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A REVIEW ON: RECENT ADVANTAGES IN HPLC: PRINCIPLES, METHOD DEVELOPMENT STRATEGIES – MODERN INNOVATIONS OF APPLICATIONS OF PHARMACEUTICAL ANALYSIS

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Abstract

Chromatographic methods play a pivotal role in modern analytical chemistry, with High-Performance Thin Layer Chromatography (HPTLC) and High-Performance Liquid Chromatography (HPLC representing two of the most widely applied separation techniques.It provides accurate and reproducible assay results, ensuring that pharmaceutical products contain the appropriate drug concentration required for therapeutic effectiveness.The composition of the mobile phase is optimized to achieve adequate separation, proper peak shape, and reproducible retention time.International regulatory authorities such as the International Council for Harmonisation (ICH), United States Food and Drug Administration (FDA), European Medicines Agency (EMA), and World Health Organization (WHO) have established harmonized guidelines to ensure analytical method suitability, validation, and lifecycle management.

INTRODUCTION

High performance liquid chromatography (HPLC) has emerged as an indispensable analytical technique in pharmaceutical sciences, owing to its high separation efficiency and accurate quantitative determination of drug substances. The progressive advancement of HPLC technology has mirrored the growing sophistication of pharmaceutical formulations and the escalating regulatory requirements for robust and precise analytical methodologies. These developments have markedly advanced pharmaceutical analysis, enabling superior resolution, sensitivity, and reproducibility relative to conventional chromatographic approaches. Within the pharmaceutical industry, HPLC is extensively applied for critical analytical tasks, including raw material quality assessment, formulation characterization, stability evaluation, and impurity profiling [1]. The technique's adaptability supports the analysis of a diverse range of chemical entities encompassing both small-molecule drugs and complex biologics. Advances in stationary phase technology, particularly the adoption of sub-2-μm particle columns and core–shell materials, have further optimized chromatographic performance by enhancing resolution and accelerating analysis.

Chromatographic methods play a pivotal role in modern analytical chemistry, with High-Performance Thin Layer Chromatography (HPTLC) and High-Performance Liquid Chromatography (HPLC representing two of the most widely applied separation techniques. Continuous advancements in these methodologies have substantially enhanced the precision, reliability, and scope of chemical analysis. HPTLC, an advanced adaptation of traditional thin-layer chromatography introduced in the 1970s, has evolved through the integration of high-quality stationary phases, automated sample handling, and sophisticated detection systems, establishing it as a standardized and robust planar chromatographic approach. Likewise, HPLC which originated in the 1960s, has undergone extensive technological refinement in terms of column materials, pumping mechanisms, and detector design. The objective of HPLC method development is to optimize analytical conditions that provide accurate and precise determination of specific analytes, which involves appropriate selection of mobile phase composition, pH, gradient conditions, column type, sample preparation procedures, and detection techniques to achieve reliable chromatographic performance [2]. Recent advances in reverse-phase HPLC have focused on overcoming limitations in interference separation, enabling rapid and reliable simultaneous analysis of combination pharmaceutical formulations such as tadalafil and losartan potassium, as discussed in this review. Recent technological progress has elevated HPLC into a highly advanced analytical platform, with innovations such as UHPLC and hyphenated HPLC–MS/MS techniques substantially improving sensitivity and enabling sub-nanogram-level detection for a wide range of compounds [3].

1. Pharmaceutical importance in HPLC

High-Performance Liquid Chromatography (HPLC) plays a vital role in the early phases of drug discovery by facilitating the analysis of newly synthesized chemical entities and natural products. It is extensively employed for the accurate identification, purity assessment, and quantitative determination of potential drug candidates. During chemical synthesis, HPLC enables continuous monitoring of reaction progress and efficient detection of impurities, intermediates, and by-products.

