International Journal of Science and Research Archive

eISSN: 2582-8185

Cross Ref DOI: 10.30574/ijsra

Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

Check for updates

High-performance liquid chromatography (HPLC): Innovations in analytical techniques and applications

Abdul Wadood Ansari *, Abhishek Yadav, Ashish Kumar Yadav, Manish Yadav and Arti

Pharmacy College, Azamgarh, Itaura, chandeshwar, Azamgarh, Uttar-Pradesh,276128.

International Journal of Science and Research Archive, 2025, 15(01), 155-166

Publication history: Received on 24 February 2025; revised on 01 April 2025; accepted on 03 April 2025

Article DOI: https://doi.org/10.30574/ijsra.2025.15.1.0929

Abstract

High-Performance Liquid Chromatography (HPLC) is a type of column chromatography that is commonly used in biochemistry and analysis to separate, identify, and quantify active chemicals. HPLC is the most often used separation technology for detecting, separating, and quantifying the drug High -Performance Liquid Chromatography (HPLC): A Comprehensive Review of Its, Principle, Detectors, And Application It works on the principle of Affinity chromatography having two phases viz: stationary and mobile phase. The constituent with lower affinity for stationary phase travels faster and vice-versa. HPLC is just one type of liquid chromatography, meaning the mobile phase is a liquid. Reversed-phase HPLC is the most common type of HPLC. The reversed-phase means the mobile phase is relatively polar, and the stationary phase is relatively non-polar. HPLC instrumentation includes a Solvent reservoir, pump, injector, column, detector, and integrator or acquisition and display system This article was written with the intention of reviewing several HPLC-related topics, including its principle, kinds, manner of separation, characteristics, instrumentation, key parameters, and numerous applications in various sectors.

Keywords: High-Performance Liquid Chromatography; Drug Quantification; Clinical Applications; Pharmaceutical Analysis

1. Introduction

In biochemistry and analysis, high-performance liquid chromatography, sometimes referred to as high-pressure liquid chromatography, is a type of column chromatography that is frequently used to separate, identify, and quantify active compounds. It is a widely used analytical method for identifying, measuring, and separating each component of a mixture. One advanced column liquid chromatography technology is HPLC [1].The HPLC procedure pushes the solvent at high pressures of up to 400 atmospheres so that the sample can be divided into distinct constituents based on variations in relative affinities. Normally, the solvent flows through the column due to gravity. HPLC typically consists of a detector that measures the retention periods of molecules, a pump that pushes the mobile phase or phases through the column, and a column that holds packing material (the stationary phase). [2] . The interactions between the stationary phase, the molecules under study, and the solvent or solvents used all have an impact on the retention time. Certain chemical or physical interactions with the stationary phase impede the addition of the samples to be examined, which are added in tiny amounts to the mobile phase stream. [3]. The kind of analyte and the makeup of the stationary and mobile phases both affect the amount of retardation. The amount of time it takes for a particular analyte to elute is known as the retention time. [4]. A common solvent is any mixture of organic liquids or water that is miscible. During the analysis, the composition of the mobile phase has been altered using gradient elution. Analyte mixtures are separated by the gradient according to the analyte's affinity for the present mobile phase. The selection of solvents, additives, and gradients is influenced by the characteristics of the analyte and the stationary phase. [5]. Simple sample fractionation and purification [6] Researchers employed traditional liquid chromatographic methods before HPLC. Liquid chromatographic methods are ineffective since solvent flow rate is dependent on gravity. It can take several

* Corresponding author: Abdul Wadood Ansari.

hours or even days to finish a separation. It was believed that gas stage partition and the study of highly polar high atomic weight biopolymers were both impracticable, even if liquid chromatography (LC) was at the time more effective. Because the solutes were thermally unstable, some organic chemists found GC to be ineffectual. As a result, it was anticipated that HPLC would soon develop due to other techniques. Cal Giddings, Josef Huber, and others predicted in the 1960s that LC could be run in the high-efficiency mode by generously lowering the pressing molecule measurement below the standard LC (and GC) level of 150 m and using pressure, in accordance with Martin and Synge's groundbreaking 1941 work. to speed up the adjustable stage. All of these expectations underwent a great deal of testing and improvement. from the 1960s to the 1970s. Early studies were carried out to enhance LC particles, and Zipax, an externally permeable chemical, was invented for the HPLC process. The 1970s saw a number of advancements in instrumentation and technology. Experts initially employed pumps and injectors to build a simple HPLC system. Gas amplifier pumps were perfect because they ran at a steady pressure. In order to maintain a steady flow and superior quantitation, check valves and release free seals were not required. Even while equipment advancements played a big part, HPLC's history mostly tells the story of how molecular technology developed. Since the advent of permeable layer particles to boost efficacy, there has been a steady trend toward smaller molecules. However, new issues surfaced as molecule sizes decreased. The inability to drive a flexible liquid through the segment and the challenge of establishing a uniform pressing of extremely thin materials are expected to be the disadvantages of the unnecessary pressure drop. Every moment the molecule size is totally decreased, another cycle of instrument development should normally occur to manage the pressure. [7]

