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 6, Issue 3, June 2026
Impact Factor: 8.2

Role of RP-HPLC in Quality Assessment of Combined Fluconazole and Ivermectin Formulations: A Comprehensive Review

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Abstract: *Fluconazole and ivermectin are widely used antifungal and antiparasitic agents, respectively, with significant therapeutic importance in the management of infectious diseases. The increasing demand for accurate quality control of pharmaceutical formulations containing these drugs has highlighted the need for reliable analytical methodologies. Among the available analytical techniques, Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) has emerged as one of the most effective and widely employed tools for the simultaneous estimation, quality assessment, and stability evaluation of pharmaceutical compounds. The present review comprehensively discusses the role of RP-HPLC in the analysis of fluconazole and ivermectin formulations. Various analytical approaches including UV spectrophotometry, HPLC, HPTLC, LC-MS/MS, and UPLC reported for the determination of these drugs are critically reviewed. Particular emphasis is placed on RP-HPLC method development strategies, selection of chromatographic conditions, validation requirements according to ICH guidelines, and stability-indicating analytical procedures. The review also highlights the application of RP-HPLC in assay determination, dissolution testing, impurity profiling, degradation product analysis, and batch-to-batch quality evaluation. Furthermore, current trends such as Quality by Design (QbD), green analytical chemistry, artificial intelligence-assisted method optimization, and hyphenated analytical techniques are discussed. The review identifies existing research gaps and future opportunities for the development of robust, environmentally sustainable, and regulatory-compliant analytical methods. Overall, RP-HPLC remains a versatile, reliable, and indispensable analytical tool for ensuring the quality, safety, and efficacy of fluconazole and ivermectin pharmaceutical formulations*

Keywords: Fluconazole, Ivermectin, RP-HPLC, Analytical Method Development, Method Validation, Stability-Indicating Method, Pharmaceutical Quality Control, Simultaneous Estimation, Chromatographic Analysis, ICH Guidelines.

I. INTRODUCTION

1.1 Overview of Pharmaceutical Quality Control

Pharmaceutical quality control is a fundamental component of drug development, manufacturing, and post-marketing surveillance. It encompasses a series of systematic procedures designed to ensure that pharmaceutical products consistently meet predefined standards of quality, safety, efficacy, and purity. The increasing complexity of modern pharmaceutical formulations has intensified the need for reliable analytical methodologies capable of evaluating active pharmaceutical ingredients (APIs), excipients, impurities, degradation products, and finished dosage forms.

Quality control testing serves as a critical checkpoint throughout the lifecycle of a pharmaceutical product. From raw material assessment to final product release, analytical testing ensures compliance with regulatory requirements established by international agencies such as the International Council for Harmonisation (ICH), United States Pharmacopeia (USP), European Pharmacopoeia (Ph. Eur.), and World Health Organization (WHO). Accurate quality assessment helps prevent therapeutic failure, adverse drug reactions, and batch-to-batch variability, thereby safeguarding patient health.

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The growing demand for combination drug products and advanced therapeutic systems has further increased the need for sophisticated analytical techniques capable of simultaneously analyzing multiple components within a single formulation. Consequently, chromatographic methods, particularly High-Performance Liquid Chromatography (HPLC), have become indispensable tools in pharmaceutical quality control laboratories worldwide. [1,2]

1.2 Importance of Analytical Method Development

Analytical method development is the process of designing and optimizing analytical procedures to accurately identify, quantify, and characterize pharmaceutical substances. A well-developed analytical method provides reliable, reproducible, and precise results while ensuring compliance with regulatory standards. Method development is particularly important in pharmaceutical analysis because drug products often contain complex mixtures of active ingredients, excipients, impurities, and degradation products that require selective and sensitive analytical evaluation.

The development of robust analytical methods contributes significantly to drug quality assurance by enabling routine analysis, stability testing, dissolution studies, content uniformity assessment, impurity profiling, and pharmacokinetic investigations. The selection of appropriate analytical conditions, including mobile phase composition, stationary phase characteristics, detection wavelength, flow rate, and sample preparation procedures, directly influences the performance of the analytical method.

Method validation is an integral part of analytical development and ensures that the developed procedure is fit for its intended purpose. Parameters such as specificity, accuracy, precision, linearity, robustness, sensitivity, and system suitability must be evaluated according to established regulatory guidelines. The implementation of validated analytical methods enhances confidence in generated data and supports regulatory submissions, product approval, and quality monitoring activities.[3,4]

1.3 Significance of Combined Drug Formulations

Combination drug formulations have gained considerable importance in modern therapeutics due to their ability to improve treatment outcomes, enhance patient compliance, and reduce healthcare costs. By incorporating two or more active pharmaceutical ingredients into a single dosage form, combination therapies can target multiple pathways involved in disease progression, thereby providing synergistic or complementary therapeutic effects.

The pharmaceutical industry has increasingly focused on developing fixed-dose combinations to address complex diseases and co-existing infections. Such formulations simplify treatment regimens by reducing pill burden and improving adherence to prescribed therapy. However, the presence of multiple active ingredients within a single formulation presents analytical challenges because each component may exhibit different physicochemical properties, stability characteristics, and chromatographic behavior.

Reliable analytical methods capable of simultaneously quantifying multiple drug substances are therefore essential for ensuring the quality and consistency of combination products. Simultaneous estimation reduces analytical time, minimizes solvent consumption, and improves laboratory efficiency while maintaining analytical accuracy and precision. Consequently, the development of advanced chromatographic methods for combination formulations remains an active area of pharmaceutical research.[5]

1.4 Role of Chromatographic Techniques in Drug Analysis

Chromatographic techniques represent one of the most powerful and versatile analytical tools available for pharmaceutical analysis. These techniques separate individual components of a mixture based on differences in their physicochemical interactions between stationary and mobile phases. Chromatography is widely utilized for qualitative and quantitative analysis, impurity profiling, stability assessment, bioanalysis, and quality control testing.

Among the various chromatographic approaches, High-Performance Liquid Chromatography (HPLC) has emerged as the preferred technique for routine pharmaceutical analysis due to its high sensitivity, selectivity, reproducibility, and versatility. Reverse Phase High-Performance Liquid Chromatography (RP-HPLC), in particular, is extensively employed because it provides excellent separation of compounds with diverse polarities and molecular structures.

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RP-HPLC offers several advantages, including rapid analysis, high resolution, low detection limits, compatibility with various detectors, and suitability for both simple and complex pharmaceutical formulations. The technique can be effectively applied to assay determination, dissolution testing, degradation studies, impurity analysis, and stability-indicating investigations. Furthermore, advances in column technology, detector systems, and software-assisted optimization have significantly enhanced the efficiency and reliability of RP-HPLC methods.

As regulatory expectations for pharmaceutical quality continue to increase, RP-HPLC remains a cornerstone analytical technique for ensuring the quality, safety, and efficacy of drug products.

