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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY: A FUTURE TOOL IN QUALITY CONTROL- A REVIEW

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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 an aim to review different aspects of HPLC, such as principle, types, instrumentation and application. High performance liquid chromatography is one of the most widely used tools to identify and quantify potency in drug substances and drug products. Analytical method development and validation are two very critical processes performed before release of a method for use in Quality Control department.

KEYWORDS: High performance liquid chromatography, instrumentation, elution, applications, mobile phase, Assay, Method development, Forced Degradation, Method Validation.

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. The amount of retardation depends on the nature of the analyte and composition of both stationary and mobile phase. The time at which a specific analyte elutes (comes out of the end of the column) is called the retention time. Common solvents used include any miscible combinations of water or organic liquids (the most common are methanol and acetonitrile). Sepration has been done to vary the mobile phase composition during the analysis; this is known as gradient elution. Despite the advances in technology, increased understanding of the analytical process and excellent softwares available for HPLC method development, there is an element of trial anderror in HPLC method development. This can be reduced if not eliminated by proper planning. This article focuses on the process of developing a robust and stability indicative RP HPLC assay method for a pharmaceutical formulation by HPLC.

Types of HPLC

Types of HPLC generally depend on phase system used in the process. Following types of HPLC generally used in analysis

Normal phase chromatography

Also known Normal phase HPLC (NP-HPLC), this method separates analytes based on polarity. NP-HPLC uses a polar stationary phase and a non-polar mobile phase. The polar analyte interacted with and is retained by the polar stationary phase. Adsorption strengths increase with increased analyte polarity, and the interaction between the polar analyte and the polar stationary phase increases the elution time.

Reversed phase chromatography

Reversed phase HPLC (RP-HPLC or RPC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. RPC operates on the principle of hydrophobic interactions, which result from repulsive forces between a polar eluent, the relatively non-polar analyte, and the non-polar stationary phase. The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand in the aqueous eluent.

Size exclusion chromatography

Size exclusion chromatography (SEC), also called as gel permeation chromatography or gel filtration chromatography mainly separates particles on the basis of size. It is also useful for determining the tertiary structure and quaternary structure of proteins and amino acids. This technique is widely used for the molecular weight determination of polysaccharides.

Ion exchange chromatography

In Ion exchange chromatography, retention is based on the attraction between solute ions and charged sites bound to the stationary phase. Ions of the same charge are excluded. This form of chromatography is widely used in purifying water, Ligandexchange chromatography, Ion-exchange chromatography of proteins, High-pH anion-exchange chromatography of carbohydrates and oligosaccharides, etc.

Bio-affinity chromatography

Separation based on specific reversible interaction of proteins with ligands. Ligands are covalently attached to solid support on a bio-affinity matrix, retains proteins with interaction to the column-bound ligands. Proteins bound to a bioaffinity column can be eluted in two ways:

- **Biospecific elution:** Inclusion of free ligand in elution buffer which competes with column bound ligand.
- **Aspecific elution:** Change in pH, salt, etc. which weakens interaction protein with column-bound substrate. Because of specificity of the interaction, bioaffinity chromatography can result in very high purification in a single step (10 - 1000-fold).

Parameters

For the accurate analysis of a compound, there are some parameters which are used as a standard for a particular compound. If there is a change occurs in the parameters the result may be affected greatly. The most commonly used parameters are internal diameter, particle size, pore size, pump pressure. For different compounds the parameters can be changed according to their nature and chemical properties.

Internal diameter

The internal diameter (ID) of an HPLC column is a critical aspect that determines quantity of analyte that can be loaded onto the column and also influences sensitivity. Larger columns are usually seen in industrial applications such as the purification of a drug product for later

use. Low ID columns have improved sensitivity and lower solvent consumption at the expense of loading capacity.

Particle size

Most traditional HPLC is performed with the stationary phase attached to the outside of small spherical silica particles (very small beads). Smaller particles generally provide more surface area and better separations, but the pressure required for optimum linear velocity increases by the inverse of the particle diameter squared.

Pore size

Many stationary phases are porous to provide greater surface area. Small pores provide greater surface area while larger pore size has better kinetics especially for larger analytes. Pore size defines an ability of the analyte molecules to penetrate inside the particle and interact with its inner surface. This is especially important because the ratio of the outer particle surface to its inner one is about 1:1000. The surface molecular interaction mainly occurs on the inner particle surface.

Pump pressure

Pumps vary in pressure capacity, but their performance is measured on their ability to yield a consistent and reproducible flow rate. Modern HPLC systems have been improved to work at much higher pressures, and therefore be able to use much smaller particle sizes in the columns (< 2 micrometres).

