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Contemporary RP-HPLC Strategies for Dual Quantification of Rosuvastatin and Ezetimibe in Combined Dosage Forms: Advances, Challenges, and Future Prospects

Abhishek Kumar Srivastava; Hridesh Singh Chauhan; Utkarsh Sharma

M Pharm at the Department of Pharmaceutical Chemistry in Advance Institute of Biotech and Paramedical Sciences. India.
Associate Professor at Department of Pharmaceutical Chemistry, Advance Institute of Biotech and Paramedical Sciences, Kanpur India.
Assistant Professor at Department of Pharmacology, Advance Institute of Biotech and Paramedical Sciences, Kanpur India.

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ABSTRACT

The increasing global burden of dyslipidemia has intensified the clinical need for efficient lipid-lowering therapies, particularly fixed-dose combinations such as Rosuvastatin and Ezetimibe. Their complementary mechanisms-HMG-CoA reductase inhibition and cholesterol absorption blockade necessitate reliable analytical strategies to ensure accurate quality assessment of combined dosage forms. Reverse-phase high-performance liquid chromatography (RP-HPLC) remains the preferred analytical platform for simultaneous quantification owing to its robustness, selectivity, and compatibility with complex pharmaceutical matrices. This review synthesizes contemporary chromatographic approaches developed for the dual estimation of Rosuvastatin and Ezetimibe, systematically evaluating method parameters including mobile phase composition, stationary phase characteristics, retention behavior, sensitivity profiles, and detection strategies. Particular emphasis is placed on physicochemical and structural factors influencing chromatographic separation, such as pKa, lipophilicity, and chromophoric properties. Advances in analytical quality by design (AQbD), eco-friendly chromatography, core-shell column technologies, and UHPLC innovations are critically discussed to highlight emerging trends that enhance resolution, reduce run time, and improve overall method efficiency. Challenges encountered in simultaneous estimation including co-elution risks, matrix interference, and low-dose analyte detection are examined alongside regulatory expectations under ICH Q2(R2) guidelines. The review concludes with future perspectives on chemometric-assisted optimization, hybrid detection systems, and evolving analytical paradigms for multi-component cardiovascular formulations. Collectively, this work provides a comprehensive scientific foundation for researchers developing next-generation RP-HPLC methods for combination drug analysis.

Keywords: Rosuvastatin, Ezetimibe, RP-HPLC, Method Validation, Combination Therapy

1. INTRODUCTION

1.1 Cardiovascular Disease Burden and Lipid-Lowering Therapies

Cardiovascular diseases (CVDs) continue to be the leading cause of mortality worldwide, accounting for approximately 17.9 million deaths annually, with a significant proportion attributable to dyslipidemia-driven atherosclerosis.[1,2] Elevated low-density lipoprotein cholesterol (LDL-C) remains one of the most prominent modifiable risk factors for CVD progression, and intensive lipid-lowering therapy constitutes the core strategy for both primary and secondary prevention.[3]

Pharmacological interventions include statins, cholesterol absorption inhibitors, fibrates, bile acid sequestrants, and PCSK9 inhibitors. Among these, statins remain the first-line therapy due to their proven efficacy in LDL-C reduction and pleiotropic benefits; however, monotherapy may be insufficient in patients with severe hypercholesterolemia or statin intolerance.[4,5] Thus, combination therapy involving agents with complementary mechanisms such as the pairing of rosuvastatin (a potent HMG-CoA reductase inhibitor) and ezetimibe (a selective NPC1L1-mediated cholesterol absorption inhibitor)—has gained prominence for achieving target lipid levels.

