IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 5, November 2025
Impact Factor: 7.67

An Analytical Study on the Formulation and Verification of HPLC Procedure

Anuja Dattatray Khatke[1], Nilesh Sevak Dhavre[2], Sanjay Kisanrao Bais[3]
Fabtech College of Pharmacy, Sangola
Correspondence Author: - Anuja Dattatray Khatke
Email: anujakhatke4510@gmail.com

Abstract: *HPLC is one of the most valuable types of column chromatography techniques used in pharmacy, pharmaceutical business, and biochemistry. It is utilized for the identification, separation, quantification, validation, and optimization of active substances. Drugs can be sorted, located, and measured using a separation process called HPLC [High Performance Liquid Chromatography]. This article focuses on the research, development, and manufacturing of innovative medications. In the human and animal studies as well. Developing and qualifying an HPLC technology requires a number of stages. It depends on the molecule's polarity, solubility, pH, PK, and other factors. Following ICH criteria for HPLC method validation provides information on performance aspects such an important performance characteristics for method validation includes trueness, repeatability, selectivity, proportionality, working intervals, minimum quantifiable concentration, and method resilience.*

Keywords: High efficiency chromatographic separation ,Compound separation chromatographically technique, protocol establishment, procedure qualification , trueness , repeatability.

I. INTRODUCTION

Analytical liquid chromatographer technique represents highly sophisticated an evaluative technique employed to isolate, determine, and quantify the constituents of a specimen that can be dissolved within a liquid medium.. Among modern analytical techniques, HPLC is regarded as one of the most trustworthy and efficient methods for evaluating both qualitative and quantitative characteristics of drugs and formulations [1]. During analysis, the mobile phase (liquid solvent) is driven at elevated pressure through a column containing a stationary phase made of porous material. Variations in how each analyte interacts with these two phases cause them to migrate at different rates, producing effective separation [2]. Compounds that exhibit stronger attraction towards the retentive medium moves more slowly within the column, whereas those with lesser attraction move through it more quickly. This technique ensures superior resolution and sensitivity, functioning under high-pressure conditions to facilitate the smooth flow of the solvent through the packed column. Since being introduced in the 1960s, HPLC has become an essential tool in pharmaceutical, environmental, and chemical laboratories because of its accuracy, reliability, and adaptability across numerous sample categories**.**

Principle

In advanced liquid chromatographical technique the prepared mixture is fed into separation chamber containing immobilized phase and a eluent is driven through under pressurized flow. Due to variable interactions between the chemical entities and both phases, the fractions migrates at a distinct rates, leading to sequential exit phase and effective segregration.

Types of HPLC

1]. Normal Phase Chromatography :-

Chromatography (HPLC) analysis is determined by the phase system used. Analytes are categorized by polarity using traditional polarity driven separation method. Polar test substances are retained in adsorption mode HPLC through

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429 Volume 5, Issue 5, November 2025 Impact Factor: 7.67

interactions between a fixed polar matrix and a hydrophobic carrier solvent. Because of this interaction, elution time rises as analytes polarity increases . NP- HPLC is a useful analytical method, especially for characterizing compound with different polarities in a variety of domains like chemistry, pharmaceuticals, and environmental study. It offers efficient separation and provides insights into sample composition based on polarity.[3,4].

2] Inverted Phase Separation (Chromatographical) method :-

In inverted reversed phase HPLC , the liquid solvent used as the flowing medium is polarity active agent or semi-polarity active component , while the packed bed is hydrophobic or nonpolar. This chromatographic method functions according to the hydrophobic attraction mechanism, where compounds with greater nonpolar characteristics interact more strongly with the stationary bed, leading to delayed elution[5].

Classification of High-Performance Liquid Chromatography:-

It can be classified into several categories based on operational scale, separation principle, and elution pattern.
According to the scale of usage, HPLC is broadly separated into analytical and preparative systems.
Different chromatographic approaches include size-exclusion, adsorptive, and affinity-based techniques.
When classified by mechanism of separation, the method includes ion-exchange and chiral-phase chromatography.
Furthermore, depending on the elution strategy, systems are categorized as isocratic or gradient elution methods.
Based on operational mode, chromatography can function under normal-phase or reverse-phase conditions..[6,7].