Instrumentation

1. Solvent delivery system
2. Pump Solvent degassing system
3. Sample injector
4. Guard column Analytical column
5. Detector Recorders and integrators

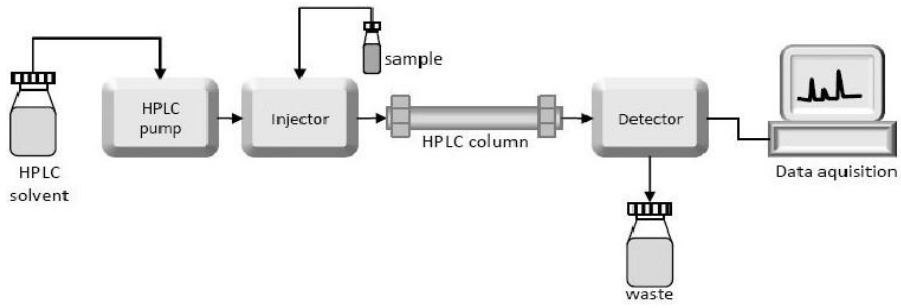

Figure 1: Parts of HPLC System.

1. Solvent delivery system

The mobile phase is pumped under pressure at a high pressure at about 1000 to 3000 psi; from one or several reservoirs and flows through the column at a constant rate. With micro particulate packing, there is a high pressure drop across a chromatography column. Eluting power of the mobile phase is determined by its overall polarity, the polarity of the stationary phase and the nature of the sample components. For normal phase separations eluting power increases with increasing polarity of the solvent but for reversed phase separations, eluting power decreases with increasing solvent polarity. Some other properties of the solvents, which need to be considered for a successful separation, are boiling point, viscosity, detector compatibility, flammability and toxicity. Optimum separating conditions can be achieved by making use of mixture of two solvents.

2. Pump

The most important component of HPLC in solvent delivery system is the pump, because its performance directly effects the retention time, reproducibility and detector sensitivity. The particle size of the stationary phase is 5-10 μm. So resistance to flow is observed. This is the reason that high pressure is required and this is provided by using pumps. The different types of pumps include: Constant pressure pump which uses a constant pressure to the mobile phase; the flow rate through the column is determined by the flow resistance of the column and any other restrictions between the pump and the detector outlet. Constant flow pump generates a given flow of liquid, so that the pressure developed depends on the flow resistance.

Constant pressure pumps

The simplest type of constant pressure pump is the pressurized coil pump or gas displacement pump. consisting of some form of pneumatic device for the direct pressurization of the mobile phase with an inert gas, give a reliable pulse-free flow and have the advantage of low cost and simplicity. They are however not as accurate as constant volume pumps but can be used where flow accuracy and reproducibility are less critical. But this is now only of historical interest.

Constant Flow/Volume (Constant displacement)

Pumps If a constant flow pump is used, changes in the permeability of the system, caused by settling or swelling of the packing, or viscosity changes in the mobile phase (due to temperature fluctuations or composition changes) are compensated for by pressure changes

and the flow rate remains constant. Since flow changes cause non reproducible retention times, adversely affect resolution, and give unstable base-lines, the constant volume pump provides a more precise analysis. It is particularly useful when gradient elution is used. There are two main types of constant volume pumps: single stroke (syringe type) pumps, and reciprocating pumps having either a diaphragm or a piston. The syringe type pump consists of a syringe the plunger of which is driven by a stepping motor through a gear box. The rate of delivery from the syringe is controlled by varying the voltage on the motor. The main advantage of this type of pump is that it is capable of providing a pulse-free flow at high pressure and the flow rate is independent of the operating pressure, if the compressibility of the liquid is ignored. Its main disadvantage is that it has a finite solvent capacity. Dual syringe systems found in some instruments are of course expensive, but with suitable gradient formers they also provide the gradient elution capacity. Due to its high cost and lack of flexibility this type of pump is little used in today's commercial instruments.

Check valves

These are present to control the flow rate of solvent and back pressure. Pulse dampeners These are used to dampen the pulse observed from the wavy baseline caused by the pumps.

Solvent degassing system

The constituents of the mobile phase should be degassed and filtered before use because several gases are soluble in organic solvents. When solvents are pumped at high pressure, gas bubbles are formed which will interfere with the separation process. Numerous methods are employed to remove the dissolved gases in the mobile phase. They include heating and stirring, vacuum degassing with an aspirator, filtration through 0.45 filters, vacuum degassing with an air-soluble membrane, helium purging, ultra-sonication or purging or combination of these methods. HPLC systems are also provided an online degassing system, which continuously removes the dissolved gases from the mobile phase.

3. Sample injector

Two means for analyte introduction on the column are injection in to a flowing stream and a stop flow injection. Several devices are available either for manual or auto injection.

a. Septum injectors- for injecting the sample through a rubber septum.
b. Stop flow- in which the flow of the mobile phase is stopped for a while and the sample is injected through a valve device.

c. Rheodyne injector (loop valve type) - it is the most popular type. This has a fixed volume loop like 20-50 µl or more.