1.1. Principle

A small volume of liquid sample is injected into a tube filled with tiny particles, known as the stationary phase, which has a diameter of 3 to 5 microns (μm). The sample's constituent parts are then moved down the packed tube (column) using a liquid (mobile phase) that is forced through the column by high pressure that is supplied by a pump. The column packing, which entails different chemical and/or physical interactions between their molecules and the packing particles, keeps these components apart. The amount of these separated components is measured by a low-through device (detector) at the tube's (column's) exit. This detector's output is referred to as a "HPLC." Although LC and HPLC function similarly in theory, HPLC is significantly faster, more efficient, more sensitive, and easier to use. The previous method of simple liquid chromatography still finds use for preparatory reasons, even if HPLC still receives the majority of the credit for the analytical side [8]

2. High performance liquid chromatography types:

The use of a phase system in the process is a common factor in HPLC types. The types of HPLC listed below are commonly employed in analysis. [9] [10] [11] [12] [13]

2.1. Exchange of ions Chromatography

In ion-exchange chromatography, retention is driven by the attraction between solute ions and charged sites attached to the stationary phase. Ions with the same charge are excluded. Protein ion-exchange chromatography, protein ligand-exchange chromatography, high-pH anion-exchange chromatography of carbohydrates and oligosaccharides, and other water purification procedures commonly use this kind of chromatography. In ion-exchange chromatography, retention is driven by the attraction between solute ions and charged sites attached to the stationary phase. This method is almost always used with ionic or ionizable materials. The sample will be more strongly drawn to the ionic surface and take longer to elute if its charge is larger.

2.2. Normal Phase Chromatography

This method separates analytes based on their polarity and is also referred to as Normal Phase HPLC (NP-HPLC). In NP-HPLC, both polar stationary phase and non-polar mobile phase are employed. The polar analyte was interacted with and held in place by the polar stationary phase. Stronger adsorption forces are produced by more polar analyte, and the elution duration is prolonged by the interaction of the polar analyte with the polar stationary phase.

In NP-HPLC, the mobile phase is non-polar, but the stationary phase is polar. The polar stationary phase will retain analytes that are significantly more polar in a combination of components to be separated for a longer period of time than those that are comparatively less polar. As a result, the least polar component will elute first. Hydrogen bonding and dipole-dipole interactions are the main attracting factors.

2.3. Reversed phase chromatography

Changed phases The mobile phase of HPLC (also known as RP-HPLC or RPC) is aqueous and somewhat polar, while the stationary phase is non-polar. RPC focuses on the theory of hydrophobic interactions, which are caused by repulsive forces between a polar eluent, the relatively non-polar analyte, and the non-polar stationary phase. The analyte's affinity for the stationary phase is proportional to the contact surface area surrounding its non-polar region after it has attached to the ligand in the aqueous eluent.

In RP-HPLC, the mobile phase is either polar or somewhat polar, whereas the stationary phase is non-polar. The hydrophobic interaction theory forms the basis of RP-HPLC. Analytes that are relatively less polar in a mixture of components will be retained by the non-polar stationary phase for a longer amount of time than analytes that are significantly more polar. The component that is more polar will elute first. 11. Somewhat hydrophobic compounds can be separated with excellent recovery and resolution using reversed phase chromatography.

2.4. Size exclusion chromatography

SEC is a kind of chromatography that mainly uses size to separate particles. It is sometimes referred to as gel permeation chromatography or gel filtration chromatography. Determining the quaternary and tertiary structures of proteins and amino acids is aided by it. This method can be used to find the molecular weight of polysaccharides. SEC, sometimes referred to as gel permeation chromatography or gel filtration chromatography, is primarily used to separate particles according to size. After the sample has been simply screened or filtered, material with precisely controlled pore sizes is placed into the column. While smaller molecules enter the pores of the packing particles and elute later, larger molecules move through the column more quickly. The molecular weight of polysaccharides is commonly ascertained using this technique**.**

2.5. Affinity chromatography

In affinity chromatography, a substance known as an affinity ligand is covalently bonded to a solid support. Antibodies, enzyme inhibitors, cofactors, coenzymes, and other compounds that preferentially and reversibly attach to analyte molecules in the sample are examples of affinity ligands. The idea is that the substrate (or occasionally a coenzyme) is covalently attached to a support medium (such as cellulose beads) to form the stationary phase. This exposes the reactive groups that are necessary for enzyme binding. All of the otter proteins will elute in the column's void volume as the protein mixture passes through the chromatography column, but the proteins with a binding site for the immobilized substrate will bind to the stationary phase.

Only the molecules that attach to the affinity ligand preferentially are retained after the sample passes through the column. The mobile phase moves through the column with non-binding molecules. By altering the mobile-phase conditions, the retained analytes can be eluted once the undesirable molecules have been eliminated. Following their self-bonding, they need to be extracted from the bonded stationary phase using a different solvent with a high separation capacity. It is primarily helpful for protein and other biomolecule separation. [14]

3. Mode of separation

HPLC offers two types of separation depending on the eluent's composition. [15] [16]

- **Gradient elution:** The gradient form of separation includes variable eluent composition. This technique significantly increases a system's separation power, mostly as a result of a drop in peak width and a rise in apparent efficiency. Peak width fluctuates in direct proportion to the rate at which the composition of the eluent changes.

