Working and Application of High Performance Liquid Chromatography (HPLC)

Umar Farooq
M.Phil Chemistry Riphah International University Islamabad
Email: farooqumar7272@gmail.com

Muhammad Asif
M.Phil. Chemistry Riphah International University Islamabad
Email: asif19176@gmail.com

Ayesha Kiran
M.Phil. Analytical Chemistry Superior University Lahore
Email: ayeshakiran420@gmail.com

Khalid Iqbal
M.Phil. Chemistry Riphah International University Islamabad
Email: khalidkallu19@gmail.com

Received on: 25-04-2024 Accepted on: 26-05-2024

Abstract

Chromatography has existed for a long time and is still used to separate compounds. Analyzing a broad variety of application areas is made possible by high-performance liquid chromatography (HPLC). In this article, we will go over the basics of high-performance liquid chromatography (HPLC), including how it works, what parts make up an HPLC system, and what variables impact the accuracy of a test. This new advanced HPLC category uses the same fundamental theory and methods as its predecessors but boasts superior chromatographic performance. This paper aims to provide a comprehensive overview of UPLC, including its principles, instruments, and applications.

Keywords: High Performance Liquid Chromatography, Working and Application of(HPLC)

What is HPLC?

HPTLC

Combining the skill of chromatography with speed and low cost, H.P.T.L.C. is a constructive qualitative analytical approach. Its improved resolution and shorter runtime represent a giant leap forward for the TLC concept. High-performance liquid chromatography (HPLC) is a method for separating substances. It begins by injecting a small amount of fluid sample into a tube packed with tiny particles called the stationary phase. Then, a pump is used to force liquid, called the mobile phase, down the packed tube, called the column, and each sample

component is moved down the column. The packing of the column separates the components from each other through chemical and physical interactions between their molecules and the packing particles. At the end of the column, a flow-through device called a detector detects the amount of each separated component. This detector produces what is known as a "liquid chromatogram" as an output.
Except for the rate, LC and HPLC are functionally equivalent.

The essential steps involved are,
• Preparing the sample
• Choosing the chromatographic layers
• Choosing the plates
• Washing the plates before use
• Conditioning the samples
• Applying the samples after conditioning
• Mobile phase
• Developing the chromatography
• Detecting spots
• Scanning and documenting the process.

1. SAMPLE PREPARATION:
• A highly concentrated solution is required since a small sample quantity is required for operation. Silica gel pre-coated plates are ideal for normal phase chromatography, but only when employing non-polar volatile solvents. The material is typically dissolved in polar solvents for reversed-phase chromatography.

SELECTION OF CHROMATOGRAPHIC LAYERS
• Layers of H.P.T.L.C. are accessible as pre-coated silica gel, which is extensively used as an adsorbent due to its very tiny particle size.

PLATES:-
• These plates look just like regular T.L.C. plates. In this context, ultrafine-grained silica gel is an effective adsorbent. Particle size is a valuable tool for improving sensitivity and resolution. A 200 mm layer of 4–5 mm silica gel is created using an inert binder to make plates. Sizes ranging from 5x7.5 cm to 20x20 cm are used. The coating material is a silica gel F254 with a 6-millimeter pore size and a fluorescent indicator. T.L.C. plates have a particle size of 5–20 mm, whereas H.P.T.L.C. plates have a particle size of 4–8 mm.

1. PRE-WASHING:-
• Water vapors or volatile contaminants can't be retained on plates; thus, washing them is necessary. Using methanol, the plates are cleaned.

2. CONDITIONING:-
• After washing, put the plates in the oven at 120 degrees Celsius for 15 to 20 minutes.

3. SAMPLE APPLICATION
A maximum of a 1 mm diameter sample spot should be administered.
• Self-loading capillary is one of many sample spotting procedures; it allows for applying tiny volumes of samples to the plate. The surface is glass tubing with platinum-iridium tubing fused to one end.

4) PRECONDITIONING:
Saturation is unnecessary for mobile phases with low polarity. On the other hand, strongly polar mobile phases need saturation.

5) MOBILE PHASE:
Considering the solvent solubility of the analytic absorbent layer and the chemical characteristics of the solute, the solution of the suitable mobile phase is a process of trial and error.

