IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 3, May 2024

A Review on High Performance Liquid Chromatography (HPLC)

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Abstract: *High-performance liquid chromatography (HPLC) is an important qualitative and quantitative technique, generally used for the estimation of pharmaceutical and biological samples. It is the most versatile, safest, dependable, and fastest chromatographic technique for the quality control of drug components. This article was prepared with a review different aspects of HPLC, such as principle types, instrumentation, and application. High- Performance Liquid Chromatography has played a significant role in clinical laboratories for the separation and quantitation of biomarkers in different body fluids. The development of HPLC involves four basic steps; scouting, optimization, robustness testing, and validation. The technique is used to analyze drugs and medicines for their purity and to maintain the highest standards for pharmaceutical products with the end goal of helping patients with medical issues. Method of validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Validation High-Performance Liquid Chromatography method as per ICH guidelines covers all the performance characteristics of validation, like Accuracy, precision, specificity, linearity, range, limit of detection, limit of quantification, robustness, system suitability the testing. The limitation of High-Performance Liquid Chromatography methods of development, public health importance, and validation is the automated process becomes complicated, has low separation power, and is expensive but High-Performance Liquid Chromatography is the modern diagnostic technique is used in all sectors.*

Keywords: HPLC, Chromatography, Mobile phase

I. INTRODUCTION

High Performance Liquid Chromatography (HPLC) was developed in the early 1960's. Today it has grown into an essential tool for the modern analytical laboratory and it has replaced gas chromatography (GC) for a variety of analyses. HPLC is a technique that is usually covered in undergraduate courses devoted to instrumental analytical methods. In its applications to food analysis, the technique has gained increased acceptance mainly because it met two basic factors, namely: i) the need for a wide range of rapid analyses for nutrients; and ii) the need for methods that can be easily automated. In spite of those notable advantages, the integration of HPLC in the food laboratory has been slow compared to other areas like pharmaceutical chemistry and forensic toxicology. This might be due to factors such as the complexity of the matrices found in most food systems and the very low levels at which many of the components of interest are found in foodstuffs. However, today a multitude of analyses performed by HPLC exist and are recognised by the Association of Official Analytical Chemists. The technique certainly holds a promising future for food analysis. **[1]**

HPLC

- High performance liquid chromatography or commonly known as HPLC is an analytical technique used to separate, identify or quantify each component in a mixture.
- 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.

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- 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.
- Basic terms of HPLC.
- Eluent – means mobile phase only.
- Elute- mobile phase and solute.
- Effulents – means mobile phase leaving the column.[**2-3]**

PRINCIPLE

Principle of High-Performance Liquid Chromatography (HPLC) 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**. [6]**

Types of HPLC

Different Types of HPLC Columns Used in Analysis

- Different types of HPLC columns are used in analysis of different pharmaceutical compounds according to their nature and column separation capacity.
- Columns the main component in HPLC because the column is responsible for the separation of the sample components. The sample passes through the column with the mobile phase and separates in its components when it comes out from the column.
- Generally, silica gel is filled in the high performance liquid chromatography columns because of its particle size and orosi that helps in separation of components and silica gel is also an inert material that does not react with mobile phases.
- Therefore silica columns can be used to analyze the compounds of different chemical natures. The material filled in the HPLC columns is known as a stationary phase.
- There are different types of chromatography columns on the basis of their composition and method of are described separation. Some of them here,

Normal Phase Columns:

1) Normal Phase HPLC Columns
2) Reverse Phase Columns
3) Ion Exchange Columns
4) Size Exclusion Columns

Normal Phase HPLC Columns:

This type of columns has more polar stationary phase than the mobile phase. The packing material of the column should be more polar than the mobile phase and this condition is fulfilled by the silica that is polar material. But water is more polar than the silica, therefore, water is not used and methylene chloride, hexane and chloroform or a mixture of these with diethyl ether is used as mobile phase. • Separation of the sample components occurs on the basis of the polarity of the sample components. Sample components having more polarity interact more with polar stationary phase resulting in separation from the less polar component that interacts with less polar mobile phase. Silica columns are widely used in

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the pharmaceutical analysis. The chromatography column packing in which normal phase columns are used is known as Normal Phase Chromatography.

Reverse Phase HPLC Columns:

In reverse phase columns as its name states, it is reverse of the normal phase columns. It has a non-polar or less polar stationary phase than the more polar mobile phase. Bonded hydrocarbons like C8 and C18 and other non-polar hydrocarbons are used as stationary phase in reverse phase columns while aqueous organic solution like water-methanol or water-acetonitrile mixture is used as mobile phase.