The injector has two modes, i.e., load position when the sample is loaded in the loop and the inject mode, when the sample is injected.. An injector for a HPLC framework should give infusion of the fluid specimen inside the scope of 0.1 mL to 100 mL of volume with high reproducibility and under high pressure (up to 4000 psi). Automatic injector is a microprocessor-controlled version of the manual universal injector. Usually, up to 100 samples is capable of being loaded on to the auto injector tray. The system parameters such as flow rates, volume to be injected, gradient, run time, etc. are selected, stored in memory and sequentially executed on consecutive injections.

4. Guard column

Guard column has very small quantity of adsorbent and improves the life of the analytical column. It also acts as a prefilter to remove particulate matter, if any, and other material. Guard column has the same material as that of analytical column. It does not contribute to any separation but is necessarily used before the analytical column to protect & increase lifetime of column. Operator usually slurry or dry packs short guard column regularly with same or similar packing used in analytical column (old column material) are used for guard columns. However, the particle size of the packing material is bigger in size than analytical column in order to avoid the pressure drop in the HPLC system.

Analytical column

Columns are typically made of polished stainless steel, glass, and polyethylene or poly ether ether ketone (latest). Column length is around 50 mm to 300 mm and has an internal diameter across of somewhere around 2mm to5 mm. They are generally loaded with a stationary phase with a particle size of 3 µm to 10 µm. 1gm of stationary phase provides surface area ranging from 100- 860 sq.m. with an average of 400sq.m.

5. Detector Recorders and Integrators

There are several ways of detecting when a substance has passed through the column. Generally UV spectroscopy is attached, which detect the specific compounds. Many organic compounds absorb UV light of various wavelengths. The amount of light absorbed will depend on the amount of a particular compound that is passing through the beam at the time.

Recorders and Integrator Recorders: the signals from the detector after amplification (if necessary) are recorded as a series of peaks, each one representing a compound in the mixture. Baseline and the peaks are recorded with respect to time. Retention time for all the peaks can be found from the recordings. The area under the peak is proportional to the amount of substance passed through detector, and this area can be calculated automatically by the computer linked to the display. Integrator: improved version of recorders by which signals from the detector are gathered on graph recorders or electronic integrators that fluctuate in many-sided quality and in their capacity to process, store and reprocess chromatographic data. The computer integrates the response of the detector to each component and places it into a chromatograph that is easy to read and interpret.

Application

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.

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.

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.

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.

Other applications of HPLC

Other applications of HPLC includes

Pharmaceutical applications

- 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

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

Bio-monitoring of pollutant forensics

- 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 Clinical

- 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.

Food and Flavor

- 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.

Advantages

1. HPLC has many advantages over other methods of chromatography.
2. It has made significant contribution to the growth of analytical science and its diverse application in pharmaceuticals, environmental, forensics, foods, polymers and plastics, clinical fields etc.

3. HPLC provides a highly specific, reasonably precise, and fairly rapid analytical method for a plethora of complicated samples.
4. HPLC is capable of tackling macromolecules.
5. It is profoundly suitable for most 'pharmaceutical drug substances'. It offers an efficient means of analysis pertaining to 'labile natural products'.
6. HPLC allows the dependable analysis of a good number of products including biochemical, metabolic products, non volatile substance, polar compounds etc.
7. Preparation and introduction of sample is easy and simple in HPLC. Resolution of compounds and speed of separation is high. HPLC software is capable of reporting precise and accurate results.
8. Sensitivity of detectors used is high. A large number of stationary phases and columns can be used to suit different ranges of application. Recording and storage of information is easy.
9. The columns operated carefully under controlled conditions without overloading can be resed for significant period of time.
10. HPLC coupled with mass spectrophotometers and FT- IR system have improved efficacy. Along with hyphenated techniques.
11. HPLC have been used to analyse impurities in pharmaceutical formulations.

Disadvantages

1. HPLC is considered one of the most important techniques of the last decade of the 20th century. Despite of the several advantages there are certain limitations also.
2. Limitations include price of columns, solvents and a lack of long term reproducibility due to proprietary nature of column packing. Others include: Complexity of separation of certain antibodies specific to the protein.
3. The cost of developing an HPLC apparatus for assay or method of separation of individual components is tremendous.
4. Due to the speed of the HPLC and its reliance on the different polarities; two compounds with similar structure and polarities can exit the chromatographic apparatus at the same time (co-elution).
5. This is difficult in detecting compounds.
6. Low sensitivity of some compounds towards the stationary phase in the columns is difficult.