- **Isocratic elution:** One characteristic of the isocratic mode of separation is constant eluent composition, which indicates that equilibrium conditions in the column and the actual velocity of compounds flowing through the column are constant. The component's peak capacity is modest, and the longer it is left on the column, the wider the resulting peak becomes.

3.1. The elements that affect HPLC performance: [17]

- **Diameter inside:** An HPLC column's internal diameter (ID) is a crucial factor that affects sensitivity and establishes how much analyte can be placed onto the column. Larger columns are typically found in industrial

settings, such as when a drug product is being purified for eventual use. Low ID columns sacrifice loading capacity in exchange for increased sensitivity and reduced solvent use.

- **Particle size**: The stationary phase is typically affixed to the exterior of tiny, spherical silica particles (very tiny beads) in the majority of conventional HPLC procedures. Although the pressure needed for optimal linear velocity rises by the inverse of the particle diameter squared, smaller particles typically offer greater surface area and better separations.

- **Pump pressure**: Although the capacity of pumps varies, their performance is evaluated by their ability to produce a consistent and repeatable low rate. Modern HPLC systems have been enhanced to operate at much higher pressures, allowing for the use of much smaller particle sizes in the columns.

- **Temperature:** The temperature has an impact on the HPLC's proper operation; most HPLC columns can operate at room temperature or at a temperature of 25 to 35 ◦ c, but there are some exceptional cases that call for a higher temperature.

- **Pore size**: To increase surface area, many stationary phases are porous. bigger holes have superior kinetics, particularly for bigger analytes, while smaller pores offer more surface area. The size of the pores determines how well the analyte molecules can enter the particle and interact with its inner surface. Given that the outer particle surface to its inner one has a 1:1000 ratio, this is very crucial. The inner particle surface is where the majority of the surface molecule interaction takes place.

3.2. HPLC PARAMETER

There are some factors that are utilized as a standard for a specific compound in order to accurately analyze it. A modification in the parameters could have a significant impact on the outcome. Internal diameter, particle size, pore size, and pump pressure are the most often utilized metrics. The parameters can be altered for various compounds based on their chemical makeup and characteristics.

- **Time of retention**: The interval of time between the injection point and peak maxima's emergence is known as the retention time. Another definition of it is the amount of time needed for half of a component to elute from a column. Minutes and seconds are used to measure it.
- **Separation factors** the ratio of the partition coefficients of the two components to be separated is known as the separation factor.
- **Resolution:** The degree of separation between two components and the baseline separation attained is measured by resolution. Rs 2 (Rt, Rt.)/ww
- **Height Equivalent to a Theoretical Plate** refers to a hypothetical or imaginary unit of column in which the equilibrium of the solute distribution between the stationary phase and mobile phase has been reached. It may also be referred to as the column's functional unit. The efficiency of separation can be described by a theoretical plate of any height. The column is more effective if the HETP is lower.
- **Efficiency:** The theoretical plates represent a column's efficiency.
  - n = 16 Rt/w
  - Where there are no theoretical plates Retention duration
  - W = is the base peak width.
- **Retention time**: The amount of carrier gas needed to elute 50% of the component from the column is known as the retention volume. It is the result of a low rate and retention time.
- **Asymmetry factor**: The center of a chromatographic peak should be symmetrical. However, in reality, the peak exhibits tailing or fronting and is not symmetrical due to a few causes. Saturation of the stationary phase causes fronting, which can be prevented by employing a smaller sample size. More active adsorption sites cause tailing, which can be removed with support pretreatment. AF = b/a (b, a computed by 5% or 10% of the peak height) can be used to calculate the asymmetry factor (0.95 to 1.05). The higher concentrated sample, high injection volume, and column deterioration all contribute to broad peaks.

4. Instrumentation

The required equipment consists of a high-pressure pump, an injector for sample entry, a stationary phase-containing column, a detection, and a recorder. [18] [19]

4.1. Injection of the sample

Sample fluid can be injected using septum injectors. An injector (sample manager or autosampler) provides the capability to introduce [inject] the sample into the continuously circulating mobile phase stream that transfers the sample onto the HPLC column. Repeatable results can be obtained by combining a loop injector with a new, advanced rotary valve. The usual range of sample amounts is 5–20 microliters. Sample fluid can be injected using septum injectors. It is possible to inject the sample when the mobile phase is flowing or stopped. Repeatable results can be obtained by combining a loop injector with a new, advanced rotary valve.

4.2. Pump

A high-pressure pump (solvent delivery system or solvent manager) generates and measures a mobile phase flow rate, which is often expressed in milliliters per minute. The pump draws the mobile phase from the solvent reservoir, pushes it into the column, and then sends it to the detector. The operating pressure is influenced by the column's size, particle size, flow rate, and mobile phase composition. Flow rates in HPLC usually range from one to two minutes. The normal design of a pump can handle pressures between 6000 and 9000 psi (400 and 600 bar).

The pump draws the mobile phase from the solvent reservoir, pushes it into the column, and then sends it to the detector. 42000 KPa is the operating pressure of the pump. The size of the particles in the column, the flow rate, and the makeup of the mobile phase all affect this operating pressure.