Separation of sample components in reverse phase columns also occurs on the basis on the polarity of the sample components but it happens just opposite of the normal phase HPLC columns, therefore, this type of chromatography is known as Reverse Phase Chromatography.

Ion Exchange HPLC Columns:

The compounds those can easily ionize are analyzed using these columns. Stationary phase in these columns remains acidic or basic having negative or positive charge while mobile phase is a polar liquid as the salt solution in water. Separation of molecules occurs on the basis of the attractive ionic force between molecules and the charged stationary phase. Due to the exchange of ions during the separation of sample components, it is known as lon Exchange Chromatography.

Size Exclusion HPLC Columns:

Porous stationary phase in these columns allows the separation of the components according to their size. Combination of polymers like polysaccharides and silica is used as stationary phase in these columns. Small sample molecules penetrate in the pores of stationary phase while the large molecules penetrate partially into the pores. • Therefore the large molecules of the sample elute first than the small molecules and this chromatography is called Size columns are generally not used in the analysis of pharmaceutical compounds.

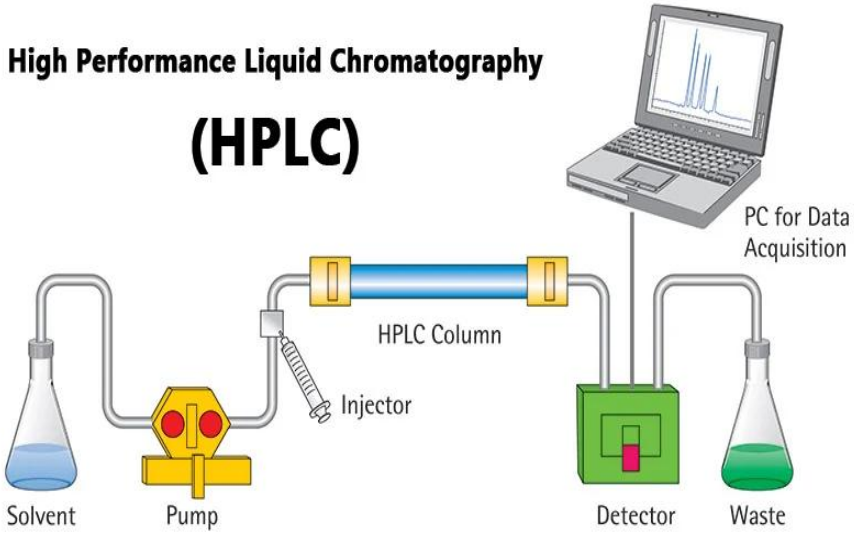

Fig.1: Instrumentation of High-Performance Liquid Chromatography (HPLC) Instrumentation of HPLC

The Pump:

The development of HPLC led to the development of the pump The pump is positioned in the most upper stream of the liquid chromatography system and generates a flow of eluent from the solvent reservoir into the system. High-pressure generation is a "standard" requirement of pumps besides which, it should also to be able to provide a consistent pressure at any condition and a controllable and reproducible flow rate. Most pumps used in current LC systems

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generate the flow by back-and-forth motion of a motor-driven piston (reciprocating pumps). Because of this piston motion, it produces "pulses".

Injector:

An injector is placed next to the pump. The simplest method is to use a syringe, and the sample is introduced to the flow of eluent. The most widely used injection method is based on sampling loops. The use of the auto sampler (auto-injector) system is also widely used that allows repeated injections in a set scheduled-timing.

Column:

The separation is performed inside the column. The recent columns are often prepared in a stainless steel housing, instead of glass columns. The packing material generally used is silica or polymer gels compared to calcium carbonate. The eluent used for LC varies from acidic to basic solvents. Most column housing is made of stainless steel since stainless is tolerant towards a large variety of solvents.

Detector:

Separation of analytes is performed inside the column, whereas a detector is used to observe the obtained separation. The composition of the eluent is consistent when no analyte is present. While the presence of analyte changes the composition of the eluent. What detector does is to measure these differences. This difference is monitored as a form of an electronic signal. There are different types of detectors available.

Recorder:

The change in eluent detected by a detector is in the form of an electronic signal, and thus it is still not visible to our eyes. In older days, the pen (paper)-chart recorder was popularly used. Nowadays, a computer-based data processor (integrator) is more common. There are various types of data processors; from a simple system consisting of the in-built printer and word processor while those with software that are specifically designed for an LC system which not only data acquisition but features like peak-fitting, baseline correction, automatic concentration calculation, molecular weight determination, etc.

