

A Review on High Performance Liquid Chromatography

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Abstract: High performance liquid chromatography (HPLC) has been widely used for years as an analytical method and is a key tool for the separation and analysis of pharmaceutical drugs, for drug monitoring and for quality assurance and life science research. HPLC is commonly used for Separation and analysis of non-volatile compounds. Due to the flexibility of this technique numerous applications have been adopted for routine use in the pharmaceutical industries, research laboratories, analytical laboratories, clinical laboratories and also colleges. In order to make a qualitative assessment of the compound and quantitative analysis of the compound in Pharmaceuticals products such as aspirin, ibuprofen, paracetamol, Salts like sodium chloride and potassium phosphate, Organic chemicals like polymers (e.g. polystyrene, polyethylene), Proteins like egg white or blood protein, Heavy hydrocarbons like asphalt or motor oil, Many natural products such as ginseng, herbal medicines, plant extracts and Thermally unstable compounds such as trinitrotoluene (TNT) and also clinical chemistry HPLC is regularly used technique

Keywords: High performance liquid chromatography, Pharmaceutical impurity profiling, Pharmaceutical quality control, stationary phase, pharmaceutical drugs

I. INTRODUCTION

HPLC is an abbreviation for High Performance Liquid Chromatography. "Chromatography" is a technique for separation, "chromatogram" is the result of chromatography, and "chromatograph" is the instrument used to conduct chromatography. Among the various technologies developed for chromatography, devices dedicated for molecular separation called columns and high-performance pumps for delivering solvent at a stable flow rate are some of the key components of chromatographs.

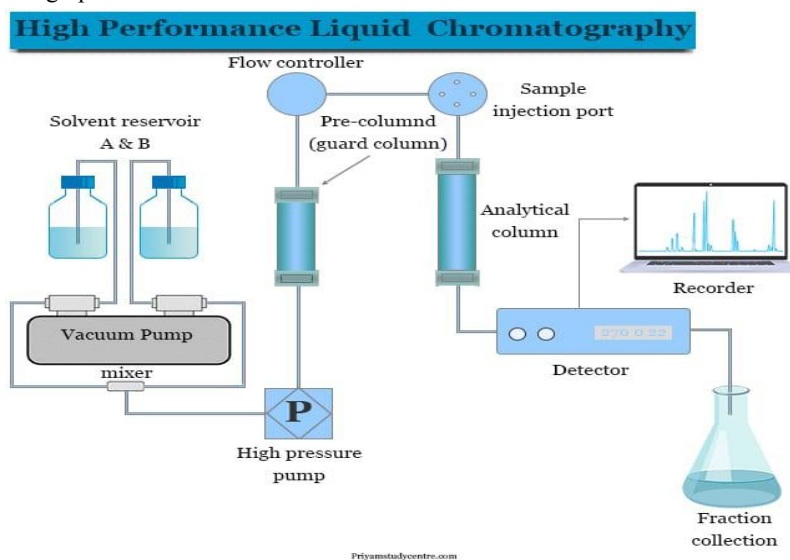


Fig 1:- High Performance Liquid Chromatogram

As related technologies became more sophisticated, the system commonly referred to as High Performance Liquid Chromatography, simply became referred to as "LC". Nowadays, Ultra High Performance Liquid Chromatography (UHPLC), capable of high-speed analysis, has also become more wide-spread. Only compounds dissolved in solvents can be analyzed with HPLC. HPLC separates compounds dissolved in a liquid sample and allows qualitative and quantitative analysis of what components and how much of each component are contained in the sample. It works on the principle of hydrophobic interactions hence the more nonpolar the material is, the longer it will be retained. Reversed-phase HPLC is by far the most popular mode chromatography. Almost 90% of all analyses of low molecular weight samples are carried out using RP- HPLC. The subsequent table provides a summary of basic analysis parameters such as Drug, column type, mobile phase composition, flow rate and detector type of a huge number of pharmaceutical drugs⁽¹⁾

TYPES OF HPLC²

Depending on the stationary phase system, the HPLC technique is of the following types:

1. Normal Phase HPLC (NP-HPLC): This method utilise a polar stationary phase and a non-polar mobile phase for the separation of mixture components on the basis of polarity. Silica is generally used as the stationary phase and hexane, methylene chloride, chloroform, diethyl ether, and mixtures of these are the selected mobile phases.
2. Reverse Phase HPLC (RP-HPLC or RPC): This method utilise a non-polar stationary phase and an aqueous, moderately polar mobile phase. RPC is based on the principle of hydrophobic interactions, resulting from repulsive forces between a polar eluent, the relatively non-polar component, and the non-polar stationary phase.
3. Size-Exclusion HPLC (SEC) or Gel Permeation or Filtration Chromatography: This method involves separation of particles based on their size. It is also used to determine the tertiary and quaternary structures of proteins and amino acids. This method is highly useful for the determination of molecular weight of polysaccharides.
4. Ion-Exchange HPLC: In this method, retention occurs according to the attraction between the solute ions and charged sites bound to the stationary phase, excluding the similarly charged ions. This chromatography is widely used in purifying water. ligand exchange chromatography, ion-exchange chromatography of proteins, high- pH anion exchange chromatography of carbohydrates and oligosaccharides,

INSTRUMENTATION OF HPLC³

- Instrumentation: Ultra-high pressure LC (UHPLC) going mainstream.
- Columns : Sub-2 μm , sub-3 μm core-shell and hybrid particles; novel bonding chemistries; hydrophilic interaction chromatography (HILIC); immobilized polysaccharide chiral phases; columns for biomolecules and biopharmaceuticals.
- Others: Liquid chromatography – Mass Spectrometry (LC/ MS) - particularly HighResolution MS (HRMS) or Hybrid MS; Charged Aerosol Detector (CAD); Automated Method Development Systems (AMDS) yet comprehensive update of important HPLC developments, with each topic supported by brief descriptions of practical benefits/applications, critical commentaries from a user's perspective, and key references