4.3. The Detector

All of the molecules that elute (come out) of the column can be identified (detected) by the detector. The quantity of molecules in the sample is measured by a detector so that the chemist may analyze them quantitatively. The detector outputs to a computer or recorder the liquid chromatogram, often known as the graph of the detector response. A drug can be identified in a variety of ways after it has passed through the column. Usually, the specific compounds are found using UV spectroscopy. Various wavelengths of UV light are absorbed by a variety of biological materials. Light absorption depends on the quantity of a particular material passing through the beam at any given moment.

4.4. Column

It is the place where the separation really takes place. For the separation, the column contains the chromatographic packing material needed. It is referred to as the stationary phase since the column hardware holds this packing material in situ. The tube is made of stainless steel. 5 to 25 cm long and 2 to 4.6 cm in diameter. Either the entire packing material or just a small portion of it is permeable.

Clean stainless steel is usually used to construct columns, which typically have an internal diameter of 2 to 5 mm and a length of 50 to 300 mm. Usually, they have a stationary phase with molecules that are between three and ten meters in size. It is common to hear about microbore segments, which are columns with inner diameters less than 2 mm. The mobile phase and column temperatures should ideally be consistent throughout the experiment.

4.5. Detector

The detector can recognize (detect) any molecule that attracts (emerges) from the column. The quantity of molecules in the sample is measured by a detector so that the chemist may analyze them quantitatively. As an output to a computer or recorder, the detector generates the liquid churnutogram, or graph of the detector response.

A drug can be identified in a variety of ways after it has passed through the column. Usually, the specific compounds are found using UV spectroscopy. Various wavelengths of UV light are absorbed by a variety of biological materials. The quantity of a certain material passing through the beam at any given moment will dictate how much light is absorbed.

The chromatographic column's HPLC detector, which is placed toward the end of the column, separates the analytes as they elute. UV spectroscopy, fluorescence, mass spectrometric, and electrochemical identification are widely employed detecting. There are several methods to determine if a material has passed through the column. Usually, the specific chemicals are identified using UV spectroscopy. A variety of UV light wavelengths are absorbed by numerous natural compounds. The amount of a certain substance that is now flowing across the beam will impact how much light is absorbed.

5. Interpreting the output from detector:

A component of the minute that passed through the detector and absorbed UV light is represented by each peak in the output, which is captured as a sequence of spikes. The computer attached to the display cam automatically calculates the area under the peak, which is proportional to the amount of substance that passes through the detector.

5.1. Sample reservoir

A glass container holds the contents of the mobile phase. The mobile phase, or solvent, in HPLC is made up of polar and non-polar ligand components. Depending on the composition of the sample, different polar and non-polar solvents will be chosen.

5.2. Data collection device

The detector's signals can be captured by graph recorders or electronic integrators, which differ in their multifarious quality and capacity to analyse, store, and reprocess chromatographic data. The indicator's reaction to each component is coordinated by the PC, which then puts it into a chromatograph that is simple to read.

An HPLC device's schematic illustration usually includes a locator, pumps, and a sampler.

The sampler carries the sample into the column after introducing it into the mobile phase stream. The pumps transport the mobile phase through the column. Considering a quantitative study of the example components, the detector generates a signal that is proportional to the size of the sample component that emerges from the segment.

A digital microchip and software that also gives information manage the HPLC apparatus. A synthetic slope in the portable stage can be produced by a few mechanical pump models in an HPLC system that can mix different solvents in amounts that vary over time. A column broiler that considers altering the temperature at which the partition happens is also included in most HPLC equipment.

5.3. Operation

In order to isolate and analyse the sample blend, a discrete small volume (usually measured in millilitres) is added to the mobile phasestavam that is permeating the collar. Sample segments pass through the segments at different speeds due to particular physical interactions with the adsorbent (also known as the stationary stage). The velocity of each component depends on its mobile phase and chemical composition. The time it takes for a particular It elutes (rises up out of the column) at the analyte time. A distinguishing normal for a particular analyte is the retention time determined under specific conditions [20] [21] .

Many columns containing adsorbents with varying molecular sizes and surface properties are available ("surface science"). Higher operational pressure, or "backpressure," is required when using packing materials for small molecules, and this often enhances chromatographic resolution, or the degree of separation between successive analytes rising up out of the column. Sorbent particles can have either a polar or hydrophobic character. The most common examples of a basic mobile phase are acetonitrile and methanol, however any miscible mixture of water with various natural solvents can be utilized. Certain HPLC systems use water-free mobile phases. The aqueous part of the mobile phase may contain salts or acids (such as formic, phosphoric, or trifluoroacetic corrosive) to help separate the components of the sample. The mobile phase's composition can either be kept constant ("isocratic elution mode") or changed ("inclination elution mode") during the chromatographic analysis. Components of the sample whose propensities for the stationary stage are not substantially different can usually be separated by isocratic elution. In gradient elution, the mobile phase's structure usually varies from low to high eluting quality. High eluting quality leads to quick elution, and analyte maintenance durations are a strong measure of the mobile phase's eluting quality.