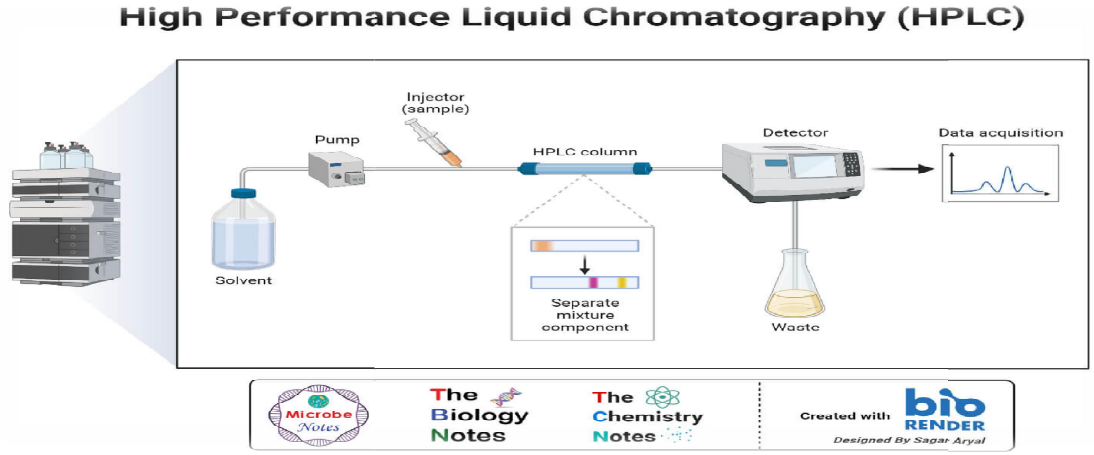

Figure 1. A schematic diagram of HPLC instrument.

Main parts of the HPLC System:-

- Mobile phase delivery module
- Pressure – generating pump
- Solvent degasser or air removal unit
- Sample introduction system
- Protective pre- column
- Primary analytical column
- Signal detector
- Computerized data acquisition and integration unit

1] The Pump

The pump is the most crucial part of the HPLC solvent delivery system since it is directly affects to the detector sensitivity, repeatability, and retention duration. The stationary phase particle size ranges from 5 to 10 µm. Thus, the

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429 Volume 5, Issue 5, November 2025 Impact Factor: 7.67

flow resistance is seen. This is the reason behind the need for high pressure, which pump supply. There is various kind of pumps :-

ⅰ . Constant pressure pump

ⅱ . Constant flow pump

2] The solvent degassing system

Since a number of gases are soluble in organic solvents, the components prior to use, the mobile phase should undergoes filtration and degassing to remove impurities and air bubbles that could interfere with chromatographic performance . The separation process will be hampered by the gas bubbles that occurs when the solvents are poured at high pressure. To eliminate dissolved gases in the mobile phase.

3]. Sample injection system

The sample injection system adds or introduce either a sample solution or a standard solution to the mobile phase. Analytes can be introduced to the column using either a stop flow injection or an injection into a flowing stream. There are numerous devices that can be injected manually or automatically.

4]. Guard column

The guard column prolongs the life of the analytical column while containing a very minimal amount of adsorbent. Additionally, it serve as a prefilter to exclude other materials and any particle debris. It is employed before the analytical column in order to protect the column's lifespan, although it does not contribute to any separation.

5]. Primary Analytical column

HPLC columns are commonly made from durable materials such as glass, stainless steel, polyethylene, or the more advanced polyether ether ketone (PEEK). These columns usually possess an inner diameter of 2–5 mm and a length that ranges from 50 mm to 300 mm. They are packed with stationary-phase particles of 3–10 µm in size, offering a surface area between 100 and 860 m^2/g, displaying a median of approximately 400 meter square/g. analytical shaft with internal width below 2 millimeter are identified as microbore types. To ensure accurate chromatographic performance, the heat level of both the packed bed tube and the moving medium should remain stable throughout the assessment. The functional chemistry of the stationary zone is ascertained in the manner of separation. Normal-section chromatography utilizes hydroxylated silanol groups, while reverse-phase chromatography employs hydrophobic ligands such as C18 (octadecylsilane), C8 (octylsilane), C4 (butylsilane), cyanopropyl (CN), and amino (NH_2) groups for effective separation.