1.5 Need for Simultaneous Estimation of Fluconazole and Ivermectin

Fluconazole is a widely used triazole antifungal agent that inhibits fungal cytochrome P450 enzymes involved in ergosterol biosynthesis, thereby disrupting fungal cell membrane integrity. It is commonly prescribed for the treatment of candidiasis, cryptococcal infections, and various systemic fungal diseases. Due to its favorable pharmacokinetic profile and broad-spectrum antifungal activity, fluconazole remains one of the most frequently utilized antifungal medications globally.

Ivermectin is a broad-spectrum antiparasitic agent belonging to the avermectin class. It exerts its pharmacological action by binding to glutamate-gated chloride channels in parasites, leading to paralysis and eventual death of the organism. Ivermectin is widely used for the treatment of parasitic infections such as onchocerciasis, strongyloidiasis, scabies, and other helminthic diseases.

The growing interest in combination therapies and multi-component pharmaceutical formulations has created a need for reliable analytical methods capable of simultaneously estimating fluconazole and ivermectin. The significant differences in their physicochemical properties, polarity, solubility, and chromatographic behavior make simultaneous analysis particularly challenging. Traditional single-analyte methods are often time-consuming and resource-intensive when applied to combination products.

Therefore, the development and application of RP-HPLC methods capable of simultaneously determining fluconazole and ivermectin are essential for routine quality control, stability testing, dissolution studies, and regulatory compliance. Such methods facilitate efficient analysis while maintaining the accuracy, precision, and reliability required for pharmaceutical quality assessment. [6,7]

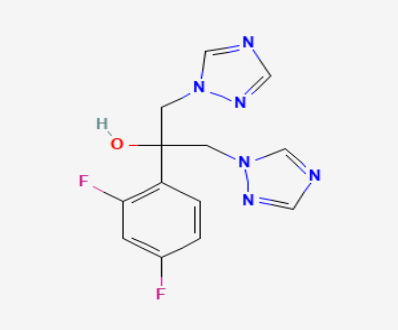
1.6 Objectives of the Review

The primary objective of this review is to provide a comprehensive overview of the role of Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) in the quality assessment of combined fluconazole and ivermectin formulations. The review aims to summarize current knowledge regarding analytical method development, chromatographic optimization strategies, and validation approaches used for the simultaneous estimation of these drugs.

II. DRUG PROFILE

2.1 Fluconazole

Chemical Structure



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Fluconazole is a synthetic triazole antifungal agent belonging to the azole class of antifungal drugs. The molecule contains two triazole rings attached to a fluorinated aromatic ring and a hydroxyl-substituted carbon atom. The presence of triazole moieties contributes significantly to its antifungal activity by interacting with fungal cytochrome P450 enzymes involved in ergosterol biosynthesis.

IUPAC Name

2-(2,4-Difluorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl)propan-2-ol

Molecular Formula and Molecular Weight

- Molecular Formula: $C_{13}H_{12}F_2N_6O$
- Molecular Weight: 306.27 g/mol

Mechanism of Action

Fluconazole acts by selectively inhibiting fungal cytochrome P450-dependent enzyme lanosterol 14α-demethylase. This enzyme is responsible for converting lanosterol into ergosterol, an essential component of fungal cell membranes. Inhibition of ergosterol synthesis results in altered membrane permeability, disruption of cellular functions, and inhibition of fungal growth. Due to its selective affinity for fungal enzymes over mammalian enzymes, fluconazole exhibits favorable therapeutic efficacy and safety.

Pharmacokinetics

Fluconazole exhibits excellent oral bioavailability, often exceeding 90%, and its absorption is not significantly affected by food intake. The drug is widely distributed throughout body fluids and tissues, including cerebrospinal fluid, making it effective in the treatment of systemic fungal infections. Plasma protein binding is relatively low, approximately 11–12%. Fluconazole undergoes minimal hepatic metabolism and is primarily eliminated unchanged through renal excretion. The elimination half-life is approximately 30 hours, allowing once-daily dosing in most therapeutic regimens.

Therapeutic Applications

Fluconazole is extensively used for the treatment and prevention of fungal infections, including:

- Oropharyngeal candidiasis
- Esophageal candidiasis
- Vulvovaginal candidiasis
- Cryptococcal meningitis
- Systemic candidiasis
- Dermatophytic infections
- Prophylaxis in immunocompromised patients

Its broad-spectrum antifungal activity and favorable pharmacokinetic profile have established fluconazole as a first-line therapy for numerous fungal infections.

Official Compendial Methods

Official monographs for fluconazole are available in major pharmacopoeias, including:

- United States Pharmacopeia (USP)
- British Pharmacopoeia (BP)
- European Pharmacopoeia (Ph. Eur.)
- Indian Pharmacopoeia (IP)

These monographs describe validated analytical procedures, including HPLC-based assay methods, impurity profiling, identification tests, and dissolution studies for routine quality control.

2.2 Ivermectin

Chemical Structure

Ivermectin is a semi-synthetic derivative of avermectins, which are macrocyclic lactones produced by fermentation of *Streptomyces avermitilis*. Commercial ivermectin is a mixture predominantly composed of 22,23-dihydroavermectin

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B1a and a smaller proportion of 22,23-dihydroavermectin B1b. The molecule possesses a complex macrocyclic ring system with multiple hydroxyl and ether functional groups that contribute to its antiparasitic activity.

IUPAC Name

(4aR,5S,6S,6aR,8aS,9R,10S,11R,12S,12aR,12bS)-5,6,6a,7,8,8a,9,10,11,12,12a,12b-Dodecahydro-11-hydroxy-5-(2-hydroxypropan-2-yl)-9-(tetrahydro-2H-pyran-2-yloxy)-4a,8a-dimethyl-3,14-dioxo-4H,5H-furo[3,4-f][1,3]dioxacyclopentadecine

Molecular Formula and Molecular Weight

- Molecular Formula: $C_{48}H_{74}O_{14}$
- Molecular Weight: 875.10 g/mol

Mechanism of Action

Ivermectin exerts its antiparasitic effect by selectively binding to glutamate-gated chloride ion channels present in the nerve and muscle cells of parasites. Activation of these channels increases chloride ion permeability, resulting in hyperpolarization, paralysis, and eventual death of the parasite. The drug exhibits high selectivity for parasite-specific channels, contributing to its favorable safety profile in humans.

Pharmacokinetics

Ivermectin is absorbed following oral administration, with absorption enhanced by fatty meals. Due to its lipophilic nature, the drug demonstrates extensive tissue distribution. It exhibits high plasma protein binding, primarily to albumin. Ivermectin undergoes hepatic metabolism predominantly through cytochrome P450 enzymes and is eliminated mainly through fecal excretion. The elimination half-life ranges between 12 and 36 hours depending on formulation and patient characteristics.

Therapeutic Applications

Ivermectin is widely employed in the treatment of various parasitic infestations, including:

- Onchocerciasis
- Strongyloidiasis
- Scabies
- Pediculosis (lice infestation)
- Lymphatic filariasis
- Ascariasis
- Other helminthic infections

Its broad-spectrum antiparasitic activity and favorable safety profile have made it one of the most widely used antiparasitic agents worldwide.