The structure of the mobile phase is determined by the force connecting the stationary stage and a number of crample components, or "analytes" (also known as eleratiing hydrophobic connections in turned around stage HPLC). Based on their preference for each, analytes split between the stationary and mobile stages. during the detachment procedure of the sample. This procedure is continuous rather than gradual, yet it is comparable to what happens during a liquid-liquid extraction. As the mobile stage gets more packed in acetonitrile (in a verutile period of better sluting quality), more hydrophobic components will chute (fall off the column) later in this scenario employing a water aceronitrile angle [22] [23]

6. HPLC detector

In HPLC, a detector is positioned at the system's end. Analyzing the solution that is eluting from the column is its job. The electronic signal that emerges from each component of the mixture is proportional to the concentration of each individual analyte component [24] [25]

6.1. The characteristics of the detectors used in HPLC

- All of the mixture's components must exhibit responsiveness.
- The response must not be impacted by temperature changes.
- It needs to be able to track even lower levels.
- The signal must be consistent and repeatable
- It must react linearly to the analyte's
- The peaks shouldn't get wider.
- It must not be harmful [26].
- he eluent composition (gradient) must not affect it.
  - The peaks shouldn't get wider.

6.2. Types of detectors

6.2.1. U.V detectors

- Predicated on molecular electronic transitions.
- The most popular kind of LC detector.
- The Hg lamp's fixed wavelength is 254 nm (π = > π^*).
- The adjustable wavelength can be chosen for monochromators, filters, or specific wavelengths still only able to use one wavelength.
- Limitations of solvents with UV-vis abs.
- Z-shaped, low-through detectors (V, 1 ~ 10 μL and b, 2 ~ 10 mm) The spectrophotometer is more adaptable.

6.2.2. Refractive index detectors

- The almost ubiquitous but subpar detection threshold
- Allows light to pass between the sample and reference compartments.
- The light beam that travels through the compartments is recorded as zero when the solvent composition is the same.
- The light beam will be shifted from the detector by changes in the refractive index when a solute is present in the sample compartment.
- 10 ng of solute is the limit of detection (LOD).

6.2.3. Detectors that use electrochemistry

- Predicated on the analyte's amperometric reaction to an electrode that is typically maintained at a constant voltage.
- Because the reaction is dependent on a surface phenomenon rather than a bulk feature of the solution (such as UV-vis absorbance) an electroactive analyte may be extremely sensitive.
- ease of use, convenience, and broad applicability
- The Teflon thin-layer flow cell is 50 μm thick and has a volume of 1 to 5 μL Indictor E: Pt, Au, C
- Multi-electrode: concurrent detection or indication of sample purity

6.2.4. Fluorescence detectors:

- After the sample absorbs incident light, the fluorescence rays it emits are measured.
- The light utilized for excitation is produced by a xenon arc lamp.
- Only appropriate for substances that exhibit fluorescence.
- Extremely sensitive and precise.
- Under fluorescent stimulation, certain molecules are unstable.

6.2.5. IR detectors

FTIR or a filter instrument

- Comparable cell (b, 0.2 ~ 1.0 mm and V, 1.5 ~ 10 μL)
- Limit: unique optics and no appropriate solvent
- Spectrum records of flowing systems that are comparable to the diode array system can be obtained using FT-IR.
- Alcohols and water can significantly interfere with the detection of solutes.

The limit of detection (LOD) is 100 ng.

6.2.6. Mass detectors

- **Universal detector:** An MS detector first ionizes the chemical that is eluting from the HPLC column, then measures its mass and/or breaks the molecule up into smaller, compound-specific fragments.
- Because a compound's mass spectrum is unique to it and functions similarly to a fingerprint, the MS detector can occasionally identify the component directly.

6.2.7. Data collection devices

Chart recorders and electronic integrators, which differ in complexity and capacity to analyse, store, and reprocess chromatographic data, can be used to gather signals from the detector. The detector's reaction to each component is combined by the computer and displayed in an easily readable and interpretable chromatograph. Often referred to as the data system, the computer not only manages every module of the HPLC instrument but also uses the signal from the detector to calculate the quantity of sample (quantitative analysis) and the time of elution (retention time) of the sample components (qualitative analysis) [10].

7. Various application of HPLC

Through the use of HPLC, one can learn about a compound's identity, quantity, and resolution. The term preparative HPLC refers to the separation and purification of substances. This is in contrast to analytical HPLC, where the main objective is to learn more about the sample substance.

7.1. Identification

HPLC is typically utilized for compound assays. The known sample's peak on the chromatograph should be clearly visible due to the assay's settings. The identifying peak should have a reasonable retention period and be easily distinguishable from unrelated peaks at the detection levels where the test will be conducted.

7.2. Purification

Purification is the process of extracting the desired chemical from a mixture of chemicals or contaminants. Every material showed a unique peak at particular chromatographic conditions. Only the pure desired compound can be collected or extracted if the impurities and compounds travel through the column in sufficiently diverse ways.

7.3. Chemical separation

Since different compounds migrate at different rates depending on the column and mobile phase utilized, the choice of stationary phase and mobile phase has a significant impact on the extent or degree of separation.