6) CHROMATOGRAPHIC DEVELOPMENT:
The most well-known procedure in H.P.T.L.C. is the linear development method, which involves placing the plate vertically in a solvent solution in an appropriate container. Chromatograms may be created from either side of the solvent, typically supplied by capillary action.
Chromatographic development may also use other techniques, such as circular development, anti-circular devices, and multiple development.

7) DETECTION OF SPOT:
As soon as the development is finished, the plates are removed from the chamber and allowed to dry to eliminate any remaining mobile phase. Iodine vapor in an iodine chamber is a standard method of detection.

8) SCANNING AND DOCUMENTATION:
Data recording and storage devices, as well as computers, are part of the H.P.T.L.C. equipment. Computers display the discovered spots as peaks after scanning the development of H.P.T.L.C. plates in specific UV regions using the equipment. The scanner takes a bond and turns it into a peak, the height or area of which is proportional to the local concentration of the material. The area beneath the point and the heights of the mountains are measured.

Principle of HPLC
With high-performance liquid chromatography (HPLC), the analyte (sample) is distributed between the mobile phase (eluent) and the stationary phase (column packing material) according to the separation principle. The analyte's chemical structure determines the amount of retardation experienced by molecules as they travel through the stationary phase. The unique intermolecular interactions with the packing material define sample molecules' "on-column" duration. This means that various components of a sample elute at multiple rates. In this way, the elements of the sample are separated.
Once the analytes have exited the column, a detection device (such as a UV detector) can

identify them—a chromatogram results from the signals' conversion and recording by a software data management system. Further processing of the mobile phase is possible once it has passed the detecting unit.

Column:
To separate a sample's relevant components, the column's stationary phase—sometimes called the "heart of the chromatograph"—uses several physical and chemical characteristics. The tiny particles within the column cause the high backpressure at typical flow rates.
• Due to the column's resistance, the mobile phase must be forced via the pump, which results in a high internal pressure inside the chromatograph.

4. Detector:
• The detector may see the individual molecules that elute from the column.
The chemist may conduct a quantitative analysis of the sample components with the help of a detector, which measures the quantity of these molecules.
• The liquid chromatogram, a graph showing the detector's reaction, is created when the detector sends an output to a recorder or computer.

5. Computer:
• The computer, also known as the data system, controls all of the HPLC instrument's modules and performs quantitative analysis by determining the amount of elution time (retention time) and the components' amounts (quantitative analysis) based on the signal from the detector.

APPLICATIONS OF HPTLC:
- Testing for Stability, Identity, and Purity in Pharmaceuticals; Content Uniformity
- Herbs - Authentication; Evaluation of stability; Identifying counterfeit herbs; Screening for indicator chemicals
- Medical - Lipides; research on metabolism; drug screening; monitoring of doping
- Food and Feed - Analyzing additives (such as vitamins) and pesticides; conducting stability tests; quality control
- Cosmetics-Recognition of Primary Ingredients; Examination of additives (colorants, preservatives, etc.); detection of illicit substances
- Biological technology - Enzyme characterization (component profiles); Proteomics (HPLC-MS coupling); Process optimization and development;
- Keeping an eye on processes: validating cleaning,
- Analysis of water, soil, and residue in the environment
- Chemical analysis and dyestuff testing are part of forensic science.

The four most common methods for separating chemicals are as follows:
1. Chromatography in reversed-phase
2. Adsorption and normal-phase chromatography
3. Chromatography based on ions
4. Chromatography via size exclusion

(1) Reversed-Phase Chromatography (RPC)

• Non-polar organic solvents (e.g., methanol, acetonitrile) and water (as a buffer) comprise the mobile phase, whereas non-polar column packings include C18, C8, C3, phenyl, and others.

To this day, RPC remains the most often used method. This setting is used by more than 90% of chromatographers.

• RPC is adaptable since it may be used for polar, non-polar, ionizable, and ionic compounds.

Gradient elution is often used for samples that include various chemicals. The process starts with a mobile phase that is primarily water-based and gradually increases the amount of organic solvent added over time.

Eluting potent chemicals, the organic solvent boosts the solvent's potency.

2) Normal Phase or Adsorption Chromatography

LC Columns

• Here, the mobile phase is non-polar (such as hexane, isooctane, methylene chloride, or ethyl acetate). At the same time, the column packing is polar (such as silica gel, cyanopropyl-bonded, amino-bonded, etc.).