Official Compendial Methods

Official analytical methods for ivermectin are available in:

- United States Pharmacopeia (USP)
- British Pharmacopoeia (BP)
- European Pharmacopoeia (Ph. Eur.)
- Indian Pharmacopoeia (IP)

These compendial methods generally employ chromatographic techniques such as HPLC for assay determination, impurity evaluation, and stability assessment.

2.3 Rationale for Combined Formulation

Therapeutic Advantages

Combination drug therapy has become an important strategy for improving therapeutic outcomes and patient compliance. The combination of fluconazole and ivermectin offers the possibility of simultaneously managing fungal and parasitic infections, particularly in regions where co-infections are prevalent. The use of a single dosage form may reduce pill burden, improve adherence to treatment regimens, and simplify disease management.

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The complementary pharmacological actions of these drugs enable broader therapeutic coverage. While fluconazole targets fungal pathogens through inhibition of ergosterol synthesis, ivermectin acts against parasites by disrupting neuromuscular transmission. Their distinct mechanisms of action minimize the likelihood of pharmacodynamic overlap and may provide synergistic clinical benefits in specific patient populations.

Clinical Significance

Patients with compromised immunity are often susceptible to both fungal and parasitic infections. Simultaneous administration of fluconazole and ivermectin may therefore provide a practical therapeutic approach in selected clinical settings. Furthermore, the increasing interest in multidrug therapy has encouraged the development of analytical methods capable of assessing such combinations accurately and efficiently.

The availability of reliable analytical procedures is essential for ensuring dosage accuracy, therapeutic effectiveness, and product quality throughout the product lifecycle. Simultaneous estimation methods contribute significantly to routine quality control and regulatory compliance.

Marketed Products and Emerging Applications

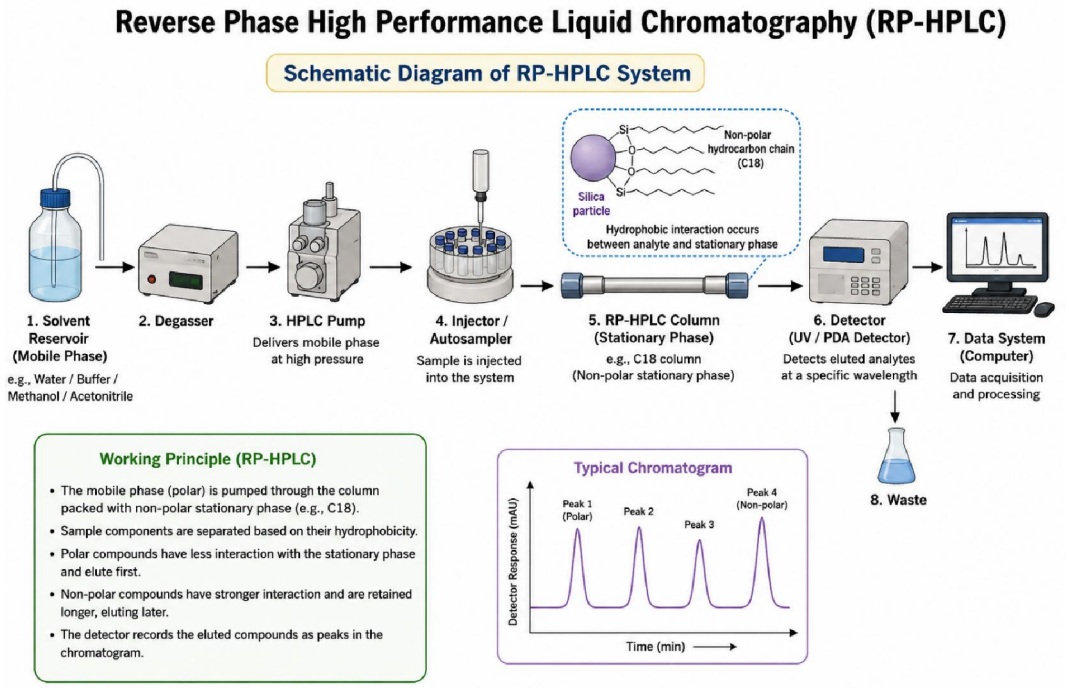
Although fixed-dose combinations of fluconazole and ivermectin are relatively limited compared with other therapeutic combinations, growing interest in combination therapies has stimulated research into their formulation and analytical evaluation. Emerging pharmaceutical development efforts are focused on improving therapeutic convenience, reducing treatment duration, and enhancing patient compliance.

The increasing investigation of novel dosage forms, including tablets, capsules, and advanced drug delivery systems containing multiple active ingredients, underscores the need for robust analytical methodologies capable of simultaneous determination of fluconazole and ivermectin.[8-10]

III. REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (RP-HPLC)

3.1 Principles of RP-HPLC

Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) is one of the most widely utilized analytical techniques in pharmaceutical analysis. The technique is based on differential partitioning of analytes between a nonpolar stationary phase and a relatively polar mobile phase. Compounds with greater hydrophobicity interact more strongly with the stationary phase and therefore exhibit longer retention times.



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In RP-HPLC, separation is achieved through a combination of hydrophobic interactions, adsorption phenomena, and partitioning mechanisms. Variations in molecular structure, polarity, ionization characteristics, and hydrophobicity influence retention behavior, allowing effective separation of complex mixtures. RP-HPLC is particularly advantageous for pharmaceutical applications because it provides excellent resolution, reproducibility, sensitivity, and versatility.

3.2 Instrumentation

Solvent Reservoir

The solvent reservoir stores mobile phase components and supplies them to the chromatographic system. High-purity solvents are utilized to minimize baseline noise and analytical interference.

Pump

The pump delivers the mobile phase at a controlled and constant flow rate under high pressure. Modern HPLC pumps are capable of generating pressures exceeding several hundred bars while maintaining excellent flow-rate precision.

Injector

The injector introduces a precise volume of sample into the mobile phase stream. Automated autosamplers improve analytical reproducibility and throughput.

Column

The chromatographic column is the core component of the HPLC system. RP-HPLC commonly employs C18-bonded silica columns due to their excellent retention characteristics and broad applicability for pharmaceutical compounds.

Detector

The detector monitors analytes as they elute from the column. The generated signal is proportional to analyte concentration and enables qualitative and quantitative analysis.

Data Processing System

Chromatographic software records, processes, and interprets detector signals. It performs peak integration, calibration, quantification, system suitability evaluation, and report generation.

3.3 Types of HPLC Detectors

UV Detector

UV detectors measure analyte absorbance at selected wavelengths and are the most commonly used detectors in pharmaceutical analysis due to their simplicity, sensitivity, and cost-effectiveness.

PDA Detector

Photodiode Array (PDA) detectors simultaneously monitor multiple wavelengths and provide spectral information useful for peak purity assessment and compound identification.

Fluorescence Detector

Fluorescence detectors offer superior sensitivity for compounds capable of fluorescence emission and are valuable for trace-level analysis.

Mass Spectrometric Detector

Mass spectrometric detectors provide molecular weight and structural information, making them highly valuable for impurity profiling, metabolite identification, and bioanalytical studies.