7.4. Other application

There are numerous uses for the HPLC in the domains of pharmacy, forensics, the environment, and clinical.

7.4.1. Pharmaceutical Applications

- **HPLC technique for simultaneous measurement of thimerosal and aluminium in medicines and vaccines**

the creation and approval of a simple and useful chromatographic method for the simultaneous identification, separation, and quantitative assessment of the adjuvant aluminum and the preservative thimerosal in drugs and

vaccines. TM and Al can be identified. When postcolumn derivatization and dithizone are utilized as complexing agents, the RP-HPLC technique is used concurrently. The process outlined here can be applied to drugs, immunizations, and other products that contain TM and A [27].

- **A HPLC method for measuring human insulin in pharmaceutical preparations has been developed and validated**

A simple and reliable HPLC technique with diode array detection was developed and validated to ascertain the presence of human insulin in pharmaceutical preparations. The method was verified for linearity, accuracy, precision, sensitivity, and stability. As a result, pharmaceutical items containing human insulin can have their quality periodically checked using this technology [28].

7.5. Environmental Applications

- **Pesticide analysis using Solid Phase Micro Extraction—HPLC (SPME-HPLC)**

One important and diverse category of agricultural and environmental species is pesticides. High efficiency, unique selectivity, and high sensitivity separation techniques are often required for their detection in complex environmental matrices (e.g., water, soil, sludge, sediments, etc.), feed and food, and formulations. Because they induce cancer, pesticides (organophosporus, organochlorine, carbamate, dithiocarbamate, etc.) are dangerous to humans when they ascend the food chain. Residual analysis was done to find out how much and what kind of pesticides and their metabolites were remained in food when it was consumed. The combination of SPME with HPLC is a commonly used analytical technique. This offers numerous benefits over conventional sampling techniques, which require larger samples, take longer, and use solvents. The SPME-HPLC approach can be used to identify polar carbamate pesticides in clean water samples [29].

- **Chromatographic techniques for identifying the active ingredients and their impurities in pesticide formulations**

To detect the active chemicals and their contaminants in pesticide formulations, HPLC can be a highly useful analytical method. Since fake plant protection products have the potential to harm the environment, the food commerce, public health, and agricultural yields, quality control is essential to ensuring the products' efficacy and quality. Since contaminants affect the safety, stability, and quality of plant protection products, it is becoming more and more crucial to identify and quantify them in order to regulate them [30].

7.6. Forensic Applications

- **Development of analytical techniques for micellar liquid chromatography with direct injection to identify monitorable substances in serum and urine**

Therapeutic medication monitoring is a common practice in clinical trials. It is necessary to measure the drug concentrations in biological fluids. The well-established method of micellar liquid chromatography (MLC), a subset of reverse phase-high performance liquid chromatography (RP-HPLC), has been effectively used to analyze these matrices. MLC is used to identify numerous drug classes in serum and urine, including analgesics, bronchodilators, anticonvulsants, antiarrhythmics, tricyclic antidepressants, and selective serotonin reuptake inhibitors. The main advantages of this approach are its direct injection capabilities and efficient chromatographic elution, even with the complexity of biological fluids. They found the MLC-procedures to be reliable, economical, environmentally friendly, safe, selective, sensitive enough, and efficient. As a result, this is an excellent choice for monitoring purposes for identifying pharmaceuticals in serum and urine [31].

- **Direct drug glucuronide detection using HPLC in biological matrices**

The identification of a drug and its glucuronide metabolite or metabolites is essential for the interpretation of forensic and clinical toxicology. Until recently, glucuronides were recognized by either cleaving the glucuronide with an enzyme (such -glucuronidase) to produce the parent molecule, which was then detected, or by derivatizing to a more volatile or detectable counterpart. The direct detection of the glucuronide conjugates using HPLC resolves the significant disadvantages of techniques utilizing enzymatic cleavage processes and/or derivatization. Consequently, HPLC offers a straightforward method of identifying the glucuronides of many potentially abused drugs in human biological matrices when used in conjunction with a range of detectors.

These methods will make it possible to identify parent compounds and estimate metabolite amounts long after the drug has left the systemic circulation. These methods have developed into invaluable tools for forensic and clinical toxicology [32].

7.7. Food and Flavour Applications

- **Purine analysis of fish oil supplements using a single HPLC approach that has been developed and validated**

Foods strong in purines, such as meats, seafood, plants high in purines, and animal protein, should be avoided on a daily basis by gout sufferers. In order to test the naturally occurring purines guanine, purine, theobromine, and adenine precisely, an efficient RP-HPLC method was developed and verified. Both seafood and fish oil frequently contain these purines. It takes around 20 minutes to quantify all four purines in fish oil using the analytical method. The RP-HPLC technique provides a simple, efficient, and repeatable way to analyze naturally occurring purines in fish oil [33]

- **Recent advancements in phenolic food compound separation using HPLC**

Phenolic compounds, a significant class of natural products, are the main bioactive ingredients of many medicinal and nutritious plants. They have been found to provide a number of health benefits, including anti-inflammatory, anti-cancer, and antioxidant properties. Most of the time, plants contain phenolic chemicals in the form of many analogues with similar physicochemical properties and structures. The significance of rapid, accurate, and sensitive analytical techniques for their examination in diverse food samples is growing from a nutritional standpoint. HPLC is the most popular separation technique for these applications. The usage of contemporary techniques such as ultrahigh-pressure liquid chromatography (UHPLC), hydrophilic interaction liquid chromatography (HILIC), and multidimensional liquid chromatography (LC) is growing. Phenolic compounds in food samples can be chemically analyzed using these techniques [34].