Quantitative and Qualitative Analysis of Active Pharmaceutical Ingredients (APIs)

HPLC is extensively used for the qualitative identification and quantitative determination of active pharmaceutical ingredients in bulk drugs and dosage forms. It provides accurate and reproducible assay results, ensuring that pharmaceutical products contain the appropriate drug concentration required for therapeutic effectiveness. HPLC is routinely applied to various dosage forms such as tablets, capsules, injections, suspensions, and transdermal systems. Its high separation efficiency enables clear resolution of APIs from excipients, impurities, and degradation products, ensuring precise and reliable analysis [4,5].

Impurity Profiling and Purity Assessment Impurity profiling is essential for ensuring the safety and quality of pharmaceutical products. Impurities may arise during synthesis, storage, or degradation and can affect drug efficacy and safety. HPLC is a highly sensitive and selective technique used to detect, identify, and quantify trace-level impurities.

Stability Testing and Shelf-Life Determination

Stability testing is performed to evaluate the chemical stability and shelf life of pharmaceutical products under various environmental conditions, including temperature, humidity, light, and pH. HPLC is widely used in stability studies to monitor drug degradation and identify degradation products. Stability-indicating HPLC methods can distinguish between intact drug molecules and their degradation products, providing accurate information on drug stability [6].

Bioanalysis and Pharmacokinetic StudiesHPLC plays a critical role in pharmacokinetic and bioavailability studies by analyzing drugs and their metabolites in biological samples such as plasma, serum, and urine. It provides accurate and sensitive measurement of drug concentrations, enabling the evaluation of pharmacokinetic parameters such as absorption, distribution, metabolism, and excretion (ADME).

Dissolution Testing and Drug Release Studies

Dissolution testing is an important quality control procedure used to evaluate drug release from pharmaceutical dosage forms. HPLC is widely employed to quantify drug release in dissolution media over time. Its high accuracy and reproducibility ensure reliable evaluation of drug release profiles [7].

Role in Quality Control and Regulatory ComplianceHPLC is a fundamental tool in pharmaceutical quality control laboratories. Method validation ensures accuracy, precision, specificity, and reliability of analytical results, supporting compliance with regulatory requirements.

Application in Herbal Drug Standardization

HPLC is widely used in the analysis and standardization of herbal medicines. It enables identification and quantification of bioactive phytoconstituents

1. Selection of mobile phase in HPLC

The selection of an appropriate mobile phase is one of the most critical factors in the development and optimization of High-Performance Liquid Chromatography (HPLC) methods. The composition of the mobile phase has a direct impact on chromatographic performance parameters, including retention behaviour, resolution, selectivity, peak symmetry, and analysis time. The mobile phase acts as the transport medium that carries analytes through the stationary phase, enabling their separation based on differences in physicochemical properties such as polarity, molecular structure, and interaction with the stationary phase. Therefore, careful optimization of the mobile phase is essential to ensure accurate, reliable, and reproducible analytical results.

In reverse-phase HPLC (RP-HPLC), which is widely employed in pharmaceutical analysis, the mobile phase generally consists of a mixture of an aqueous component and an organic solvent. The aqueous phase may include purified water or buffered solutions, while commonly used organic solvents include methanol, acetonitrile, and tetrahydrofuran. These organic modifiers play an important role in controlling analyte retention and improving separation efficiency by adjusting the polarity and elution strength of the mobile phase. Among these solvents, acetonitrile is frequently preferred due to its low viscosity, high elution strength, and compatibility with ultraviolet detection, which contribute to improved peak resolution and reduced system backpressure.Buffer systems are often incorporated into the mobile phase to maintain a stable pH environment, which is particularly important for ionizable pharmaceutical compounds [8].

HPLC Method development

High-Performance Liquid Chromatography (HPLC) method development is a structured and systematic process designed to establish reliable and efficient analytical procedures for the separation, identification, and quantification of components within a sample. This process ensures adequate resolution, sensitivity, accuracy, and reproducibility of analytical results. Effective method development also involves evaluating factors that influence analyte retention, peak shape, and selectivity, thereby enabling precise and consistent analysis [9].