3.4 Factors Affecting RP-HPLC Separation

Mobile Phase Composition
pH of Mobile Phase
Flow Rate
Column Selection
Temperature
Detection Wavelength

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These parameters significantly influence retention behavior, peak symmetry, resolution, theoretical plate count, and overall chromatographic performance. Careful optimization of these variables is essential for successful method development and validation.

IV. ANALYTICAL CHALLENGES IN SIMULTANEOUS ESTIMATION OF FLUCONAZOLE AND IVERMECTIN

4.1 Physicochemical Differences

Fluconazole and ivermectin possess markedly different molecular structures, polarity profiles, and molecular weights. These differences significantly affect chromatographic retention and make simultaneous separation challenging.

4.2 Solubility Considerations

Fluconazole exhibits relatively high aqueous solubility, whereas ivermectin is highly lipophilic and poorly soluble in water. This disparity complicates sample preparation and mobile phase optimization.

4.3 Stability Issues

Both drugs may undergo degradation under specific environmental conditions such as heat, light, oxidation, and pH variation. Stability-indicating analytical methods are therefore necessary for accurate quality assessment.

4.4 Matrix Interference

Excipients and degradation products present in pharmaceutical formulations can interfere with analyte detection and quantification. Effective chromatographic separation is required to eliminate such interferences.

4.5 Peak Resolution Challenges

Due to differences in retention characteristics and chemical properties, obtaining satisfactory peak resolution for both analytes within a reasonable analysis time can be difficult. Optimization of mobile phase composition, pH, flow rate, and stationary phase selection is therefore critical for successful simultaneous estimation.[11,12]

V. LITERATURE REVIEW OF ANALYTICAL METHODS

5.1 UV Spectrophotometric Methods

Principle

UV-visible spectrophotometry is one of the most widely used analytical techniques for quantitative determination of pharmaceutical compounds. The method is based on the absorption of ultraviolet or visible radiation by molecules containing chromophoric groups. The amount of absorbed radiation is directly proportional to the concentration of the analyte according to Beer-Lambert's law. Due to its simplicity, low cost, and rapid analysis, UV spectrophotometry remains a popular choice for routine quality control of pharmaceutical products.

Reported Methods

Several UV spectrophotometric methods have been reported for the estimation of fluconazole and ivermectin either individually or in combination with other drugs. Fluconazole exhibits significant absorption in the UV region owing to the presence of aromatic and triazole rings, making direct spectrophotometric estimation feasible. Various researchers have developed zero-order, derivative, area-under-curve, ratio spectra, and multicomponent analysis methods for its determination in pharmaceutical formulations.

Ivermectin, owing to its limited UV absorption characteristics, often requires derivatization or specialized analytical conditions for spectrophotometric analysis. Several studies have employed derivative spectroscopy and chemometric approaches to improve sensitivity and selectivity in ivermectin determination.

Advantages and Limitations

Advantages

- Simple and rapid analysis
- Low operational cost
- Minimal sample preparation
- Suitable for routine quality control
- High sample throughput

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Limitations

- Limited selectivity in multicomponent formulations
- Susceptibility to interference from excipients
- Lower sensitivity compared to chromatographic methods
- Inability to separate degradation products and impurities
- Unsuitable for stability-indicating studies

Because of these limitations, UV spectrophotometric methods are generally employed for preliminary screening and routine assay determination rather than comprehensive pharmaceutical quality assessment.

5.2 HPLC Methods

Conventional HPLC

Conventional High-Performance Liquid Chromatography has become one of the most reliable analytical techniques in pharmaceutical analysis. The technique offers excellent separation capability, high sensitivity, and reproducibility for qualitative and quantitative determination of pharmaceutical compounds. Numerous conventional HPLC methods have been reported for the assay determination of fluconazole and ivermectin in bulk drugs, pharmaceutical formulations, and biological matrices.

Early analytical methods employed normal-phase and reverse-phase chromatographic systems with UV detection. These methods provided acceptable analytical performance; however, longer analysis times and lower column efficiencies limited their applicability in high-throughput environments.

RP-HPLC Methods

RP-HPLC is the most extensively utilized chromatographic approach for pharmaceutical quality control. Numerous RP-HPLC methods have been reported for the determination of fluconazole and ivermectin individually and in combination with other therapeutic agents.

Most reported RP-HPLC methods employ C18 stationary phases with mobile phases consisting of phosphate buffers, ammonium acetate buffers, methanol, or acetonitrile. Detection wavelengths generally range between 210 and 260 nm depending on the analyte and detector configuration. These methods provide excellent accuracy, precision, linearity, and robustness suitable for routine quality control applications.

The increasing popularity of RP-HPLC can be attributed to:

- Excellent separation efficiency
- High reproducibility
- Compatibility with diverse pharmaceutical compounds
- Regulatory acceptance
- Suitability for method validation according to ICH guidelines

Stability-Indicating RP-HPLC Methods

Stability-indicating RP-HPLC methods are specifically designed to separate active pharmaceutical ingredients from their degradation products. Such methods are critical for assessing drug stability, shelf-life, and formulation integrity.

Several studies have reported stability-indicating RP-HPLC methods for fluconazole and ivermectin involving forced degradation under acidic, alkaline, oxidative, thermal, and photolytic conditions. These methods demonstrate the ability to accurately quantify intact drug substances in the presence of degradation products, thereby ensuring compliance with regulatory requirements.

The increasing emphasis on quality by design (QbD) and lifecycle management has further highlighted the importance of stability-indicating chromatographic methods in pharmaceutical analysis.

5.3 HPTLC Methods

High-Performance Thin-Layer Chromatography (HPTLC) is an advanced form of thin-layer chromatography that offers improved resolution, sensitivity, and reproducibility. HPTLC methods have been employed for quantitative determination of numerous pharmaceutical compounds, including antifungal and antiparasitic agents.

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HPTLC provides several advantages such as simultaneous analysis of multiple samples, low solvent consumption, and relatively simple instrumentation. Several researchers have reported HPTLC methods for the estimation of fluconazole and related antifungal agents using silica gel plates and densitometric detection.

Although HPTLC is suitable for routine quality control, its sensitivity and precision are generally lower than those of HPLC-based techniques. Consequently, HPTLC is often considered a complementary analytical tool rather than a primary method for pharmaceutical quality assessment.

5.4 LC-MS/MS Methods

Liquid Chromatography coupled with Tandem Mass Spectrometry (LC-MS/MS) represents one of the most powerful analytical platforms currently available. The combination of chromatographic separation with mass spectrometric detection provides exceptional sensitivity, specificity, and structural information.

LC-MS/MS methods have been extensively utilized for:

- Pharmacokinetic studies
- Bioequivalence investigations
- Therapeutic drug monitoring
- Impurity profiling
- Metabolite identification

For fluconazole and ivermectin, LC-MS/MS methods offer extremely low detection limits and excellent selectivity in complex biological matrices such as plasma, serum, and urine. The technique enables accurate quantification even at trace concentration levels.

Despite its superior analytical performance, LC-MS/MS requires expensive instrumentation, highly trained personnel, and significant maintenance costs, limiting its routine use in many quality control laboratories.