1.2 Rationale for Combination Therapy: Rosuvastatin + Ezetimibe

Rosuvastatin and ezetimibe offer complementary pharmacodynamics: rosuvastatin suppresses endogenous cholesterol biosynthesis, while ezetimibe inhibits intestinal absorption of dietary and biliary cholesterol.[6] This dual mechanism produces superior LDL-C reduction, improved non-HDL cholesterol, and better cardiovascular outcomes compared with statin monotherapy at equivalent doses.[7,8]

Clinical trials such as **IMPROVE-IT** have demonstrated that ezetimibe added to statin therapy significantly reduces CVD risk in high-risk populations.[9] Additionally, combination therapy improves adherence by reducing pill burden and enables attainment of more stringent lipid targets recommended by current international guidelines.[10]

The increasing utilization of fixed-dose combinations (FDCs) of rosuvastatin–ezetimibe in global markets has consequently created a robust need for reliable analytical methodologies capable of simultaneous quantification of both drugs in bulk and formulated products.

1.3 Analytical Need for Simultaneous Estimation

Combined dosage forms containing rosuvastatin and ezetimibe require rigorous quality control to ensure potency, stability, and bioequivalence. The physicochemical differences between the two molecules—rosuvastatin being hydrophilic and ionizable, whereas ezetimibe is lipophilic with poor aqueous solubility—pose analytical challenges for simultaneous measurement.[11]

Regulatory expectations (ICH Q2(R1)) demand validated analytical methods that demonstrate specificity, sensitivity, linearity, accuracy, precision, and robustness.[12] Although spectrophotometric and chemometric methods have been proposed, **RP-HPLC** remains the gold standard owing to its superior resolution, reproducibility, and adaptability for forced-degradation (stability-indicating) studies.[13]

An optimized RP-HPLC method enables:

- sharp and well-resolved peaks for both analytes,
- reduced run time and solvent consumption,
- suitability for routine QC and stability testing,
- application in dissolution studies and pharmacokinetics.

1.4 Role of RP-HPLC in Drug Combination Analysis

Reverse-phase HPLC (RP-HPLC) is the most extensively reported technique for concurrent quantification of rosuvastatin and ezetimibe, owing to its versatility in handling compounds with differing lipophilicity. C18 columns, acidified mobile phases, and optimized organic modifiers (acetonitrile or methanol) are frequently employed to achieve effective peak separation.[14-16]

Key analytical advancements include:

- **Use of volatile buffers** (e.g., ammonium acetate/formate) compatible with LC-MS.
- **pH-controlled separation** improving peak symmetry for rosuvastatin (pKa 4.0–4.6).
- **Gradient elution programs** enabling reduced analysis time.
- **Stability-indicating methods** capable of resolving degradation products under stress conditions (acid/base hydrolysis, oxidation, photolysis, thermal degradation).[17]

RP-HPLC also supports simultaneous dissolution profiling, which is essential for ensuring uniform drug release from fixed-dose combinations. The increasing trend toward quality-by-design (QbD) approaches has further refined robustness and method operability ranges for these assays.

1.5 Scope of the Review

This review synthesizes contemporary RP-HPLC methodologies developed for dual quantification of rosuvastatin and ezetimibe, highlighting innovations in chromatographic conditions, mobile phase chemistry, forced-degradation behavior, and method validation attributes. It critically examines analytical challenges associated with their physicochemical disparities and summarizes QbD-driven strategies, green chromatography initiatives, and LC-MS compatible approaches.

Furthermore, key gaps in literature are identified, with recommendations for future work focusing on enhanced sensitivity, eco-friendly separations, hyphenated techniques, and greater suitability for regulatory submissions. By integrating advances, limitations, and prospective trends, the review aims to guide formulation scientists and analytical chemists in selecting or designing optimized RP-HPLC methods for combination lipid-lowering therapeutics.