Evaluation of Physicochemical Properties of the Analyte

The first step in HPLC method development involves a comprehensive assessment of the physicochemical characteristics of the analyte, such as molecular structure, polarity, molecular weight, solubility, dissociation constant (pKa), and chemical stability.

Selection of Chromatographic Mode

The selection of an appropriate chromatographic mode is primarily based on the polarity and chemical nature of the analyte. Reverse-phase HPLC (RP-HPLC) is the most widely employed mode in pharmaceutical analysis due to itsversatility and compatibility with a broad range of drug molecules [10].

Selection of Stationary Phase

The stationary phase plays a crucial role in achieving efficient separation of analytes. Silica-based columns bonded with nonpolar functional groups, such as C18, C8, and phenyl phases, are commonly used in pharmaceutical analysis. C18 columns are most frequently preferred due to their high resolution, reproducibility, and wide applicability [11].

Selection and Optimization of Mobile Phase

The composition of the mobile phase is optimized to achieve adequate separation, proper peak shape, and reproducible retention time [12].

Selection of Detection Wavelength

The detection wavelength is selected based on the maximum absorbance (λmax) of the analyte, which is usually determined using UV–Visible spectroscopic analysis. Selection of an appropriate wavelength ensures maximum sensitivity and accurate quantification while minimizing interference from excipients, impurities, or degradation products.

Optimization of Chromatographic Conditions

Chromatographic parameters such as flow rate, column temperature, injection volume, and mobile phase composition are systematically optimized during method development. These parameters significantly influence retention time, peak shape, resolution, and overallanalytical efficiency. Proper optimization ensures consistent performance, improved separation, and reproducibility of the chromatographic method [13].

Selection of Elution Mode

The choice between isocratic and gradient elution depends on the complexity of the sample. Isocratic elution, where the mobile phase composition remains constant throughout the analysis, is suitable for simple mixtures containing analytes with similar polarity.

System Suitability Testing

System suitability testing is performed to ensure the proper functioning and performance of the chromatographic system before analysis. Key parameters evaluated include retention time, theoretical plate count, resolution, and tailing factor [14].

Method Validation

Method validation is carried out to confirm the reliability, accuracy, and consistency of the developed HPLC method. Validation is performed according to internationally accepted regulatory guidelines established by the International Council for Harmonisation. The validation process includes evaluation of parameters such as accuracy, precision, specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), and robustness. Method validation ensures that the developed analytical method is suitable for its intended pharmaceutical application [15].

Application of the Developed Method

Once validated, the developed HPLC method can be applied to routine pharmaceutical analysis. It is widely used for quantitative estimation of drug substances, impurity profiling, stability studies, dissolution testing, and quality control of pharmaceutical formulations.

5. HPLC METHOD VALIDATION

High-Performance Liquid Chromatography (HPLC) method validation is a crucial step in analytical method development to ensure that the procedure is suitable for its intended purpose and produces reliable, accurate, and reproducible results. Validation provides documented evidence that the analytical method consistently meets predetermined performance criteria for the identification and quantification of pharmaceutical compounds.

Specificity

Specificity is an important validation parameter that demonstrates the ability of the HPLC method to accurately measure the analyte in the presence of other components such as impurities, degradation products, and excipients. It ensures that the analyte peak is clearly separated and free from interference.

Linearity and Range

Linearity refers to the ability of the analytical method to produce detector responses that are directly proportional to the concentration of the analyte over a specified range. The correlation coefficient, slope, and intercept are evaluated to determine linearity, and a correlation coefficient (R^2) of 0.999 or greater is generally considered acceptable. The range represents the interval between thelowest and highest concentrations at which the method demonstrates acceptable accuracy, precision, and linearity [16].

Accuracy

Accuracy indicates the closeness of agreement between the experimental results and the true value of the analyte. It is determined by recovery studies in which known amounts of analyte are added to the sample matrix at different concentration levels, such as 80%, 100%, and 120%. The percentage recovery is calculated, and acceptable recovery values typically fall within the range of 98% to 102%, confirming the accuracy and reliability of the method.