6]. Detectors:-

A detector positioned in downstream of the column to detects the compounds that are eluted from the column. When a material has traveled through the column, there are a number of methods for detecting it. The characteristics of the substance to be separated determine which detector is utilized.

UV Detectors:-

This kind of detectors is the most widely used since it possesses high sensitivity, exhibits a wide dynamic response range , is largely insensitive to verifying temperature condition , and works well with gradient elution. It keeps track of substances that absorbs visible or ultraviolet light. UV of absorbing electromagnetic radiation at different wavelengths. The extent of light absorption depends on the concentration and nature of the substance currently passing through the light beam. Wavelength at which absorption occurs is more than 200nm, as long as the molecule possesses.

DOI: 10.48175/IJARSCT-30064

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429 Volume 5, Issue 5, November 2025 Impact Factor: 7.67

Differential refractive detector:-

Differential refractometers serve as universal detectors in HPLC systems. To enhance the UV models. These detectors measures the refractive index difference comparing a reference channel of containing the uncontaminated mobile phase the column eluent. Detection occurs whenever there is a measurable changes in refractive index between the solute and the mobile medium, enabling the identification of virtually any compound are the HPLC detectors that are closest to a universal detector. All eluting zones with RI distinct from the refined clarified solvent stream are recorded. The more powerful the signal, the larger the distinction between the eluents and sample light bending indices .

Fluorescence detectors:-

This detector is capable of detecting compounds with the fluorescence or compounds with fluorescing derivatives with excellent sensitivity and specificity. Compared to UV detection, the sensitivity could be up to 1000 times higher. The cell is exposed to light of the appropriate wavelength, and radiation with greater wavelength is observed at an angle of ninety degrees. Using a somewhat large cell (20ml or more) increases the luminous strength and thus, the detection capability. Simple units have a defined wavelength range for fluorescent light detection.

Photo diode array transducers :-

The (DAD), is sometimes referred to as the photo diode array (PDA),has the ability to measures the whole wavelength range in real times, which could have the additional benefits. A configured row of separate photo detecting elements fabricated on a semiconductors IC device for spectral measurement is termed as a photodiode array detector.