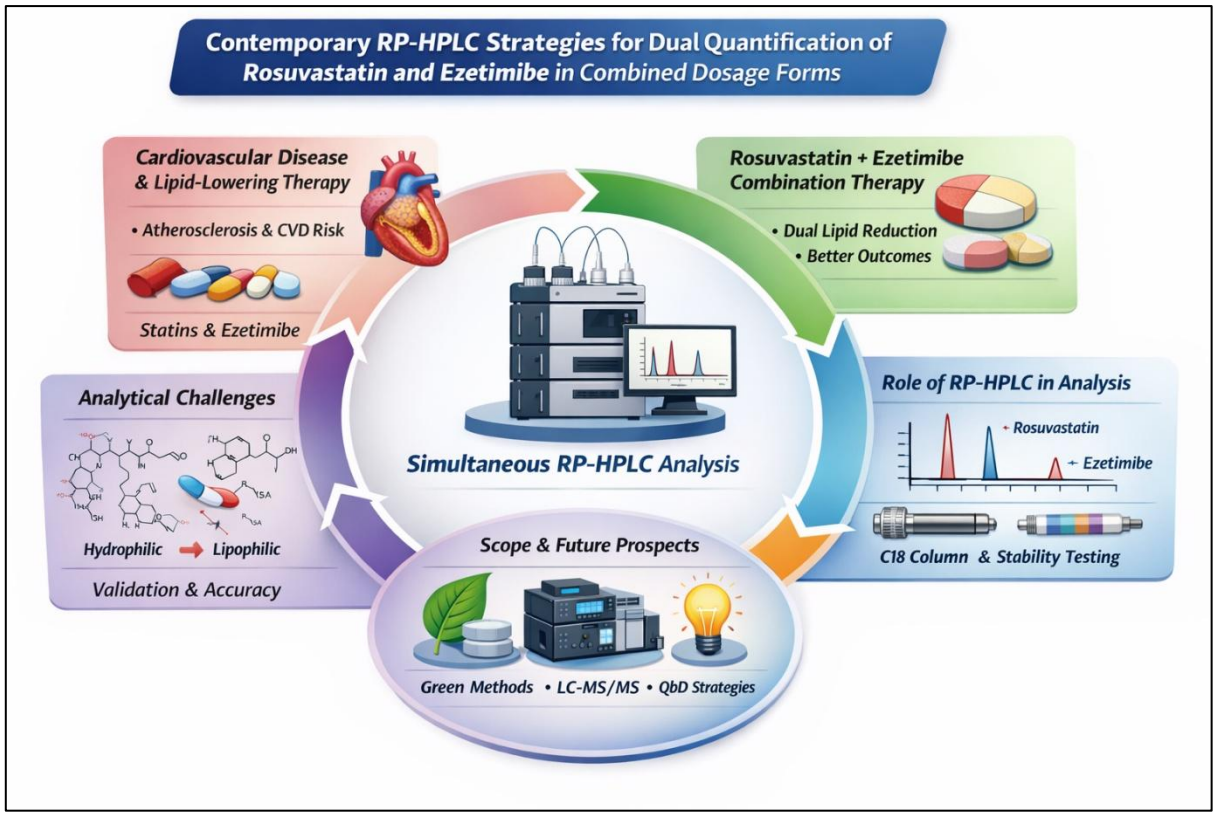

Fig 1 : Integrated schematic summarizing disease burden, combination-therapy rationale, analytical challenges, and RP-HPLC approaches for dual quantification of rosuvastatin and ezetimibe.

2. Pharmacological Overview of Rosuvastatin and Ezetimibe

2.1 Rosuvastatin: Mechanism, PK/PD, and Chemical Characteristics

Rosuvastatin is a synthetic, highly potent HMG-CoA reductase inhibitor that suppresses endogenous cholesterol biosynthesis by competitively blocking the conversion of HMG-CoA into mevalonate, the key precursor in the cholesterol synthesis pathway.[18] This inhibition results in significant upregulation of hepatic LDL receptors, which enhances LDL-C clearance and contributes to substantial reductions in circulating atherogenic lipoproteins. Pharmacokinetically, rosuvastatin exhibits nearly 20% oral bioavailability, peak plasma concentrations within 3–5 hours, and minimal hepatic metabolism, with CYP2C9 being the primary pathway.[19] Its hydrophilic nature and selective uptake through OATP1B1/1B3 transporters confer hepatoselectivity, allowing enhanced LDL-lowering efficacy with reduced systemic exposure. Pharmacodynamically, rosuvastatin reduces LDL-C by 45–63% across therapeutic doses and exhibits pleiotropic benefits, including improved endothelial function and reduction of vascular inflammation through modulation of CRP and oxidative stress.[20]