5.5 UPLC Methods

Ultra-Performance Liquid Chromatography (UPLC) is an advanced chromatographic technique that utilizes columns packed with sub-2 µm particles. The reduced particle size enhances chromatographic efficiency, resulting in improved resolution, shorter analysis times, and reduced solvent consumption.

UPLC methods have gained considerable attention for pharmaceutical analysis because they provide:

- Faster separations
- Higher sensitivity
- Improved peak capacity
- Reduced solvent usage
- Increased laboratory productivity

Recent studies have demonstrated the successful application of UPLC methods for assay determination, impurity profiling, and stability assessment of antifungal and antiparasitic drugs. UPLC is increasingly being adopted in pharmaceutical industries as a modern alternative to conventional HPLC.

VI. RP-HPLC METHOD DEVELOPMENT STRATEGIES

6.1 Selection of Stationary Phase

The stationary phase plays a critical role in chromatographic separation. Appropriate column selection significantly influences retention behavior, resolution, peak symmetry, and analysis time.

C18 Columns

C18 columns are the most widely used stationary phases in RP-HPLC. Their hydrophobic octadecylsilane surface provides excellent retention and separation of a broad range of pharmaceutical compounds. Most reported methods for fluconazole and ivermectin utilize C18 columns because of their versatility and reproducibility.

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Core-Shell Columns

Core-shell columns contain a solid core surrounded by a porous outer layer. These columns offer improved efficiency, reduced band broadening, and shorter analysis times compared to conventional fully porous particles. Their use has become increasingly popular in modern pharmaceutical analysis.

Monolithic Columns

Monolithic columns consist of a continuous porous structure that facilitates high permeability and low back pressure. These columns allow rapid separations at higher flow rates while maintaining satisfactory chromatographic performance.

6.2 Selection of Mobile Phase

Buffer Systems

Buffers maintain a stable pH throughout chromatographic analysis and improve reproducibility. Commonly used buffers include:

- Phosphate buffer
- Ammonium acetate buffer
- Ammonium formate buffer
- Acetate buffer

Selection of an appropriate buffer depends on analyte pKa, detector compatibility, and method objectives.

Organic Modifiers

Organic solvents modify the elution strength of the mobile phase and influence analyte retention.

Common organic modifiers include:

- Acetonitrile
- Methanol
- Tetrahydrofuran (THF)

Acetonitrile is generally preferred due to its low viscosity, excellent peak shape, and strong elution capability.

Gradient vs Isocratic Elution

Isocratic Elution

The mobile phase composition remains constant throughout analysis. This approach is simple and suitable for analytes with similar retention characteristics.

Gradient Elution

The mobile phase composition changes during analysis. Gradient methods are particularly useful for separating compounds with significantly different polarities and retention behaviors, such as fluconazole and ivermectin.

6.3 Optimization Parameters

Resolution

Resolution is a measure of the degree of separation between adjacent chromatographic peaks. A resolution value greater than 2.0 generally indicates complete baseline separation.

Tailing Factor

The tailing factor evaluates peak symmetry. Symmetrical peaks are essential for accurate quantification and method reproducibility.

Retention Time

Retention time indicates the duration required for an analyte to elute from the chromatographic column. Appropriate retention ensures effective separation from interfering substances.

Theoretical Plates

Theoretical plate count represents column efficiency. Higher plate counts indicate better chromatographic performance and sharper peaks.

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6.4 Detection Wavelength Selection

Selection of an appropriate detection wavelength is essential for achieving maximum sensitivity and selectivity. UV spectra of analytes are typically recorded to identify wavelength regions with significant absorbance.

For simultaneous estimation of fluconazole and ivermectin, wavelengths in the lower UV region are commonly selected because both analytes exhibit adequate absorbance. The final wavelength is chosen based on sensitivity, baseline stability, and detector response.

VII. METHOD VALIDATION ACCORDING TO ICH GUIDELINES

Method validation establishes that an analytical procedure is suitable for its intended purpose. According to ICH Q2(R2), validation involves systematic evaluation of multiple performance characteristics.

7.1 System Suitability Testing

System suitability tests verify the proper functioning of the chromatographic system before analysis. Parameters include retention time, resolution, tailing factor, and theoretical plate count.

7.2 Specificity

Specificity assesses the ability of the method to accurately measure analytes in the presence of impurities, degradation products, and formulation excipients.

7.3 Linearity

Linearity evaluates the relationship between analyte concentration and detector response over a specified concentration range.

7.4 Accuracy

Accuracy represents the closeness of measured values to the true value and is commonly evaluated through recovery studies.

7.5 Precision

Repeatability

Repeatability measures variation under identical operating conditions over a short period.

Intermediate Precision

Intermediate precision evaluates variability between analysts, instruments, laboratories, or different days.

7.6 Robustness

Robustness determines the ability of the method to remain unaffected by small deliberate variations in analytical conditions.

7.7 Ruggedness

Ruggedness assesses the reproducibility of analytical results under normal operational variations.

7.8 Limit of Detection (LOD)

LOD represents the lowest concentration of analyte that can be detected but not necessarily quantified accurately.

7.9 Limit of Quantification (LOQ)

LOQ represents the lowest concentration that can be quantified with acceptable precision and accuracy.[13,14]

7.10 Solution Stability

Solution stability studies evaluate the stability of analytical samples and standard solutions during storage and analysis.

Table: Acceptance Criteria for Validation Parameters

Validation Parameter	Acceptance Criteria
System Suitability	Resolution > 2.0
Tailing Factor	≤ 2.0
Theoretical Plates	> 2000
Linearity	$r^2 \geq 0.999$
Accuracy	98–102% Recovery
Precision	%RSD ≤ 2.0
Robustness	%RSD ≤ 2.0

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 6, Issue 3, June 2026
Impact Factor: 8.2

Ruggedness	%RSD ≤ 2.0
LOD	Method Dependent
LOQ	Method Dependent
Solution Stability	Assay Variation ≤ 2%

VIII. STABILITY-INDICATING RP-HPLC METHODS

8.1 Importance of Stability Studies

Stability studies are an essential component of pharmaceutical development and quality assurance. These studies evaluate the ability of a drug substance or drug product to maintain its chemical, physical, microbiological, therapeutic, and toxicological properties throughout its shelf life. The primary objective of stability testing is to establish appropriate storage conditions, retest periods, expiration dates, and packaging requirements that ensure product quality and patient safety.

Drug substances are susceptible to degradation due to environmental factors such as temperature, humidity, light exposure, oxidation, and pH variations. Degradation can lead to loss of potency, alteration of therapeutic efficacy, and formation of potentially harmful degradation products. Therefore, pharmaceutical manufacturers are required to perform comprehensive stability studies to demonstrate product integrity during storage and distribution.

Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) has become the preferred analytical technique for stability studies because of its excellent resolution, sensitivity, reproducibility, and ability to separate active pharmaceutical ingredients from degradation products. Stability-indicating RP-HPLC methods provide reliable information regarding degradation pathways and product stability under various stress conditions.