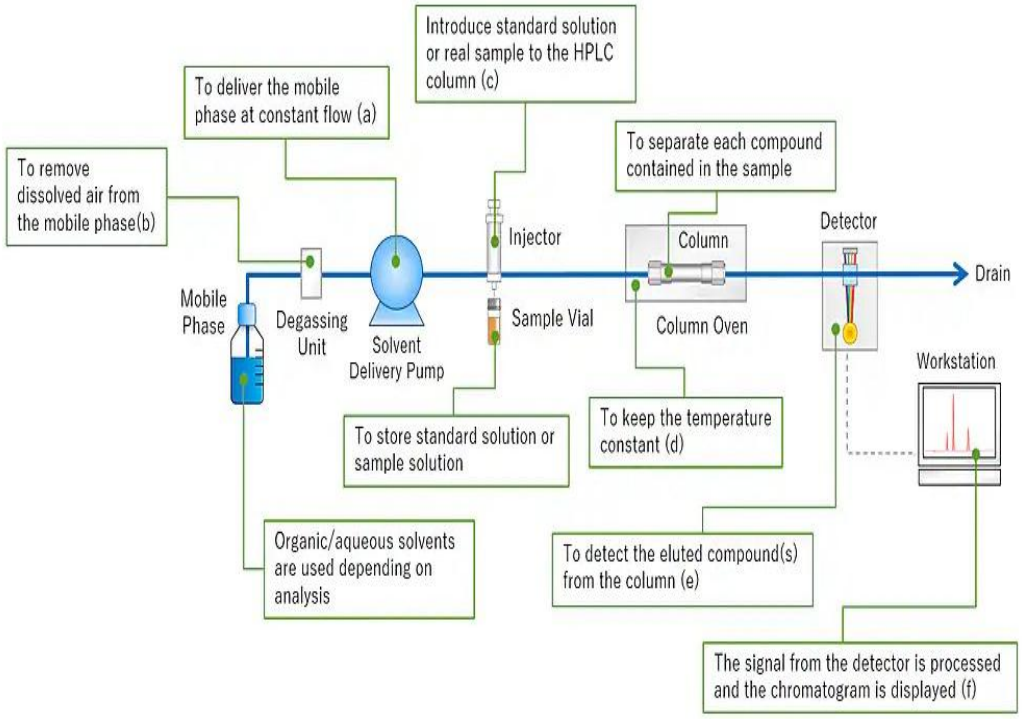

Fig 2 :- HPLC instrumentation names and its work.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 5, November 2025
Impact Factor: 7.67

Development Workflow for HPLC Techniques:

- Understanding the core chemical properties of the molecule being studied.
- Selecting the instrumental and chromatographic parameters needed for separation.
- Outlining the methodological framework for analysis.
- Preparing and processing the sample before chromatographic injection.
- Adjusting conditions for maximum method performance.
- Verifying the credibility and consistency of the final method.

Understanding the core chemical properties of the molecule being studied.:-

In the process of developing an analytical technique, the chemical and physical attributes of a drug compound play a vital role. Prior to establishing a method, it is essential to evaluate parameters such as solubility, polarity, dissociation constant (pKa), and pH of the substance. The polarity of a compound serves as a key physical factor that aids analysts in the process of deciding the most appropriate dispersing liquid system and proportion of the passing chemical fluid. The solubility behavior of a molecule is directly influenced by its polarity. For instance, non-polar liquid reagent namely benzol keep seperate with polar solvents like water. Compounds tend to dissolve in solvents of similar polarity — reflecting the general principle of "like dissolves like."

Selection of Diluents and Mobile Phase the choice of a suitable diluent or moving phase depends on its ability to dissolve the component under study and its chemical compatibility, ensuring that none of the liquid components react with the substance being evaluated. In HPLC method design, acidity index and dissociation index considerations play a crucial role. The pH denotes the basic species -10 negative logarithm of hydronium ion concentration. Adjusting the ionization level for ionizable compounds allows the generation of well-defined and symmetrical separation science related peaks, contributing to improved sensitivity, minimal injection-to-injection variability, and stable retention times—all essential for accurate quantitative analysis. [8,9].

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 5, November 2025
Impact Factor: 7.67

II. SELECTION OF CHROMATOGRAPHIC CONDITION

During the initial phase of method setup, a preliminary set of component discrimination factor —including the type of detector, the column, and the moving phase—is selected to obtain the first trial chromatograms. Reversed-phase systems, typically employing a C18 sorbent and UV absorbance detection, are commonly used. At this stage, the analyst must decide whether the separation will rely on an program or a gradient profile, as each mode offers distinct advantages depending on the specific characteristics and separation requirements of the compounds present.

2.1. Choosing the Column

Selecting the appropriate chromatographic column represents the first and most essential stage in HPLC method formulation. A reliable and high-performance column is fundamental for establishing a robust and consistent analytical system. Columns should demonstrate long-term stability and consistent performance to avoid irregularities in sample retention times or peak behavior during analytical runs.

In most cases, reversed-phase columns such as C8 and C18 are suggested, as they are made from high-purity, low-acid silica materials that provide optimal resolution for alkaline or basic compounds. [10]

The column's bonded phase properties, silica quality, and geometric parameters such as diameter and length are the main considerations during selection. Owing to their outstanding surface chemistry and physical robustness, silica-based materials are the preferred packing medium in current HPLC column manufacturing. [11]

2.2. Buffers selection

The required pH determines the choice of buffer. Within RP based proficiency using siliceous material based sorbent packaging, the typical acceptable proton activity measure span is 2 to 8. as ionic stabilizers govern hydrogen potential scale is ideally at their proton release index is close to the desired acidity level. The common guideline it is advisable to choose a buffer system whose dissociation constant (Pka) is no more than two units lower than of the acidity level of the intended mobile phase. General factors to take into account while choosing a buffer :-

Compared to acetonitrile or THF, Phosphate dissolves better in methanol than in the water.

Certain hygroscopic salt buffers can causes chromatographic changes, among these increased peak skewing alkaline compounds and potentially different selectivities.