Precision

Precision reflects the reproducibility of the analytical method under normal operating conditions. Precision is expressed as the relative standard deviation (RSD), and values less than or equal to 2% indicate acceptable reproducibility of the method.

Limit of Detection and Limit of Quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) are important parameters that indicate the sensitivity of the analytical method. LOD represents the lowest concentration of analyte that can be detected but not necessarily quantified, whereas LOQ represents the lowest concentration that can be quantified with acceptable accuracy and precision.

Robustness

Robustness evaluates the reliability of the method when small, deliberate variations are introduced into chromatographic conditions such as flow rate, mobile phase composition, pH, and column temperature. A robust method remains unaffected by these minor changes and produces consistent and reliable results, indicating its suitability for routine analytical applications [17,18].

System Suitability

System suitability testing is conducted to verify that the chromatographic system is functioning properly before analysis. It ensures adequate performance of the HPLC system and the reliability of analytical results.

REGULATORY GUIDELINES FOR HPLC METHOD DEVELOPMENT

High-Performance Liquid Chromatography (HPLC) method development is a fundamental component of pharmaceutical analysis and must be performed in accordance with internationally accepted regulatory standards. These standards ensure that analytical methods produce reliable, accurate, precise, and reproducible results suitable for pharmaceutical quality control and regulatory submissions.

ICH Regulatory Framework

The ICH guidelines serve as the primary global reference for analytical method development and validation. The ICH Q2(R1) guideline outlines the essential validation characteristics required to confirm the reliability and performance of analytical methods. These parameters include specificity, linearity, accuracy, precision, detection limit, quantitation limit, robustness, and system suitability [19]. Evaluation of these characteristics ensures that the developed HPLC method can selectively and accurately quantify the analyte in the presence of impurities, degradation products, and excipients.

FDA Regulatory Requirements

The FDA provides detailed guidance for analytical method development and validation to support regulatory submissions, including New Drug Applications (NDA) and Abbreviated New Drug Applications (ANDA). The FDA recommends the development of stability-indicating HPLC methods capable of evaluating drug identity, strength, purity, and quality. The analytical method must demonstrate acceptable specificity, accuracy, precision, and robustness. Additionally, proper documentation, system suitability testing, and compliance with Good Manufacturing Practice (GMP) are mandatory to ensure regulatory approval and data integrity.

EMA Regulatory Considerations

The EMA follows harmonized regulatory standards aligned with ICH guidelines. EMA recommendations emphasize validated analytical methods for impurity profiling, stability testing, and quality control of pharmaceutical products. Reliable HPLC methods are essential for ensuring product safety, quality, and regulatory compliance within the European pharmaceutical regulatory system.

WHO Guidelines for Analytical Method Development

The WHO provides technical guidance for analytical method validation and quality control testing. WHO guidelines focus on ensuring method suitability, instrument calibration, system performance verification, and proper validation procedures.

Role of GLP and GMP in Method Development

Compliance with Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP) is essential during HPLC method development and validation. These practices ensure proper instrument qualification, standardized operating procedures, trained personnel, and data integrity. Adherence to these regulatory standards improves analytical reliability and ensures compliance with regulatory expectations.

Documentation and Regulatory Compliance

Proper documentation is a critical requirement for regulatory-compliant HPLC method development. Regulatory authorities require detailed records of method development studies,validation protocols, validation reports, chromatograms, calibration data, and system suitability results. Comprehensive documentation ensures traceability, transparency, and reproducibility, and supports regulatory review and approval.

6. ECONOMIC CONSIDERATIONS OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Economic considerations play a crucial role in the selection and application of High-Performance Liquid Chromatography (HPLC) in pharmaceutical analysis. Compliance with international regulatory requirements established by organizations such as the International Council for Harmonisation (ICH), U.S. Food and Drug Administration (FDA), and World Health Organization (WHO) further emphasizes the importance of HPLC in pharmaceutical laboratories.