Chemically, rosuvastatin possesses acidic functional groups (pKa ~4.0–4.6), including a sulfonamide and carboxylic acid moiety, which strongly influence its ionization behavior and chromatographic retention under RP-HPLC conditions.

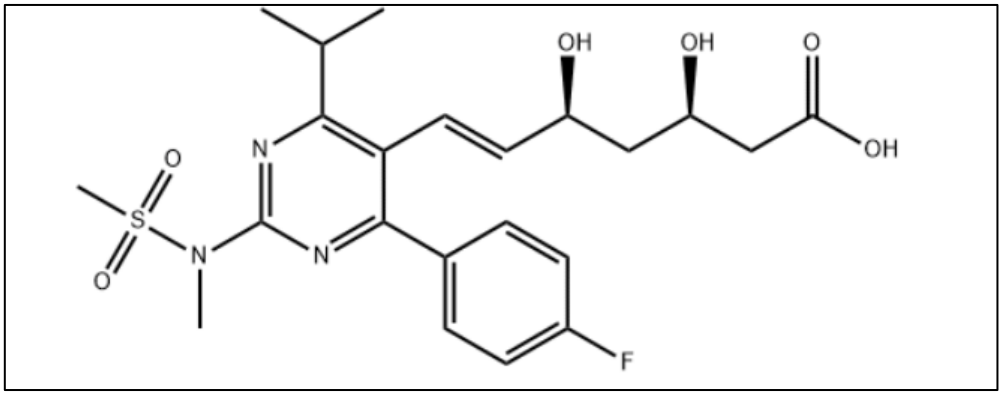

Fig 2: Rosuvastatin Chemical Structure

2.2 Ezetimibe: Mechanism, PK/PD, and Chemical Characteristics

Ezetimibe acts through a complementary mechanism by selectively inhibiting the Niemann–Pick C1-Like 1 (NPC1L1) transporter located on enterocytes, thereby reducing intestinal absorption of dietary and biliary cholesterol.[21] This effect decreases hepatic cholesterol pools and promotes compensatory LDL receptor expression, amplifying the LDL-lowering action when co-administered with statins. Ezetimibe demonstrates rapid absorption and extensive conversion to its pharmacologically active glucuronide metabolite, which undergoes enterohepatic recirculation and sustains prolonged cholesterol-lowering effects.[22] The drug shows minimal involvement of CYP pathways, reducing interaction potential.

Chemically, ezetimibe is lipophilic and contains multiple aromatic rings and a β-lactam core, contributing to its poor aqueous solubility and preferential retention on non-polar stationary phases. Its neutral ionization profile under acidic chromatographic conditions necessitates higher organic solvent proportions for adequate elution in RP-HPLC methods.

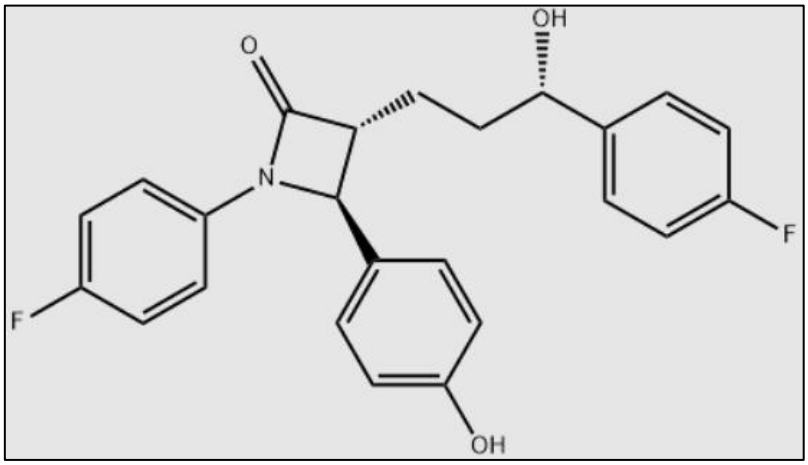

Fig 3 : Ezetimibe Chemical Structure

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2.3 Molecular and Physicochemical Properties Influencing Chromatography

