larger ID. Today, the vast majority of applications of HPLC use such columns. However, column technology is not stagnant. Narrow capillaries are attracting attention due to improved chromatographic resolution and compatibility with MS detection. Also, the pillar and post columns on a chip are showing promise for high durability and efficiency.

As usual, column technology leads instrument development. Thermo Fisher Scientific (TFS) introduced the Vanquish product platform that features four analytical systems plus nine special purpose instruments designed for nano niche instruments, such as inverse gradient and heart cutting.

The Vanquish Core has specifications that are competitive with legacy analytical technology (~4 mm ID columns packed with 3, 5 or 10 um packing). The specifications for the Horizon and Flex instruments are compatible with UHPLC column technology. The Neo is designed for the emerging nano flow column technology.

Microflow LC is also attracting attention for at-site applications. AXcend Corp introduced upgrades to their very compact capillary LC/MS. It is designed for Process Analytical Technology (PAT) in pharmaceuticals to BTEX in various aqueous matrices. One huge advantage of capillary LC instruments is the dramatic reduction of mobile phase that is required for analysis and also post-run disposal.



APPLICATIONS

The information that can be obtained using HPLC includes identification, quantification, and resolution of a compound. Preparative HPLC refers to the process of isolation and purification of compounds. This differs from analytical HPLC, where the focus is to obtain information about the sample compound.

1) Chemical Separations:- It is based on the fact that certain compounds have different migration rates given a particular column and mobile phase, the extent or degree of separation is mostly determined by the choice of stationary phase and mobile phase.

2) Purification:- Purification is defined as the process of separating or extracting the target compound from a mixture of compounds or contaminants. Each compound showed a characteristic peak under certain chromatographic conditions. The migration of the compounds and contaminants through the column need to differ enough so that the pure desired compound can be collected or extracted without incurring any other undesired compound.

3) Identification:- Generally assay of compounds are carried using HPLC. The parameters of this assay should be such that a clean peak of the known sample is observed from the chromatograph. The identifying peak should have a reasonable retention time and should be well separated from extraneous peaks at the detection levels which the assay will be performed.

4) HPLC is used across a broad range of industries like drug development in pharma/biopharma. Precision medicine and gene therapy in proteomics research. Monitoring quality control of raw materials and finished goods in product manufacturing.

5) Quality control testing of drug

6) It qualitative and quantitave analysis.

7) Stability studies.

8) In analysis of biological fluids.

9) HPLC is used for qualitative and quantitative analysis Separation of complex molecules Prepare huge quantities of pure materials To analyze the purified compounds for trace contaminant Self-life determinations of pharmaceutical product.

CONCLUSION

- It can be concluded from the entire review that HPLC is a versatile, reproducible chromatographic technique for the estimation of drug products. It has wide applications in different fields in term of quantitative and qualitative estimation of active molecules.

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