Ultra-high pressure Liquid Chromatography (UHPLC):-

The "revolution" in ultra-high pressure LC (UHPLC) began in 1997 with the proof-of-concept study by Professor James Jorgenson , followed by the first commercial system introduced in 2004 . Today, the transformation from HPLC to UHPLC is mostly complete with all major manufacturers having some type of UHPLC offerings. Detailed reviews of UHPLC systems, columns and applications are available elsewhere . Fundamentals, benefits, potential issues and best practices of UHPLC in pharmaceutical analysis are well documented .

The benefit of faster analysis of UHPLC

In a method transfer case study for a drug product.

Using a geometrical scaling of column and operating parameters from HPLC to UHPLC, a reduction of analysis time up to tenfold with similar resolution, is not unusual.

This benefit of “faster analysis with good resolution” provides the primary incentive for most users to consider the purchase of the more expensive UHPLC equipment.

APPLICATION OF HPLC⁴

HPLC industry Application

There is a wide variety of applications throughout the process of creating a new drug from drug discovery to the manufacture of formulated products that will be administered to patients. This Process to create a new drug can be divided into 3 main stages ;-

1. Drug discovery
2. Drug development
3. Drug manufacturing.
4. Pharmaceutical applications:
5. Tablet dissolution study of the pharmaceutical dosage form.
6. To control drug stability, Shelf-life determination.
7. Identification of active ingredients.
8. Pharmaceutical quality control.
9. Tablet dissolution of pharmaceutical dosage forms.

Food and Flavor analysis

1. Rapid screening and analysis of components in nonalcoholic drinks.
2. Measurement of quality of soft drinks and water.
3. Sugar analysis in fruit juices.
4. Analysis of polycyclic compounds in vegetables.
5. preservative analysis.
6. Multiresidue analysis of lots of pesticides in food samples by LC triple quadrupole MS.

Environmental applications:-

1. Detection of phenol compounds in drinking water.
2. Identification of diphenhydramine in sedimented samples.
3. Bio-monitoring of pollutant.

II. HPLC SYSTEM INFORMATION ⁶

1. Hardware: Dionex-Thermo Fisher Ultimate 3000 w/ auto-sample(HPLC #2), P-680 pumpTCC-100 Column. Compartment, Variable Wavelength Detectors.
1. Consumables: Analytical column Bio-Rad Aminex HPX-87H.(order#125-0140) (ion.exclusion); Guardcolumnis a Bio-Rad Micro-Guard Cation-H cartridges (order.#1250129);.column typically operates at 50°C.
2. The column type is specific to the analytes. The 87H column is a hydrogen-form column used for analysis of carbohydrates in solution with carboxylic acids, volatile fatty acids, short chain fatty acids, alcohols, ketones, and many neutral metabolic by-products. Most often used for organic acid analysis, this column is also useful for fermentation monitoring, biological fluid analysis, and acetylated sugar separations.
3. High-performance liquid chromatography (HPLC) formerly referred to as high pressure liquid chromatography, Is a technique in analytical chemistry use to separate , identify , and quantify each component in a mixture.

III. NEW TREND HPLC STUDY⁷

Today, chromatography is the backbone of separation science and is being used in all research laboratories and pharmaceutical industries of the world. The journey of chromatography was started first by Mikhail Tswett. (Russian botanist) in 19031. Chromatographic process can be defined as separation technique involving mass-transfer between

stationary phase and mobile phase. In these chromatography techniques, HPLC is one of the chromatographic techniques, which is mostly used analytical technique. In This method stationary phase can be a liquid or a solid phase. HPLC utilizes a liquid mobile phase to separate the components of a mixture. Highperformance liquid chromatography (HPLC) is the term used to describe liquid chromatography in which the liquid mobile phase is mechanically pumped through a column that contains the stationary phase. An HPLC instrument, therefore, consists of an injector, a pump, a column, and a detector⁽⁷⁾

IV. CONCLUSION

High performance liquid chromatography is just the premier technique for Trace analysis of organic and inorganic compounds. Determination of trace compounds is very important in pharmaceutical, biological, toxicology and environmental studies since even a trace substance can be harmful or poisonous.

HPLC is applied for molecular weight determination, in analytical chemistry, pharmaceutical and drug science, clinical sciences, food technology, and consumer products, combinatorial chemistry, polymer chemistry, environmental chemistry and green chemistry.

The role of HPLC in the pharmaceutical industry is very vital particularly in pre formulation, process development, during formulation development and drug discovery and to verify drug purity. All the work which has it being done in pharmaceutical substances, Preparation of pure compounds, trace analysis, food safety where we have to analyze for pesticides and toxic chemicals founds in food and food products all of these things are done routinely and daily rapidly by high performance liquid chromatography or GC.

An important role of chromatography is the QC of the quality of foods but of drugs controlling the raw materials and control the finished products ensuring the safety of the people, we are so dependent in the world today on chemicals, minimum synthetic chemicals made by chemist which have this mixed blessing and I think primarily of pesticides very good for agriculture, very harmful for humans if they happen to ingest these pesticides.

So, HPLC is the best separation technique for quantitative trace analysis of toxic chemicals¹⁸ ,impurities, Manufacturing of high pure products, medicinal uses, and research purpose.

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