Capital Investment

The initial capital cost of HPLC instrumentation represents a major economic factor. This includes the procurement of essential components such as pumps, injectors, detectors, columns, and data acquisition systems, along with supporting computer hardware and chromatographic software [20]. Despite the high upfront cost, the extended operational lifespan of HPLC systems, typically ranging from 8 to 12 years, ensures long-term cost effectiveness and sustained analytical performance.

Operational and Running Costs

Operational expenses are associated with routine analytical procedures and include the consumption of solvents such as methanol, acetonitrile, and buffer solutions, as well as consumables like sample vials, filters, and reagents.

Maintenance and Service Costs

Routine maintenance is essential for ensuring consistent instrument performance and preventing unexpected system failures. Maintenance activities include replacement of pump seals, injector components, and detector lamps, as well as system cleaning and calibration. Preventive maintenance programs and annual service contracts help extend instrument lifespan, reduce downtime, and minimize long-term repair costs, thereby improving overall economic efficiency [21].

Economic Aspects of Method Development and Validation

Method development and validation involve considerable economic investment due to repeated experimental trials, use of reference standards, and instrument operation time. However, validated methods provide reliable and reproducible analytical performance, reducing the risk of analytical errors, product rejection, and regulatory non-compliance [22].

Economic Benefits in Pharmaceutical Applications

HPLC contributes significantly to economic efficiency in pharmaceutical industries by ensuring accurate quality control, supporting regulatory compliance, and minimizing product rejection. It plays a vital role in drug development, stability studies, impurity profiling, and routine quality. Assurance [23]. Its reliability and reproducibility improve laboratory productivity and reduce long-term operational costs.

7.

8. QUALITY BY DESIGN (QBD) IN HPLC METHOD DEVELOPMENT

Quality by Design (QbD) is a systematic and scientific approach that emphasizes the development of analytical methods with predefined objectives and built-in quality. In High-Performance Liquid Chromatography (HPLC), QbD focuses on understanding the influence of method parameters on analytical performance to ensure method robustness, reliability, and reproducibility [24,25]. Unlike traditional trial-and-error approaches, QbD promotes a structured framework based on risk assessment, statistical optimization, and lifecycle management.

Analytical Target Profile (ATP)

The Analytical Target Profile (ATP) defines the intended purpose and required performance characteristics of the analytical method. It establishes predefined criteria such as accuracy, precision, specificity, sensitivity, and quantification range necessary for reliable analyte determination.

Critical Quality Attributes (CQAs)

Critical Quality Attributes (CQAs) are measurable chromatographic parameters that determine the suitability and reliability of the analytical method. In HPLC, CQAs typically include retention time, resolution, peak symmetry, theoretical plate count, and peak area. These attributes directly influence method accuracy, precision, and selectivity, and therefore must be carefully monitored and controlled [26].

Critical Method Parameters (CMPs)

Critical Method Parameters (CMPs) are experimental variables that significantly affect the performance of the analytical method and influence CQAs. Common CMPs in HPLC include mobile phase composition, pH, flow rate, column temperature, stationary phase characteristics, and detection wavelength.

Risk Assessment

Risk assessment is an essential component of the QbD approach and is used to identify potential sources of variability that may affect method performance [27].

Design of Experiments (DoE)

Design of Experiments (DoE) is a statistical methodology used to evaluate the effects of multiple method parameters and their interactions on analytical performance. DoE allows efficient optimization of chromatographic conditions while minimizing experimental trials.

Method Optimization and Establishment of Design Space

Method optimization involves systematic adjustment of critical method parameters to achieve optimal analytical performance.[28] The design space is defined as the range of method conditions within which the analytical method consistently produces acceptable results. Operating within this design space ensures method robustness, flexibility, and regulatory compliance without requiring additional regulatory approval.

Advantages of QbD in HPLC Method Development

The application of QbD principles in HPLC method development provides several advantages, including enhanced method robustness, improved understanding of method variables, reduced variability, and minimized risk of method failure. QbD facilitates regulatory flexibility, reduces the need for frequent method revalidation, and ensures consistent analytical performance over the method lifecycle.