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 noise and causes an unstable baseline. 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.

Column Heater:

The LC separation is often largely influenced by the column temperature. In order to obtain repeatable results, it is important to keep consistent temperature conditions. Also for some analysis, such as sugar and organic acid, better resolutions can be obtained at elevated temperatures (50 to 80°C). Thus columns are generally kept inside the column oven (column heater). [**4-5**]

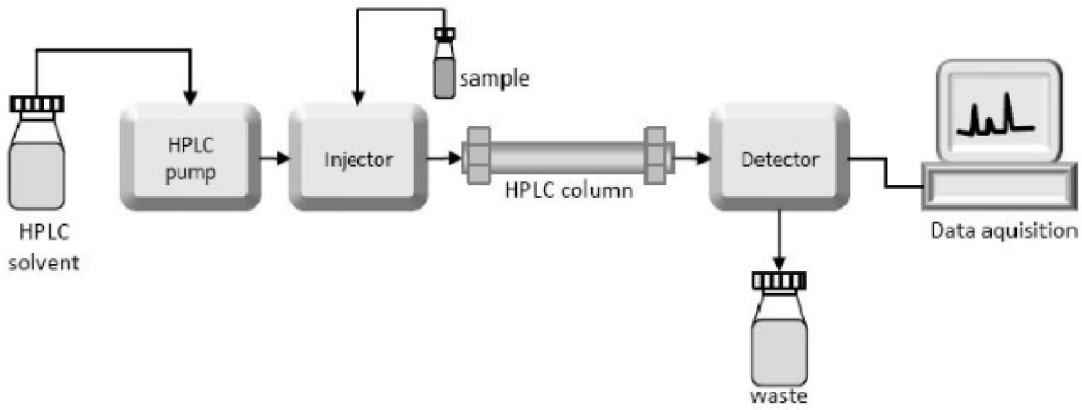

Fig. 2 HPLC basic instrumentation

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Advantages of High-Performance Liquid Chromatography (HPLC)

- Speed
- Efficiency
- Accuracy
- Versatile

And extremely precise when it comes to identifying and quantifying chemical components
Limitations:
Cost: Despite its advantages, HPLC can be costly, requiring large quantities of expensive organics.
Complexity
HPLC does have low sensitivity for certain compounds, and some cannot be detected as they are irreversibly adsorbed.
Volatile substances are better separated by gas chromatography.

Applications:

- Drug Discovery
- Clinical Analysis Proteomics
- Forensic Chemistry
- Drug Metabolism study
- Environmental chemistry
- Diagnostic studies
- Cosmetic analysis
- Determination of Green Florescent Protein
- Structural Determination
- Pharmaceutical Applications
- Identification of Bile Acid Metabolite.

Clinical Applications:

- Biochemical Genetics
- qualitative and quantitative analysis
- Therapeutic Drug Monitoring. [**7-8]**

Other applications of HPLC:

Other applications of HPLC includes

Pharmaceutical applications [9-12]

- Tablet dissolution study of armaceutical dosages form.
- Shelf-life determinations of parmaceutical products
- Identification of active ingredients of dosage forms
- Pharmaceutical quality control

Environmental applications [13-16]

- Detection of phenolic compounds in Drinking Water
- Identification of diphenhydramine in sedimented samples
- Bio-monitoring of pollutant

Forensics [17-19]

- Quantification of the drug in biological samples.
- Identification of anabolic steroids in serum, urine, sweat, and hair
- Forensic analysis of textile dyes.
- Determination of cocaine and metabolites in blood

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Clinical [20-23]

- Quantification of ions in human urine Analysis of antibiotics in blood plasma.
- Estimation of bilirubin and bilivirdin in blood plasma in case of hepatic disorders.
- Detection of endogenous neuropeptides in extracellular fluids of brain.

Foodand Flavor [24]

- Ensuring the quality of soft drink and drinking water.
- Analysis of beer.
- Sugar analysis in fruit juices.
- Analysis of polycyclic compounds in vegetables.
- Trace analysis of military high explosives in agricultural crops.

II. CONCLUSION

The proposed HPLC method provide simple, specific, precise, accurate, and reproducible quantitative analysis for simultaneous analysis of esomeprazole and tadalafil in pharmaceutical formulation.

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