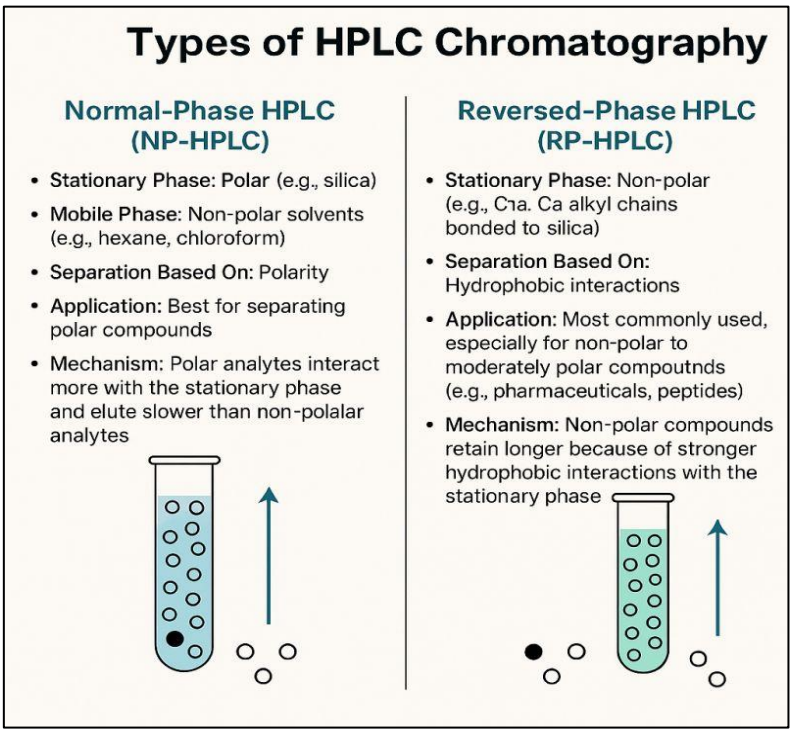

Fig 4 : Types of HPLC Chromatography

The simultaneous chromatographic separation of rosuvastatin and ezetimibe is strongly influenced by their contrasting physicochemical signatures. Rosuvastatin's hydrophilicity, acidic pKa, and ionizable polar groups make it sensitive to mobile-phase pH and prone to early elution. In contrast, ezetimibe's high lipophilicity and neutral character yield delayed retention and broader peaks at low aqueous solubility conditions. These differences require careful balancing of mobile-phase composition, organic modifier ratios, and pH control to achieve sharp, well-resolved peaks for both analytes.

Table 1. Key Physicochemical Differences Affecting RP-HPLC Separation

Property	Rosuvastatin	Ezetimibe	Chromatographic Implication
Hydrophilicity	High	Low	Causes early elution vs. delayed elution
pKa	4.0–4.6	Weakly acidic/neutral	Affects ionization and peak symmetry
Aqueous Solubility	Good at acidic pH	Very poor	Requires high organic content
Structure	Polar sulfonamide + COOH	Aromatic + β-lactam	Different retention mechanisms
LogP	~2.2	~4.7	Widely separated retention times

2.4 Analytical Challenges Due to Structural and Functional Difference

The chemical and functional discrepancies between the two molecules introduce several analytical challenges during simultaneous RP-HPLC quantification. Their divergent solubility profiles and ionization behaviors often result in imbalance in retention times, where rosuvastatin elutes too quickly while ezetimibe retains excessively without optimization of mobile-phase polarity. Forced-degradation patterns further complicate method development, as rosuvastatin undergoes oxidative degradation whereas ezetimibe shows susceptibility to alkaline hydrolysis, requiring a robust stability-indicating method to resolve all degradation products.