8.2 ICH Stability Guidelines

The International Council for Harmonisation (ICH) has established comprehensive guidelines for stability testing of pharmaceutical products. Key stability-related guidelines include:

- ICH Q1A(R2): Stability Testing of New Drug Substances and Products
- ICH Q1B: Photostability Testing
- ICH Q2(R2): Validation of Analytical Procedures
- ICH Q14: Analytical Procedure Development

These guidelines emphasize the importance of stress testing and stability-indicating analytical methods to ensure that pharmaceutical products remain safe and effective throughout their intended shelf life.

Forced Degradation Studies

Forced degradation studies involve exposing pharmaceutical compounds to extreme stress conditions to accelerate degradation and identify potential degradation products. These studies assist in understanding degradation mechanisms and establishing the specificity of analytical methods.

Acid Hydrolysis

Acid hydrolysis studies are conducted by exposing drug substances to acidic conditions, typically using hydrochloric acid. This stress condition evaluates the susceptibility of the drug molecule to acid-catalyzed degradation. Acid hydrolysis is particularly useful for identifying degradation pathways involving hydrolysis of labile functional groups and determining the stability of pharmaceutical formulations under acidic environments.

Base Hydrolysis

Base hydrolysis studies are performed using alkaline reagents such as sodium hydroxide. These studies assess the stability of pharmaceutical compounds under basic conditions and help identify degradation products resulting from alkaline hydrolysis. The extent of degradation depends on molecular structure, exposure time, and alkali concentration.

Oxidative Degradation

Oxidative stress testing is generally performed using hydrogen peroxide solutions. Oxidation can significantly affect drug stability by modifying susceptible functional groups such as alcohols, amines, ethers, and aromatic rings.

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Oxidative degradation studies provide valuable information regarding the oxidative stability of pharmaceutical compounds and potential impurity formation.

Thermal Degradation

Thermal degradation studies involve exposing drug substances and formulations to elevated temperatures for specified periods. These studies simulate long-term storage conditions and evaluate the effect of heat on product stability. Thermal degradation may result in chemical decomposition, physical changes, or accelerated formation of degradation products.

Photolytic Degradation

Photostability testing evaluates the effect of exposure to ultraviolet and visible light on pharmaceutical products. Certain drug molecules are sensitive to light and may undergo photochemical degradation, leading to reduced potency or formation of impurities. Photolytic studies help establish suitable packaging and storage recommendations to protect products from light-induced degradation.

Significance of Stability-Indicating Methods

Stability-indicating RP-HPLC methods play a critical role in pharmaceutical quality assessment by enabling accurate separation and quantification of active pharmaceutical ingredients in the presence of degradation products, impurities, and formulation excipients. These methods provide valuable information regarding degradation pathways, shelf-life determination, formulation optimization, and regulatory compliance.

For combined formulations containing fluconazole and ivermectin, stability-indicating methods are particularly important because both drugs may exhibit different degradation behaviors under stress conditions. A properly validated stability-indicating RP-HPLC method ensures reliable monitoring of product quality throughout its lifecycle and supports regulatory submissions.[15,16]

IX. RP-HPLC IN QUALITY ASSESSMENT OF COMBINED FORMULATIONS

9.1 Assay Determination

Assay determination is one of the primary applications of RP-HPLC in pharmaceutical quality control. The technique enables accurate quantification of active pharmaceutical ingredients within finished dosage forms. In combined formulations containing fluconazole and ivermectin, RP-HPLC provides simultaneous determination of both drugs with high precision and accuracy.

The assay results ensure compliance with pharmacopeial specifications and confirm that the formulation contains the labeled amount of each active ingredient. Reliable assay determination is essential for maintaining therapeutic efficacy and product consistency.

9.2 Content Uniformity Testing

Content uniformity testing evaluates the consistency of drug distribution among individual dosage units within a batch. Variability in drug content may result in underdosing or overdosing, potentially affecting therapeutic outcomes.

RP-HPLC is widely employed for content uniformity testing because of its excellent sensitivity and reproducibility. The technique accurately measures the concentration of active ingredients in individual tablets or capsules, ensuring compliance with pharmacopeial requirements and manufacturing quality standards.

9.3 Dissolution Testing

Dissolution testing assesses the rate and extent of drug release from pharmaceutical dosage forms. The test provides valuable information regarding formulation performance, bioavailability, and product quality.

RP-HPLC serves as the preferred analytical technique for quantifying dissolved drug concentrations during dissolution studies. Simultaneous estimation methods facilitate efficient evaluation of combination products by enabling concurrent measurement of fluconazole and ivermectin released into dissolution media.

9.4 Impurity Profiling

Impurity profiling involves identification, quantification, and characterization of impurities present in pharmaceutical products. Impurities may originate from raw materials, manufacturing processes, degradation reactions, or storage conditions.

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RP-HPLC provides excellent separation capability for impurity analysis and is routinely employed in pharmaceutical industries for impurity profiling. Accurate impurity monitoring is essential because certain impurities may affect product safety, efficacy, and regulatory compliance.

9.5 Degradation Product Analysis

Degradation products may form during manufacturing, storage, transportation, or use of pharmaceutical products. Some degradation products possess toxicological significance and therefore require careful monitoring.

RP-HPLC enables effective separation of active ingredients from degradation products, facilitating accurate assessment of product stability and safety. Stability-indicating chromatographic methods are particularly valuable for degradation product analysis in combined formulations.

9.6 Batch-to-Batch Quality Evaluation

Batch-to-batch consistency is essential for maintaining pharmaceutical quality and ensuring reproducible therapeutic performance. Variations in raw materials, manufacturing conditions, or processing parameters can affect product quality.

RP-HPLC provides a reliable analytical platform for comparing different production batches. Parameters such as assay values, impurity levels, dissolution profiles, and stability characteristics can be monitored effectively, ensuring consistent product quality throughout commercial manufacturing.

X. REGULATORY PERSPECTIVES

10.1 ICH Guidelines

The International Council for Harmonisation (ICH) provides globally accepted guidelines that harmonize pharmaceutical development and quality requirements across regulatory regions.

ICH Q2(R2)

ICH Q2(R2) outlines the validation requirements for analytical procedures. The guideline emphasizes evaluation of analytical performance characteristics including specificity, linearity, accuracy, precision, robustness, detection limits, and quantification limits.

Compliance with ICH Q2(R2) ensures that analytical methods generate reliable and scientifically sound data suitable for regulatory submissions and routine quality control.

ICH Q14 Analytical Procedure Development

ICH Q14 provides a systematic framework for analytical procedure development. The guideline encourages a science-based and risk-based approach to method development, emphasizing analytical understanding, robustness, and lifecycle management.

Implementation of ICH Q14 principles promotes development of reliable and flexible analytical procedures capable of maintaining performance throughout their lifecycle.

10.2 USP Requirements

The United States Pharmacopeia (USP) establishes legally recognized standards for pharmaceutical quality in the United States. USP monographs provide specifications for identity, assay, impurities, dissolution, and chromatographic conditions.

RP-HPLC methods developed for pharmaceutical products must comply with applicable USP requirements to ensure product quality, safety, and efficacy.

10.3 European Pharmacopoeia Requirements

The European Pharmacopoeia provides official quality standards for pharmaceutical substances and dosage forms marketed within Europe. The pharmacopoeia includes validated analytical procedures, acceptance criteria, and impurity limits that support regulatory compliance.