7. Certain compounds get absorbed or react with the chemicals present in the packing materials of the column.

CONCLUSION

The review focuses on the principle, types, instrumentation, application, advantages and disadvantages of HPLC. 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. Chromatographic separation is based on the principles of adsorption, partition, ion exchange, molecular exclusion, affinity and Chirality. HPLC is a highly assertive analytical technique which uses sophisticated technologies that have been extensively practiced from decades. Modernizations such as ultrahigh-pressure liquid chromatography, nano liquid chromatography, liquid chromatography-mass spectrometry, chiral phase separations, core-shell columns, and novel stationary phases have helped HPLC to acquire higher performance levels; in diverse factors, yielding faster speed, higher resolution, greater sensitivity, and increased precision. The practice of HPLC is restricted to analyzers, but is now widely performed by students, chemists, biologists, production workers, and other novices in academia, research, and quality control laboratories.

REFERENCES

1. Martin M., Guiochon, G. Effects of high pressures in liquid chromatography. J. Chromatogr. A, 2005; (1-2)7: 16-38.
2. Liu Y., Lee M.L. Ultrahigh pressure liquid chromatography using elevated temperature. Journal of Chromatography, 2006; 1104 (1-2): 198–202.
3. Abidi,S.L. High-performance liquid chromatography of phosphatidic acids and related polar lipids. J. Chromatogr, 1991; 587: 193-203.
4. Hearn M.T.W. Ion-pair chromatography on normal and reversed-phase systems. Adv. Chromatogr, 1980; 18: 59–100.
5. Bergh J. J., Breytenbach, J. C. Stability-indicating High-performance Liquid-chromatographic Analysis of Trimethoprim in Pharmaceuticals. J. Chromatogr, 1987; 387: 528-531.
6. Stubbs C., Kanfer, I. Stability-indicating High-performance Liquidchromato- graphic Assay of Erythromycin Estolate in Pharmaceutical Dosage Forms. Int. J. Pharm, 1990; 3(2): 113-119.

7. MacNeil L., Rice J. J., Muhammad N. Lauback R. G. Stability-indicating Liquid-chromatographic Determination of Cefapirin, Desacetylcefapirin and Cefapirin Lactone in Sodium Cefapirin Bulk and Injectable Formulations. J. Chromatogr, 1986; 361: 285-290.
8. Masoom R S, Zaid A. A and Nafisur R Analytical techniques in pharmaceutical analysis, Arabian Journal of Chemistry, Elsevier, 2012; S1409-S1421.
9. Narayudu Y Comparative Study of New Trends in HPLC: A Review, International Journal of Pharmaceutical Sciences Review and Research, 2013, ISSN 0976 – 044X.
10. Snehalakshmi R.P. A Review on Chromatography with High Performance Liquid Chromatography (HPLC) and its Functions, Research and Reviews: Journal of Pharmaceutical Analysis, Volume 4, Issue 1, March, 2015, E-ISSN: 2320-0812.
11. Prathap B, A Review - Importance of RP-HPLC in Analytical Method Development, International Journal of Novel Trends in Pharmaceutical Sciences, 2013; 3: ISSN: 2277 – 2782.
12. Santoshkumar B, A Review: HPLC Method Development and Validation, International Journal of Analytical and Bioanalytical Chemistry, 2015; 5(4): 76- 81, ISSN-2231-5012.
13. Vibha G, Development and validation of HPLC method - a review, International Research Journal of Pharmaceutical and Applied Sciences, 2012; 2(4): 17-25, ISSN-2277-4149.
14. Mukthi T, A Review on High Performance Liquid Chromatography (HPLC), Research & Reviews: Journal of Pharmaceutical Analysis, 2016; 5(2): ISSN: 2347-2340.
15. Azim M S, HPLC method development and validation, International Research Journal of Pharmacy, 2012; 4(4): 39-46, ISSN 2230-8407.
16. Pallavi N P, HPLC method development- A Review, Journal of Pharmaceutical Research & Education, 2017; 1(2): 243-260, ISSN 2456-4508.
17. Taleuzzaman M, Ultra Performance Liquid Chromatography (UPLC) - A Review, Austin Journal of Analytical and Pharmaceutical Chemistry, 2015; 2(6): 1-5. ISSN: 2381-8913.
18. Subramani P, An Overview of Liquid Chromatography-Mass Spectroscopy Instrumentation, 2014; 5(2): 47-54.
19. Anastasia Z, An overview of recent advances in HPLC instrumentation, Central European Journal of Chemistry, 2012; 10(3): 554-569.
20. Michael S, HPLC detectors: A brief review, Journal of Liquid Chromatography & Related Technologies, 2010; 33: 1130– 1150, ISSN: 1082-6076.

21. Sharma S, Gurpreet Singh, Process validation in pharmaceutical industry: an overview, journal of drug delivery and therapeutics cs, 2013; 3(4): 184-188.
22. Paula K and Campbell C, FDA, 2011 Process validation guidelines: process validation revisited, journal of validation technology autumn, 2012.