In primary core, ammonia derived salts exhibit enhanced solubility in aqueous organic solvent mixture .

After extended use , Triflouroacetic Acid degrades or loses stability . It absorb at the short UV detection band as they are prone to volatility in nature.The growth can impair chromatographic performance when it builds up on column inlets.

Phosphate buffer speeds up silica dissolution at pH values higher than 7, significantly reducing the lifespan of silica based HPLC columns. Organic buffering agents are more effective at alkaline PH conditions, typically above 7.7. in contrast, ammonium bicarbonate buffer are short lived, generally remaining stable for only 24 to 48 hours and are sensitive to pH fluctuations. Buffers is filtered by 0.2µm filter.[12].

2.3 Buffer Concentration :-

In general, a buffer concentration of 10-50mM is enough for tiny compounds. Generally speaking, you can only use up to 50% organic while using a buffer. This would rely on the buffer in question as well as its concentration. Phosphoric acid its sodium or potassium counterpart are the most widely used buffer system for RP- HPLC [13].

	Dissociation constant or useful buffering intervals	UV absorption limits (nm)
pH modifying agents		
Perflouroacetic acid	0.2	210
Formylic acidic	3.8	210
Ethanoic acid	4.8	230
Ammoniacal liquor	9.2	200

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429 Volume 5, Issue 5, November 2025 Impact Factor: 7.67

Buffers		
Azanium acetate	3.8-5.8; 8.2-10.2	205
Ammonium formiate	3.3-4.3; 8.8-9.8	210
Bi carbonate of ammonia	5.9-6.9; 8.8-9.8	200
Aquaammonia/ Ammonium hydrate	8.2-10.2	200

Fig 3 :-Buffer solution and its concentration used in HPLC

III. DESIGNING THE ANALYTICAL STRATEGY

In the early phase of RP-HPLC method development, selecting the appropriate experimental variables—including the stationary phase, mobile phase solvent system, eluent flow rate, and buffer pH—is essential. These parameters are optimized through systematic experimentation and then evaluated according to system suitability benchmarks.

Common acceptance criteria comprise a retention period exceeding five minutes, a column efficiency (measured as theoretical plates) greater than 2,000, a peak band distortion ratio is beneath 2, a discrimination power above 5, and a relative amount RSD below 2% for peak area reproducibility.

When two compounds are measured concurrently, the sensing photonic range is picked at an isobestic wavelength, ensuring equal absorbance for both substances. The linearity test confirms the working concentration range over which the analyte maintains a linear detector response. Finally, to verify the method's applicability for simultaneous analysis, a synthetic sample mixture is evaluated under the same chromatographic setup. [14,15]

IV. PREPARATION OF TEST MATERIAL

Within HPLC investigations, preparing the sample is a vital operation that ensures the generation of a consistent and injection-ready solution. The purpose of this stage is to create a well-processed fraction that aligns with the analytical method, is column-appropriate, and remains unaffected by contaminants that may distort chromatographic output.

This requires selecting a solvent medium capable of dissolving the analyte and remaining compatible with the dynamic phase, without negatively affecting peak retention or system resolution.

This initial step—beginning from collecting the material, processing it, and finally feeding it into the HPLC column [16,17].

V. METHOD REFINEMENT

In this phase, the goal is to identify potential drawbacks or weak areas of the developed analytical method and apply designed experiments to make systematic improvements. The procedure's response and stability are assessed under varied sample matrices, equipment setups, and testing conditions to verify its consistency and robustness.

This stage ensures that the analytical system remains accurate, precise, and efficient under diverse laboratory circumstances.[18].

VI. VALIDATION

The methodical process of evaluating and offering impartial proof that certain specifications for a certain intended application are fulfilled is known as validation. It entails evaluating a testing approach efficiency or reliability along with confirming such verifying it , can be satisfy particular requirements. In essence, validation gives you a comprehensive grasp of what your method can consistently deliver, especially when working with analytical techniques like High -Performance Liquid Chromatography (HPLC) that require tiny dosages or difficult circumstances [19].