RP-HPLC methods used in pharmaceutical analysis should satisfy European Pharmacopoeia requirements where applicable.

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10.4 WHO Analytical Guidelines

The World Health Organization (WHO) provides analytical guidelines aimed at ensuring the quality of pharmaceutical products worldwide, particularly in developing countries. WHO guidelines emphasize validated analytical methods, quality assurance systems, and good laboratory practices.

Compliance with WHO recommendations contributes to consistent pharmaceutical quality and improved public health outcomes globally.[17,18]

XI. CURRENT TRENDS AND FUTURE PERSPECTIVES

11.1 Quality by Design (QbD)-Based RP-HPLC

Quality by Design (QbD) has emerged as a modern approach to analytical method development. QbD emphasizes systematic understanding of analytical variables and their impact on method performance. Through experimental design and risk assessment, robust chromatographic methods can be developed with enhanced reliability and regulatory acceptance.

QbD-based RP-HPLC methods provide improved robustness, reduced method variability, and greater process understanding compared to traditional trial-and-error approaches.

11.2 Green Analytical Chemistry

Environmental sustainability has become an important consideration in pharmaceutical analysis. Green analytical chemistry aims to minimize solvent consumption, waste generation, energy usage, and environmental impact.

Current efforts focus on:

- Reducing organic solvent usage
- Employing environmentally friendly solvents
- Shortening analysis times
- Utilizing energy-efficient instrumentation

Green RP-HPLC methodologies are increasingly gaining attention as sustainable alternatives for pharmaceutical quality control.

11.3 UPLC and UHPLC Applications

Ultra-Performance Liquid Chromatography (UPLC) and Ultra-High-Performance Liquid Chromatography (UHPLC) represent significant advancements in chromatographic technology. These techniques utilize smaller particle sizes and higher operating pressures to achieve:

- Faster analysis
- Higher resolution
- Increased sensitivity
- Reduced solvent consumption

The adoption of UPLC and UHPLC continues to expand in pharmaceutical industries due to their superior analytical performance.

11.4 Artificial Intelligence in Method Optimization

Artificial Intelligence (AI) and machine learning technologies are increasingly being integrated into analytical method development. AI-driven approaches can predict chromatographic behavior, optimize experimental conditions, and reduce development time.

Potential applications include:

- Mobile phase optimization
- Retention time prediction
- Peak identification
- Automated method development
- Data interpretation

The integration of AI into chromatographic analysis is expected to transform future pharmaceutical research and quality control practices.

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11.5 Hyphenated Analytical Techniques

Hyphenated analytical techniques combine chromatographic separation with advanced detection systems. Examples include:

- HPLC-MS
- LC-MS/MS
- UPLC-MS
- HPLC-NMR

These techniques provide comprehensive analytical information, including structural characterization, impurity identification, and metabolite profiling. Their application is expected to increase significantly in future pharmaceutical investigations.

XII. RESEARCH GAPS AND OPPORTUNITIES

Lack of Reported Simultaneous Methods

Although numerous analytical methods have been reported for individual determination of fluconazole and ivermectin, relatively few studies have focused on their simultaneous estimation. This represents a significant opportunity for analytical method development and optimization.

Need for Stability-Indicating Methods

Limited information is available regarding stability-indicating methods specifically designed for simultaneous determination of fluconazole and ivermectin. Future research should focus on developing robust methods capable of separating both drugs from degradation products under various stress conditions.

Green Method Development Opportunities

Many reported analytical methods utilize large quantities of organic solvents. Development of environmentally sustainable RP-HPLC methods with reduced solvent consumption and shorter run times represents an important area for future investigation.

QbD-Based Analytical Approaches

Application of Quality by Design principles in simultaneous RP-HPLC method development remains relatively unexplored for fluconazole and ivermectin combinations. Future studies may benefit from incorporating design of experiments (DoE) and risk assessment methodologies.

Bioanalytical Applications

Further research is needed to develop sensitive and selective bioanalytical methods for simultaneous quantification of fluconazole and ivermectin in biological matrices. Such methods would support pharmacokinetic studies, bioavailability assessments, therapeutic drug monitoring, and clinical investigations. [19-25]

XIII. CONCLUSION

This review highlights the pharmaceutical significance of fluconazole and ivermectin and emphasizes the analytical challenges associated with their simultaneous determination due to differences in physicochemical properties, solubility characteristics, and chromatographic behavior. A comprehensive evaluation of reported analytical techniques indicates that RP-HPLC offers superior performance compared to conventional spectrophotometric and thin-layer chromatographic methods, particularly in terms of accuracy, specificity, and stability assessment.

The review further demonstrates that successful RP-HPLC method development depends on careful optimization of chromatographic parameters including stationary phase selection, mobile phase composition, pH, flow rate, and detection wavelength. Validation of analytical methods according to ICH Q2(R2) guidelines remains essential to ensure reliability and reproducibility of analytical results. Stability-indicating RP-HPLC methods play a crucial role in identifying degradation pathways and monitoring the integrity of pharmaceutical products throughout their shelf life.

Recent advances in chromatographic science, including Quality by Design-based method development, green analytical chemistry, UPLC/UHPLC technologies, artificial intelligence-assisted optimization, and hyphenated analytical platforms, have significantly enhanced analytical capabilities and opened new opportunities for pharmaceutical

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research. Despite these advancements, limited information is available regarding simultaneous stability-indicating methods for fluconazole and ivermectin combinations, highlighting the need for further investigation.

In conclusion, RP-HPLC continues to be an indispensable analytical technique for the quality assessment of fluconazole and ivermectin formulations. Future research should focus on the development of environmentally sustainable, QbD-based, stability-indicating, and highly efficient analytical methods that meet evolving regulatory expectations and support the advancement of pharmaceutical quality assurance.