7.8. Clinical Applications

- **For a number of therapeutic drugs, reversed phase HPLC procedures have been developed and are being used**

Cefuroxime, clindamycin, dexamethasone, dicloxacillin, doxycycline, metronidazole, oxymetazoline, paclitaxel, tobramycin, and vancomycin are among the medications that can be utilized in this process. Additionally, standard pharmaceutics methodologies are described. Reversed-phase HPLC and a hydrophobic column were used to analyze each of these drugs. This study shows that a single, simple, quick, and reasonably priced piece of technology can evaluate a variety of drugs. These methods have been proven to work well for the drug in question, and when used appropriately, gradient techniques allow for the separation and detection of many molecules, which is necessary for the experiment. With just a carefully thought-out and executed strategy, HPLC can be a powerful tool for the identification of a wide range of antibiotics [35].

- **HPLC optimization and validation of the analytical procedure for cotrimoxazole in tablet and plasma**

Using a simple and reproducible HPLC method, sulfamethoxazole and trimethoprim, also referred to as cotrimoxazole, were measured concurrently in tablet and human plasma in vitro. With good specificity, sensitivity, linearity, precision, and accuracy across the entire range of clinically significant and therapeutically achievable plasma concentrations, the recently developed analytical method for cotrimoxazole quantification in tablet and plasma samples enabled a bioequivalence trial [36].

7.9. Miscellaneous Applications

- **Recent advancements in phenolic compound HPLC separation**

Phenolic compounds are a class of naturally occurring, highly complex molecules that have a number of positive health effects. As a result, it is essential to analyze phenolics in various samples. HPLC is the basic method for phenolic separation.

However, typical HPLC methods lack sufficient resolving power when handling the complexity of phenolic fractions present in real-world materials. The need for better chromatographic throughput and resolving power is greater now than it has ever been for phenol analysis methods. Significant gains in resolution and performance have been

demonstrated by a number of important technological advancements in HPLC. High-temperature liquid chromatography (HTLC), multi-dimensional separations, ultra-high pressure liquid chromatography (UHPLC), and several other methods include novel stationary phase chemistries and morphologies. With HPLC 70, these methods can improve phenolic analysis performance.

8. Conclusion

Among the most widely used analytical methods is HPLC. It offers several advantages over conventional chromatographic techniques. Because HPLC is simple to use and efficient, it can identify and determine a wide range of natural and synthetic chemicals with accuracy and speed. Both quantitative and qualitative estimation are used in many different fields, such as clinical, food and flavor, forensic, pharmacological, and environmental, among many others. HPLC's only disadvantage is its price.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] S. G. A. A. U. V. R. M. Rao BV, " A review onstability indicating HPLC method development.," . World Journal of pharmacy and pharamceutical science , pp. 405-423, 2015.

[2] K. D. Priya Sadapha*, "Review Article on High-Performance Liquid Chromatography (HPLC) Method Development and Validation," Int. J. Pharm. Sci, pp. Pages: 23-29, , May - June 2022.

[3] B. M. Yadav V, "A REVIEW ON HPLC METHOD DEVELOPMENT AND VALIDATION.," Research Journal of life science Bioinformatics Pharmaceutical and Chemical Science, p. 166, 2017.

[4] K. B. S., "A Review: HPLC Method Development and Validation.," International Journal of Analytical and Bioanalytical Chemistry, p. X(Y)ZZ, 2015.

[5] S. R. G., "A Review on new Analytical method Development and Validation by RP-HPLC. A Review on new analytical method development," International Research Journal of Pharmaceutical and Biosciences , pp. 41-50, 2017.

[6] M. D. J. K. C. M. C. R. Khachane MA, "Pharmaceutical. Analysis and Validation:," A Review introduction International Journal of Pharmacy And Pharmaceutical Reaserch, pp. 273-298, 2021.

[7] M. Thammana, "A review on HPLC," International Journal of Research Publication and Reviews, p. 22, 2016.

[8] A. H. Ali*, "High-Performance Liquid Chromatography (HPLC): A review," ANNALS OF ADVANCES IN CHEMISTRY, 2022.

[9] B. J. C. Bergh J. J., "Stability-indicating High-performance Liquid- chromatographic Analysis of Trimethoprim in Pharmaceuticals. J. Chromatogr," International Journal of Research Publication and Reviews,, pp. 528-531, 1987.

[10] M. P. D. Laxman1, "Principles and Methods. In: Amesham Biosciences of Reversed Phase Chromatography," International Journal of Research Publication and Reviews,, pp. 6-8, 1999.

[11] M. P. D. Laxman1, "Types of HPLC. http://HPLC.chem.shu.edu/NEW/HPLC_Book/Introduction/int_t yps.html.," International Journal of Research Publication and Reviews,, 2017.