Moreover, their differing UV-absorption profiles demand careful selection of detection wavelength, as rosuvastatin absorbs strongly near 242–248 nm, while ezetimibe exhibits weaker and broader absorption bands. Column chemistry also plays a critical role; typical C18 phases may cause peak tailing for highly ionizable rosuvastatin, whereas polar-embedded phases may compromise ezetimibe retention. These complexities necessitate a well-designed chromatographic system capable of accommodating both molecular extremes without compromising resolution, precision, or analytical robustness.

3. Analytical Considerations for Simultaneous RP-HPLC Estimation

3.1 Chromophoric Characteristics and UV Absorption Profiles

The simultaneous quantification of rosuvastatin and ezetimibe requires a clear understanding of their chromophoric behavior, as both drugs possess structurally distinct UV-absorbing functional groups. Rosuvastatin exhibits strong absorption in the range of 240–250 nm due to the presence of conjugated aromatic and heterocyclic moieties. Its well-defined λmax results in highly sensitive UV detection, supporting low-level quantification in combined dosage forms. In contrast, ezetimibe displays broader and less intense UV absorption bands, attributed to its phenoxy and β-lactam structures, which exhibit limited chromophoric density.[23] Consequently, simultaneous detection often involves selecting a compromise wavelength, typically between 230–245 nm, capable of providing adequate response for both analytes. Photodiode array (PDA) detection is frequently preferred, as it allows purity assessment, peak homogeneity confirmation, and simultaneous multi-wavelength monitoring, thus improving the reliability of the simultaneous RP-HPLC assay in stability-indicating applications.[24]

3.2 Solubility, pKa, LogP, and Their Impact on Chromatographic Behavior

The physicochemical profiles of rosuvastatin and ezetimibe significantly shape their chromatographic retention and separation efficiency. Rosuvastatin possesses acidic functional groups and demonstrates good solubility in mildly acidic aqueous media, with ionization occurring near pKa values of ~4.0–4.6. This ionizable nature causes variable retention at different pH levels, necessitating strict acidic control (pH 2.5–3.0) to ensure optimal peak symmetry and suppression of secondary interactions.[25] Conversely, ezetimibe exhibits weakly acidic to neutral behavior, displays poor aqueous solubility, and possesses high lipophilicity (LogP ≈ 4.7), leading to strong retention on reversed-phase columns. The disparity in hydrophilicity (rosuvastatin) and lipophilicity (ezetimibe) requires careful adjustment of organic solvent proportion, often necessitating acetonitrile to decrease ezetimibe retention while simultaneously preventing premature elution of rosuvastatin.[26] Such physicochemical divergence constitutes the core analytical challenge in developing a harmonized RP-HPLC method capable of producing sharp, well-resolved chromatographic peaks.

3.3 Selection Criteria for Mobile Phase Components

The choice of mobile phase plays a decisive role in balancing solute retention, peak shape, and overall resolution between the two drugs. Acetonitrile is frequently preferred over methanol due to its stronger elution strength, lower viscosity, and ability to yield narrower peak widths, especially for lipophilic analytes such as ezetimibe.[27] Acidic buffers such as orthophosphoric acid, formic acid, or ammonium formate support consistent ion suppression for rosuvastatin while maintaining chemical stability for both molecules. The pH selection is critical, as rosuvastatin demonstrates peak tailing when the pH exceeds its pKa, whereas ezetimibe benefits from higher organic content for strong elution. Mobile phase optimization often involves adjusting aqueous:organic ratios, buffer molarity, and ionic strength to fine-tune the selectivity factor (α) and theoretical plate count (N). In advanced approaches, volatile buffers are used to enable LC-MS compatibility, improving method versatility for bioanalytical or trace-level estimation scenarios.[28]