REFERENCES

1. Yaseen Malik, M., Taneja, I., Raju, K. S. R., Rahaman Gayen, J., Singh, S. P., Sangwand, N. S., & Wahajuddin, M. (2017). RP-HPLC Separation of Isomeric Withanolides: Method Development, Validation and Application to In situ Rat Permeability Determination. *Journal of chromatographic science*, *55*(7), 729–735. https://doi.org/10.1093/chromsci/bmx027
2. Demirhan, T., Guksu, E., Yazar, Y., Keskin, E., Bellur Atici, E., & Özkan, S. A. (2024). Impurity assessment, development and validation of an RP-HPLC method for the determination of eleven potential impurities of eltrombopag precursor. *Journal of pharmaceutical and biomedical analysis*, *243*, 116085. https://doi.org/10.1016/j.jpba.2024.116085
3. Zheng, Y., Cao, C. Y., Guo, Z. M., Yan, J. Y., & Liang, X. M. (2024). *Se pu = Chinese journal of chromatography*, *42*(7), 646–657. https://doi.org/10.3724/SP.J.1123.2023.12003
4. Žuvela, P., Skoczylas, M., Jay Liu, J., Ba Czek, T., Kaliszan, R., Wong, M. W., Buszewski, B., & Héberger, K. (2019). Column Characterization and Selection Systems in Reversed-Phase High-Performance Liquid Chromatography. *Chemical reviews*, *119*(6), 3674–3729. https://doi.org/10.1021/acs.chemrev.8b00246
5. Wen, Q., Zhang, L., Zhao, F., Chen, Y., Su, Y., Zhang, X., Chen, P., & Zheng, T. (2023). Production Technology and Functionality of Bioactive Peptides. *Current pharmaceutical design*, *29*(9), 652–674. https://doi.org/10.2174/1381612829666230201121353
6. Papp, L. A., Szabó, Z. I., Hancu, G., Farczádi, L., & Mircia, E. (2024). Comprehensive Review on Chiral Stationary Phases in Single-Column Simultaneous Chiral-Achiral HPLC Separation Methods. *Molecules (Basel, Switzerland)*, *29*(6), 1346. https://doi.org/10.3390/molecules29061346
7. Guiard, B. P., & Gotti, G. (2024). The High-Precision Liquid Chromatography with Electrochemical Detection (HPLC-ECD) for Monoamines Neurotransmitters and Their Metabolites: A Review. *Molecules (Basel, Switzerland)*, *29*(2), 496. https://doi.org/10.3390/molecules29020496
8. Ganesh, V., Poorna Basuri, P., Sahini, K., & Nalini, C. N. (2023). Retention behaviour of analytes in reversed-phase high-performance liquid chromatography-A review. *Biomedical chromatography : BMC*, *37*(7), e5482. https://doi.org/10.1002/bmc.5482
9. Gaudin K. (2025). Potential of green solvents as mobile phases in liquid chromatography. *Journal of chromatography. A*, *1750*, 465810. https://doi.org/10.1016/j.chroma.2025.465810
10. Goel, H., Nagarajan, K., Nainwal, L. M., & Bhardwaj, S. (2025). Analytical Techniques for the Quantification and Validation of Resveratrol: A Review. *Anti-inflammatory & anti-allergy agents in medicinal chemistry*, *24*(4), 225–233. https://doi.org/10.2174/0118715230403013250901204059
11. Clogston, J. D., & Smith, M. C. (2017). Quantitation of PEG on PEGylated Gold Nanoparticles Using Reversed Phase High Performance Liquid Chromatography and Charged Aerosol Detection: Version 1.0. In *National Cancer Institute's Nanotechnology Characterization Laboratory Assay Cascade Protocols*. National Cancer Institute (US). https://doi.org/10.17917/1VHC-MJ13
12. Liu, X., Guan, F., Luo, H., Yao, B., & Tian, J. (2025). Artificial intelligence-driven discovery of bioactive peptides: Computational approaches and future perspectives. *aBIOTECH*, *7*(1), 100014. https://doi.org/10.1016/j.abiote.2025.100014
13. Rathnasekara, R., Khadka, S., Jonnada, M., & El Rassi, Z. (2017). Polar and nonpolar organic polymer-based monolithic columns for capillary electrochromatography and high-performance liquid chromatography. *Electrophoresis*, *38*(1), 60–79. https://doi.org/10.1002/elps.201600356

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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 6, Issue 3, June 2026
Impact Factor: 8.2

14. Hamed, M., Kannouma, R., Bedair, A., & Mansour, F. R. (2026). Method-at-a-Glance (MAG) as a Graphical Approach for Method Development in Reversed-Phase Liquid Chromatography of Pharmaceuticals. *Analytical chemistry*, *98*(13), 9485–9492. https://doi.org/10.1021/acs.analchem.5c07982
15. Rahman, M. S., Hiers, W., Davidson, A., Chabot, C., Riley, F., & Coutant, M. (2025). Green and white analytical chemistry: Advances, comparisons, future perspectives, and a proposal for green financing model for analytical chemistry. *Journal of pharmaceutical sciences*, *114*(11), 104005. https://doi.org/10.1016/j.xphs.2025.104005
16. Khalil, H. A., El-Sayed, N. W., Elmoslhy, R. M., & El-Yazbi, A. F. (2026). Recent advances in the analysis of the evolutionary RNA based therapeutics; a review. *International journal of biological macromolecules*, *344*(Pt 1), 150466. https://doi.org/10.1016/j.ijbiomac.2026.150466
17. Cao, G., Shen, C., Zhao, H., Li, Y., Luo, J., Chen, J., & Qiu, H. (2026). Recent advance in synthesis of monodisperse porous poly(styrene-divinylbenzene) microspheres for chromatographic separation. *Journal of chromatography. A*, *1773*, 466841. https://doi.org/10.1016/j.chroma.2026.466841
18. K, S., & M, S. (2026). A Comparative Review of Hydrophilic Interaction Chromatography(HILIC) with Traditional Chromatography in the Analysis of Aminoglycoside Antibiotics. *Critical reviews in analytical chemistry*, 1–11. Advance online publication. https://doi.org/10.1080/10408347.2025.2610328
19. Duong, Q. H., & Pegg, R. B. (2020). Quantitation of Inositol Phosphates by HPLC-ESI-MS. *Methods in molecular biology (Clifton, N.J.)*, *2091*, 31–37. https://doi.org/10.1007/978-1-0716-0167-9_2
20. Danielson, N. D., Targove, M. A., & Miller, B. E. (1988). Pre- and postcolumn derivatization chemistry in conjunction with HPLC for pharmaceutical analysis. *Journal of chromatographic science*, *26*(8), 362–371. https://doi.org/10.1093/chromsci/26.8.362
21. Escopy, S., Singh, Y., Stine, K. J., & Demchenko, A. V. (2022). HPLC-Based Automated Synthesis of Glycans in Solution. *Chemistry (Weinheim an der Bergstrasse, Germany)*, *28*(39), e202201180. https://doi.org/10.1002/chem.202201180
22. Persson, A., Tykesson, E., Ellervik, U., & Mani, K. (2022). Production and HPLC-Based Disaccharide Analysis of Xyloside-Primed Glycosaminoglycans. *Methods in molecular biology (Clifton, N.J.)*, *2303*, 173–182. https://doi.org/10.1007/978-1-0716-1398-6_15
23. Liu, C., Tian, M., Dong, W., Lu, W., Zhang, T., Wan, Y., Zhang, X., & Li, Y. (2024). SEC-HPLC analysis of column load and flow-through provides critical understanding of low Protein A step yield. *Protein expression and purification*, *216*, 106418. https://doi.org/10.1016/j.pep.2023.106418
24. Malaník, M., Jedináková, T., Stiskálková, T., Witteková, D., Chlebková, A., & Šmejkal, K. (2025). HPLC-DAD and HPLC-ELSD Profiling of Various Parts of Selected Ficus Species. *Chemistry & biodiversity*, *22*(9), e202403260. https://doi.org/10.1002/cbdv.202403260
25. Das, A., Guyer, L., & Hörtensteiner, S. (2018). Chlorophyll and Chlorophyll Catabolite Analysis by HPLC. *Methods in molecular biology (Clifton, N.J.)*, *1744*, 223–235. https://doi.org/10.1007/978-1-4939-7672-0_18