• Under 10% of the time, we do normal phase separations.

The method works well for various compounds, including those sensitive to water, geometric isomers, cis-trans isomers, class separations, and chiral compounds.

Ion exchange,

Ionic groups (such as sulfonic or tetraalkylammonium) are packed into the column, while an aqueous buffer (such as phosphate or format) serves as the mobile phase.

Twenty percent of liquid chromatography utilizes ion exchange. This method works well for separating organic and inorganic cations and anions in water.

Because they are salt in brine water, ionic dyes, amino acids, and proteins may be separated via ion exchange. Application Ion Exchange Chromatography Demonstration

Exclusion Chromatography (SEC)

1. SEC ensures the column packing material does not contact the sample chemicals. Alternatively, molecules may enter a porous medium by diffusion. Motifs are partitioned into several groups according to how their sizes compare to those of the pores. While molecules more minor than the pore opening can enter the particle and remain segregated, more giant molecules are prevented from diffusing into the particles. Primarily, large molecules elute. Substantial molecules elute secondarily.
2. Protein and polymer characterization comprise most applications for the SEC method, which 10-15% of chromatographers use.

Two ways are available:

Separation by Molecular Dynamics (SDS) in an aqueous medium (GBC), also known as gel filtration chromatography and water-based SEC.

References

1. Ali, I., Alam, S. D., Raja, R., Khan, S. A., Anjum, R., Jain, A. K., Locatelli, M. (2023). Advances in Simple and Chiral-HPLC Methods for Antiallergic Drugs and Chiral Recognition
2. Ali, I., Alam, S.D., Raja, R., Shirsath, V., Jain, A.K., Yusuf, K., Aljuwayid, A.M., Sillanpää, M. (2022). Chiral separation of β-blockers by supercritical fluid chromatography using Chiralpak-IG and Chiralpak IBN-5 columns. Chirality, 34(6), 848-855.
3. Ali, I., Raja, R., Alam, S.D., Shirsath, V., Jain, A.K., Locatelli, M. David, V. (2021). A comparison of chiral separations by supercritical fluid chromatography and high-performance liquid chromatography. Journal of Liquid Chromatography & Related Technologies, 44, (11-12), 450-463.
4. Al-Othman, Z. A., Al-Warthan, A., Alam, S. D., & Farooqi, J. A. (2014). Enantiomeric separation and simulation studies of pheniramine, Oxybutynin, Cetirizine, and brinzolamide chiral drugs on amylose-based columns, Chirality, 26(3), 136–143.
5. Asif, M., Yusuf, M., Almehmadi, Alsaiari, AA, Allahyani, M., Aljuaid M. and Alsharif, A (2024). Towards Antiviral Potential of Biomolecules Derived from Adhatod avasica as Competent Natural Molecules to Treat COVID-19 Virus Variant. Letters in Organic Chemistry, 21(5), 466-477.
6. da Costa, L. M., Oliveira de Almeida Leite, H., Kassab, N. M., & Singh, A. K. (2019). Green analytical methods for the separation of seven antihistamines: Application in separation of azelastine and related impurities in nasal solution. International Journal of Analytical Chemistry, 2019(9489723), 1-11.
7. Mechanism. Analytica, 4(1), 66-83.).
8. Tao, Q.F., Zhu, X.P. and Zhao, Y. (2007). Chiral resolution of cetirizine by capillary electrochromatography using no vancomycin hydrochloride as chiral selector," Chinese Journal of Pharmaceutical Analysis, 27(5), pp. 686–688.
9. Xiong, X., Wang, K., Tang, T., Fang, J., & Chen, Y. (2021). Development of a chiral HPLC method for the separation and quantification of hydroxychloroquine enantiomers, Scientific Reports, 11(1), 1-18.
10. Yusuf, M. (2023). Insights into the in-silico research: Current scenario, advantages, limits, and future perspectives. Life In Silico, 1(1), 13-25. https://life-insilico.com/index.php/pub/article/view/5 10.
11. Zhou, J., Luo, P., Chen, S., Meng, L., Sun, C., Du, Q., & Sun, F. (2016). Enantioseparation of six antihistamines with immobilized cellulose chiral stationary phase by HPLC. Journal of chromatographic science, 54(4), 531-535.