6.1.Method Validation

Authentication refers to the systematic process of demonstrating, through laboratory studies, that an laboratory method possesses the required performance attributes for its intended use. Any method that is newly introduced or modified

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429 Volume 5, Issue 5, November 2025 Impact Factor: 7.67

must be assessed to confirm that it can deliver consistent, exact, and repeat verified outcomes, even when performed by different analysts, in various laboratories, or on identical instruments.

The type and extent of procedural approval depend entirely on the procedure's purpose and the context in which it will be applied.

Results obtained from method validation serve as essential evidence for evaluating the accuracy, reliability, and uniformity of analytical measurements, making validation a fundamental element of any robust analytical method.

The method validation procedure depends on using equipment that is within specification, operating as intended and calibrated correctly. Analytical procedure must be validated or revalidated [20].prior to their regular implementation; when the condition under which the validated method is used alter; when the method is modified, The FDA,USP, and ICH typically recommend the following parameters [20,21] :-

1. Trueness
2. Consistency
   - Intra assay consistency
   - Moderate consistency
3. Selectivity
4. Detection Threshold
5. Quantification Threshold
6. Proportionality
7. Working interval
8. Stability or resilience

1. Trueness :- This parameter reflects the extent to which the obtained measurement agrees with the established true or reference value. It represents how correct or truthful the interpretative method .

2. Consistency :- Consistency measures the degree to which consecutive analyses of the same homogenous sample provide tightly clustered values. It includes aspects such as repeatability a moderate level of accuracy[intermediate precision]The ability to reproduce.[Reproducibility]Reliability [Repeatability] (same analyst), intermediate precision (different analysts/days), and reproducibility (different labs).

3. Selectivity :- Selectivity denotes the capacity of the method to isolate and quantify the intended analyte without interference from structurally similar compounds, by-products, contaminants, or matrix elements.

4. Detection Threshold :- The Detection Threshold is defined as the minimal The entity selected for evaluation level that produces a detectable signal, even though it may not be measurable with high exactness.

5. Quantification Threshold :- The Quantification Threshold identifies the lowest amount of The material being quantified that can be measured with reliable narrow variability and correctness, ensuring results suitable for quantitative reporting.

6. Proportionality :- Linear dependence evaluates how well the analytical response correlates with Pertaining to detailed examination increasing Examined constituent concentrations. It is typically validated using multiple calibration levels and statistical measures like slope, intercept, and correlation factor (R^2).

7. Working interval :- It refers to the span of concentrations over which the Investigative method maintains validated performance, demonstrating appropriate straight line relationship, correctness, and short term repeatability.

8. Stability or resilience :- Stability or resilience reflects the method's ability to function dependably even when slight, controlled variations are introduced to operational conditions such as temperature, pH, flow rate, or solvent composition.

Field of Application :-

Utilization HPLC is used in the food business as well as in the pharmacy, environmental, therapeutic, and forensic domains. Resolution, identity, and quantification of a substance are among the information that HPLC may provide. Additionally, it helps in purification of mixtures of substances, molecular weight measurement, and chemical separation. The foundation of chemical separation is the observation that specific chemical migrate at varying speeds

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429 Volume 5, Issue 5, November 2025 Impact Factor: 7.67

For a particular chromatographic column and mobile medium, the individual constituents of a sample are effectively resolved. The procedure of isolating or extracting a desired analyte from a mixture containing various substances or contaminants is termed purification. Under defined chromatographic parameters, every chemical entity demonstrates a unique retention signal or distinctive peak, which facilitates its identification and quantification during the analytical process. Proteins, chemicals, pharmaceuticals, and other compounds can all have their molecular weights determined thanks to molecular weight determination. Typically, HPLC is used for the assay of chemicals for identification. To facilitate the easy identification of pure chemicals.

Other application of HPLC Includes :-

Pharmaceutical applications :-

medicinal applications, such as tablet dissolution studies of medicinal dosage forms, are further uses for HPLC. stability research and shelf-life calculations. determining the dose forms' active medicinal ingredients. Pharmaceutical formulation assay and impurity analysis. quality assurance. development and research.

Forensics :-

In forensic science, HPLC is utilized for the quantitative assessment of pharmaceuticals within biological matrices. It is widely applied for the detection of anabolic steroids and related substances in biological fluids and tissues such as perspiration, hair strands, urine, and blood serum, providing critical evidence for toxicological and doping investigations. identifying whether cocaine and its metabolites are present in the blood. textile industry forensic analysis.