3.4 Column Selection: Stationary Phase Chemistry and Particle Technology

Column chemistry significantly influences retention behavior and separation selectivity in rosuvastatin–ezetimibe assays. Traditional C18 stationary phases remain the most widely utilized due to their robust hydrophobic interaction profile and compatibility with both acidic and neutral solutes.[29] However, hydrophilic interaction or polar-embedded phases are occasionally explored to address peak tailing in rosuvastatin and to refine resolution under high organic conditions. Particle size reduction—from 5 µm to sub-2 µm—enables enhanced efficiency and reduced runtime, allowing sharper peaks and improved quantification in high-throughput environments.[30] Core–shell (superficially porous) technologies further contribute to high-performance separation, offering lower backpressure and superior mass transfer characteristics. The choice of column length and internal diameter also modulates resolution and sensitivity, with shorter columns supporting rapid analysis while longer columns enhance separation robustness. Ultimately, column selection must harmonize the differential retention of both analytes without compromising method ruggedness.

3.5 Detection Strategies: UV vs PDA vs Fluorescence

UV detection remains the most accessible and practical choice for routine quality control due to its affordability and sufficient sensitivity for both drugs. However, PDA detection offers significant advantages, including spectral profiling, peak purity assessment, and simultaneous multi-wavelength monitoring—particularly beneficial when degradation products or excipient interferences are expected.[31] Fluorescence detection, though highly sensitive, is seldom applied for rosuvastatin–ezetimibe combinations because neither compound exhibits strong native fluorescence without derivatization. In modern analytical workflows,

PDA-based detection is increasingly favored for stability-indicating method development, while LC-MS or MS/MS detection is used for pharmacokinetic, degradation, or trace-level quantification where superior sensitivity and selectivity are required.[32]

3.6 Sample Preparation Techniques for Tablets and Capsules

Accurate simultaneous quantification requires efficient sample preparation to extract both analytes without inducing chemical degradation. Standard procedures involve pulverization of tablets, sonication in strong organic solvents (commonly methanol or acetonitrile), and subsequent dilution with aqueous buffer to ensure compatibility with the chromatographic system.[33] The contrasting solubilities of rosuvastatin and ezetimibe necessitate optimized solubilization protocols, often using a combination of organic solvents and mild acidic conditions to achieve complete dissolution. Filtration through 0.22 µm or 0.45 µm membranes ensures particulate removal while preventing adsorption losses. For stability-indicating studies, forced-degradation samples may require neutralization prior to dilution to avoid on-column decomposition. Ultimately, the sample preparation strategy must demonstrate recovery within acceptable ICH limits and ensure reproducibility across diverse formulation matrices.[34,35]

4.1 Summary of Published Methods (Tabulated Overview)

Published RP-HPLC methods for simultaneous estimation of rosuvastatin and ezetimibe exhibit significant diversity in chromatographic strategies, reflecting the intrinsic physicochemical differences between the two analytes.[36] Early studies predominantly relied on classical C18 columns and simple acidic mobile phases, focusing on achieving acceptable resolution rather than optimizing analytical efficiency.[37] These foundational methods established baseline conditions—such as acidic pH, moderate organic content, and UV detection near 240 nm—that continue to inform current development practices.[38]

Over time, researchers broadened methodological scope by introducing more refined chromatographic conditions aimed at enhancing selectivity and sensitivity.[39] Adjustments such as switching from methanol to acetonitrile, fine-tuning buffer concentration, and modifying elution strategies were systematically evaluated. These efforts resulted in shorter runtimes, improved peak symmetry, and better reproducibility across varying laboratory setups.[40] The literature collectively demonstrates a clear shift toward flexibility and adaptability, with modern studies emphasizing optimization for both assay and stability-indicating purposes.[41]