9. CURRENT TRENDS IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

High-Performance Liquid Chromatography (HPLC) has undergone significant technological advancements in recent years, driven by the increasing demand for rapid, sensitive, and reliable analytical techniques in pharmaceutical, biopharmaceutical, environmental, and clinical applications. Continuous improvements in chromatographic instrumentation, stationary phase materials, automation, and digital technologies have enhanced analytical performance, resolution, and reproducibility.

Ultra-High Performance Liquid Chromatography (UHPLC)

Ultra-High Performance Liquid Chromatography (UHPLC) represents a major advancement in chromatographic technology. UHPLC utilizes columns packed with sub-2 µm particle sizes and operates at higher system pressures compared to conventional HPLC. This enables improved separation efficiency, enhanced sensitivity, and significantly reduced analysis time. The increased chromatographic resolution allows better separation of structurally similar compounds and complex mixtures [29].

Automation and Intelligent HPLC Systems

Automation has become an essential feature of modern HPLC systems, improving analytical precision, reproducibility, and operational efficiency. Automated autosamplers, solvent delivery systems, and sample preparation tools reduce manual intervention and minimize human error.

Integration with Advanced Detection Techniques

The coupling of HPLC with advanced detection systems has significantly enhanced analytical sensitivity and selectivity. Hyphenated techniques such as liquid chromatography–mass spectrometry (LC-MS), diode array detection (DAD), and fluorescence detection provide detailed structural and quantitative information.

Advances in Column Technology

Significant innovations in column technology have improved chromatographic performance and method robustness. Core-shell particle columns, monolithic columns, and bio-inert stationary phases provide enhanced separation efficiency, improved peak resolution, and reduced analysis time. Bio-inert columns are particularly important for the analysis of sensitive biomolecules such as peptides, proteins, and monoclonal antibodies, as they reduce unwanted interactions and improve analytical accuracy and reproducibility.

Multidimensional Liquid Chromatography

Multidimensional liquid chromatography, particularly two-dimensional liquid chromatography (2D-LC), has emerged as a powerful technique for analyzing highly complex samples. This approach combines multiple separation mechanisms to improve peak capacity and chromatographic resolution. Multidimensional HPLC is widely applied in impurity profiling, proteomics, metabolomics, and biopharmaceutical analysis, where conventional single-dimension chromatography may be insufficient [30].

Digitalization and Advanced Data Management

Digital transformation has significantly improved the functionality of modern HPLC systems. Advanced software platforms enable automated data acquisition, real-time monitoring, and electronic data management. Cloud-based storage and integrated laboratory information management systems improve data integrity, traceability, and regulatory compliance.

Expanding Applications in Biopharmaceutical Analysis

HPLC plays a crucial role in the analysis and characterization of biopharmaceutical products, including peptides, proteins, monoclonal antibodies, and vaccines. Advances in chromatographic techniques and stationary phase materials enable accurate evaluation of purity, structural integrity, and stability of biomolecules.

CONCLUSION

High-Performance Liquid Chromatography (HPLC) continues to be one of the most powerful and indispensable analytical techniques in pharmaceutical and biopharmaceutical sciences due to its exceptional sensitivity, accuracy, precision, and reproducibility.The reliability and versatility of HPLC have ensured its widespread acceptance in both research and industrial laboratories. Significant advancements in chromatographic instrumentation, stationary phase materials, and detection technologies have greatly improved analytical performance by enhancing resolution, reducing analysis time, and increasing overall efficiency. The development of Ultra-High Performance Liquid Chromatography (UHPLC) has further strengthened chromatographic capabilities by enabling faster separations, improved sensitivity, and reduced solvent consumption.

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AUTHORSHIP CONTRIBUTIONS STATEMENT

All authors are contributed equally.

Conflicts of Interest

The author declares no conflicts of interest.

Founding

Not applicable.

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