Clinical:

Analysis of fruit liquids for sugar. Polycyclic chemicals in vegetables are analyzed. trace examination of agricultural produce containing military high explosives. checking fruits for pesticides and insecticides.

Advantages :-

Benefits Compared to alternative chromatography techniques, HPLC has numerous benefits. It has significantly aided in the development of analytical science and its wide range of applications in disciplines such as forensics, food, medicines, polymers and plastics, healthcare settings, and the environment. For a wide range of complex samples, HPLC offers an analytical approach that is highly specific, reasonably precise, and reasonably quick. HPLC has the ability to handle macromolecules. It works incredibly well with the majority of "pharmaceutical drug substances.". It provides a productive way to analyze "labile natural products.". Can be reliably analyzed using HPLC. In HPLC, sample preparation and introduction are straightforward. Compound resolution and separation speed are both high. Results from HPLC software can be reported with accuracy and precision. The employed detectors have a high sensitivity. To accommodate various application ranges. Information is simple to record and store. The columns can be rested for a considerable amount of time when they are operated carefully and under controlled conditions without overloading. The effectiveness of HPLC in conjunction with mass spectrophotometers and FT-IR systems has increased.

Disadvantages:-

Drawbacks One of the most significant methods of the final ten years of the 20th century is HPLC. Notwithstanding the many benefits, there are some drawbacks as well. The cost of the columns, the solvents, and the proprietary nature of the column packing prevent long-term reproducibility. Other factors include the difficulty of separating some protein-specific antibodies. Creating an HPLC apparatus for an experiment or a technique for separating separate components comes at a huge cost. Two chemicals with comparable structures and polarities can co-elute—leave the chromatographic system simultaneously—because of the HPLC's speed and dependence on distinct polarities. This makes compound detection challenging. It can be challenging when certain chemicals have low sensitivity to the stationary phase in the columns. Some substances react or are absorbed by the chemicals included in the column's packing materials. If mass spectrometry is not interfaced with HPLC, qualitative analysis may be restricted.

Recent Developments in High-Performance Liquid Chromatography (HPLC) :-

Advancements in analytical technology have significantly strengthened the performance and versatility of HPLC systems.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429 Volume 5, Issue 5, November 2025 Impact Factor: 7.67

HPLC–Mass Spectrometry (HPLC–MS): Integrating HPLC with mass spectrometric detection has improved both sensitivity and selectivity, allowing precise identification and quantification of complex analytes. This combination serves as an indispensable tool in modern analytical chemistry [22].

Ultra-High-Performance Liquid Chromatography (UHPLC): This upgraded version of conventional HPLC employs columns packed with finer particles, providing superior resolution, enhanced efficiency, and reduced analysis time [23].

Eco-friendly or Green HPLC: This approach aims to minimize solvent usage and lower environmental hazards by applying sustainable mobile phases, optimizing operational parameters, and reducing waste generation [24].

VII. CONCLUSION

The RP-HPLC Technique is discussed in the review. Method development and validation are ongoing, interconnected procedures that determine the measurement's performance limits and measures a parameter as intended. The separation selectivity is significantly impacted based on the picking associated with separation cartridge, ionic adjustment mixture, response sensor, spectral region and supplementary variables. Enhanced distinguishing capacity, responsiveness, economy, reduced duration, and minimal detection boundary value were the benefits of the HPLC technology. Final optimization can be achieved. According to ICH recommendations.

REFERENCES

[1].Rao B. V, Sowjanya G. N, Ajitha A, Rao Uma M. V, A Review on Stability Indicating HPLC Method Development, World Journal of Pharmacy and Pharmaceutical Sciences,2015:4(8):405-423.

[2]. Rajan H. V, A Review on Development and Validation of HPLC Method , International Journal of Current Research in Pharmacy,2015:1(2):55-68.

[3]. A Review on HPLC Method Development and Validation https://doi.org/10.1002/mas.1280110103 (accessed 06.09.25).

[4]. A Review on HPLC Method Development and Validation https://doi.org/10.1002/mas.1280080103 (accessed 08.09.25).