[12] M. Hearn, "Ion-pair chromatography on normal and reversed phase systems. Advances in Chromatography.," International Journal of Research Publication and Reviews, p. 100, 1980.

[13] R. Malviya, V. Bansal, O. Pal and P. Sharma, "High Performance liquid chromatography: a short review," J Global Pharma Technol, p. 22, 2010.

[14] M. L. D. J. S. F. Willard H, "nstrumental Methods of Analysis 7th edn,," Wadsworth Publishers, Stamford, CT, 1998..

[15] M. S. Azim, M. Mitra and P. S. Bhasin, "HPLC Method Development and Validation a Review," International Research Journal of pharmacy, pp. 39-43, 2013.

[16] A. Schellinger and P. Carr, " Isocratic and gradient elution Chromatography: a comparison in terms of speed, retention Reproducibility and quantitation. J Chromatography," International Journal of Research Publication and Reviews, pp. 253-66, 2006.

[17] A. H. Ali*, "High-Performance Liquid Chromatography (HPLC): A review," ANNALS OF ADVANCES IN CHEMISTRY, 2022.

[18] S. McCown, D. Southern and B. Morrison, "Solvent Properties and their effects on gradient elution high-performance liquid chromatography: III. Experimental findings For water and acetonitrile. J Chromatography A 1," International Journal of Research Publication and Reviews, pp. 493-509, 1986.

[19] M. P. D. Laxman1, "How Does High Performance Liquid Chromatography Work http://www.waters.com," International Journal of Research Publication and Reviews,, 2022.

[20] e. a. Harmita, "Optimation and validation of analytical method of cotrimoxazole in tablet and plasma in vitro by High performance liquid chromatography," J Bioanal Biomed, pp. 26-29, 2012.

[21] e. a. Subbaiah PR, "Method development and validation for estimation of moxifloxacin HCl in tablet dosage Form by RP-HPLC method.," Pharm Anal Acta, p. 109, 2010.

[22] e. a. Ahir KB, ". Simultaneous estimation of metformin hydrochloride and repaglinide in pharmaceutical Formulation by HPTLC-Densitometrymethod. J Chromat Separation Techniq.," International Journal of Research Publication and Reviews,, p. 166, 2013.

[23] A. Connors, "K. A Text Book of Pharmaceutical Analysis, 3rd Ed:," A Wiley Interscience publication: New York, pp. 373-400, 2005.

[24] D. K. A. D. Bhardwaj SK, "A Review: HPLC Method Development and Validation," International Journal of Analytical and Bioanalytical Chemistry, pp. 76-81, 2015.

[25] S. BK, "Instrumental methods of chemical analysis. (25th edn.);," Goel publishing house, pp. 286-385, 2006.

[26] K. PC, Pharmaceutical analysis Instrumental methods, Delhi.India: Vallah publication, 2010.

[27] M. Zaręba, P. Sanecki and R. Rawski, "Simultaneous Determination of thimerosal and aluminum in vaccines and Pharmaceuticals with the use of HPLC method," Acta Chromatographica, pp. 1-3, 2015.

[28] B. Yilmaz and Y. Kadioglu, "Development and validation of HPLC method for determination of human insulin in Pharmaceutical preparation.," Int J Pharm Sci Rev Res, p. 2, 2010.

[29] J. Aulakh, A. Malik, V. Kaur and P. Schmitt-Kopplin, " A Review on solid phase micro extraction—high performance Liquid chromatography (SPME-HPLC) analysis of pesticides," Critical Rev Anal Chem , pp. 71-85, 2005.

[30] M. Płonka, S. Walorczyk and Miszczyk.M, "Chromatographic Methods for the determination of active substances and Characterization of their impurities in pesticide formulations.," TrAC Trends Anal Chem, pp. 67-80, 2016.

[31] J. Esteve-Romero, J. Albiol-Chiva and J. Peris-Vicente, " A review On development of analytical methods to determine Monitorable drugs in serum and urine by micellar liquid Chromatography using direct injection," Anal Chimica Acta, pp. 1-6, 2016.

[32] R. Kaushik, B. Levine and W. LaCourse, "A brief review: HPLC methods to directly detect drug glucuronides in Biological matrices," Anal Chimica Acta , pp. 255-66, 2006.

[33] L. Roy, C. Harrell, A. Ryan, T. Thorsteinsson and F. Sancilio, "Development and Validation of a Single HPLC Method For Anal Purines in Fish Oil Supplements.," Food Nutr Sci, p. 1255, 2013.

[34] K. Pyrzynska and A. Sentkowska, " Recent developments in the HPLC separation of phenolic food compounds.," Crit Rev Anal Che, pp. 41-51, 2015.

[35] J. Olbrich and J. Corbett, ". Development and Utilization of Reversed Phase High Performance Liquid Chromatography Methods for a Series of Therapeutic Agents," Modern Chem Appl, p. 101, 2013.

[36] U. Mansur and J. Sartika, "Optimation and Validation of Analytical Method of Cotrimoxazole in Tablet and Plasma In Vitro by High Performance Liquid Chromatography," J Bioanalysis Biomedicine, pp. 26-9, 2012.