[5]. A Review on HPLC Method Development and Validation http://www.chemiguide.co.uk (accessed 10.09.25)

[6]. Gupta V, Jain A. D, Gill N. S, Gupta K, A Review on Development and Validation of HPLC method , International Research Journal of Pharmaceutical and Applied Sciences,2012:2(4):17-25.

[7]. Sonia K, Nappinnai M, Development and Validation of HPLC and UV-Visible Spectrophotometer Method for the Pharmaceutical Dosage Form and Biological Fluid – Review, European Journal of Biomedical and Pharmaceutical Sciences,2016:3(3):382-391.

[8].A Review on HPLC Method Development and Validation
https://www.scribd.com/doc/9508765/Physical-Properties-of Drug (accessed 12.09.25)

[9]. A Review on HPLC Method Development and Validation
https://www.xtremepapers.com (accessed 14.09.25)

[10].M. S. Charde, A. S. Welankiwar, J. Kumar, Method Development by Liquid Chromatography with Validation, International Journal of Pharmaceutical Chemistry, 2014:04(02):57-61.

[11]. Ranjit Singh, HPLC Method Development and Validation, J Pharm Educ Res, 2013:4(1):26-33.

[12]. Sabir A. M. , Molony M. , Parminder S. B, HPLC Method Development and Validation A Review, International Journal of Pharmacy:2013:4(4):39-46.

[13]. Noman A, Bukhaiti A.L, Wedad Q, Alfarga A, AbedSherif M, Mahdi A.A, And Waleed A.A, HPLC Technique Used in Food Analysis – Review, International Journal of Agriculture Innovations and Research, 2016:5(2):181-188.

[14].Sethi P. D, Introduction – High performance Liquid Chromatography, 1ST Edition, CBS Publishers & Distributors Pvt. Ltd. , New Delhi, 2001, pp.1-28

[15].A Review on HPLC Method Development and Validation, https://doi.org/10.1016/j.jchromb.2010.11.011 (accessed 16.09.25)

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429 Volume 5, Issue 5, November 2025 Impact Factor: 7.67

[16]. G. David Watson, Introduction to Pharmaceutical Analysis in Pharmaceutical Analysis , 3rd Edition, Churchill Livingstone, London: Harcourt Publishers Limited, 2012, pp. 1-765.
[17]. A. H. Beckett, J. B. Stenlake, Principles and Practices in Practical Pharmaceutical Chemistry,4TH Edition Vol I & II , CBS Publishers and Distributors Pvt. Ltd., New Delhi,2007, pp.275-290.
[18].Kayode J, Adebayo A, Effective HPLC Method Development in Journal of Health Medicine and Nursing,1st Edition Vol 12,IISTE Publications, Abuja, 2015,pp.123-133.
[19].T. Higuchi, Brochman - Hansen, Pharmaceutical Analysis in Pharmaceutical Chemistry Series, 3rd Edition, CBS Publishers and Distributors Pvt. Ltd ,New Delhi,1997, pp.310-340.
[20].T. Bhagyasree ,N. Injeti, A. Azhakesan, U. M. V. Rao , A Review on Analytical Method Development and Validation, International Journal of Pharmaceutical Research & Analysis, 2014:Vol 4:(8):444-448.
[21]. V. Kumar, R. Bharadwaj, G. G, S. Kumar, An Overview on HPLC Method Development, Optimization and Validation Process for Drug Analysis, The Pharmaceutical and Chemicals Journal, 2015:2(2):30-40.
[22].Amol V. Pore, Sanjay K. Bais, Vaibhav J. Ghutukade, Review on Recent Advancement in Herbal Technology, International Journal of Pharmacy and Herbal Technology,2024:2(1):428-439.
[23].Yogesh B. Raut, Sanjay K. Bais, Nandini Regoti, A Review on Advancement Herbal Technology, International Journal of Pharmacy and Herbal Technology,2023:1(3):105-123.
[24]. Sarfaraz Kazi, Sanjay K. Bais, Pratikasha Shinde, An Analysis of Modern Herbal Technology, International Journal of Pharmacy and Herbal Technology,2024:2(1):603-611.