This evolution is also marked by increased focus on regulatory compliance. Contemporary methods frequently incorporate forced degradation data, peak purity evaluation through PDA detection, and statistical validation under ICH guidelines.[42] As a result, modern RP-HPLC methods not only quantify both drugs efficiently but also satisfy stringent quality-control expectations required for routine analytical use in pharmaceutical environments.[43]

4.2 Mobile Phase Compositions Used Across Studies

Mobile-phase composition is one of the most variable parameters across published methods, with researchers experimenting extensively to balance the contrasting retention behaviors of rosuvastatin and ezetimibe.[44] Many early methods utilized methanol due to its widespread availability and compatibility with reversed-phase systems, but its higher viscosity often resulted in broader peaks and longer analysis times.[45] As methodologies matured, acetonitrile became the dominant organic phase due to its superior elution strength and ability to improve column efficiency.[46]

Acidic aqueous buffers are critical components in nearly all published methods. Orthophosphoric acid, ammonium formate, and formic acid are most frequently used to stabilize pH and suppress ionization of rosuvastatin, thereby enhancing peak shape and retention consistency.[47] In contrast, ezetimibe's lipophilicity requires higher proportions of organic solvent to prevent excessive retention, necessitating fine balancing of aqueous and organic phase percentages.[48]

Recent studies have increasingly employed gradient elution strategies to improve separation of ezetimibe from hydrophobic degradation products and formulation excipients.[49] Other advancements include the use of volatile buffers to enhance MS compatibility and adoption of low-pH mobile phases (<3.0) to ensure consistent ion suppression.[50] Collectively, the literature demonstrates that careful selection and optimization of the mobile phase remain central to achieving reliable, high-resolution chromatographic performance.[51]

4.3 Column Types, Dimensions, and Operating Conditions

C18 stationary phases dominate published methods due to their broad applicability and reliable performance for both hydrophilic and lipophilic compounds.[52] Most early studies utilized 250 × 4.6 mm columns packed with 5 µm particles, providing adequate

resolution but at the cost of long analysis times.[53] As analytical practices evolved, shorter columns and smaller particle sizes were introduced to meet modern expectations for high throughput and improved separation efficiency.[54]

Advancements in column technology—such as core–shell (superficially porous) particles—have dramatically improved efficiency in more recent studies.[55] These columns offer enhanced mass transfer, reduced backpressure, and narrower peak widths, providing a distinct advantage in separating rosuvastatin and ezetimibe despite their divergent hydrophobicity profiles.[56] Some studies also explored polar-embedded phases or C8 columns to address specific retention challenges, though such modifications require careful optimization of mobile-phase pH and organic strength.[57]

Temperature control is another critical factor influencing column performance. Operating temperatures between 30–40°C improve peak shape by reducing solvent viscosity and enhancing mass-transfer kinetics.[58] Similarly, flow-rate adjustments (typically 0.8–1.2 mL/min) allow analysts to balance resolution and runtime. Together, the literature clearly shows that column selection and operational parameters play a decisive role in producing consistent and robust chromatographic outcomes.[59]

4.4 Retention Time Patterns and Peak Resolution Consistency

Retention-time trends across studies consistently indicate that rosuvastatin elutes earlier than ezetimibe due to its hydrophilic structure and limited hydrophobic interaction with the stationary phase.[60] Most reported retention times for rosuvastatin lie between 2–5 minutes, depending on mobile-phase composition and column efficiency.[61] In contrast, ezetimibe generally demonstrates longer retention—frequently between 5–10 minutes—owing to its markedly higher lipophilicity.[62]

Despite these predictable trends, achieving consistent resolution remains a challenge. Variations in pH, buffer concentration, and organic solvent ratios can significantly affect selectivity, particularly near rosuvastatin's pKa range.[63] Studies that maintain mobile-phase pH below 3.0 report the best peak shapes for rosuvastatin, while also ensuring adequate hydrophobic interaction for elution of ezetimibe. This pH-controlled optimization is a recurring theme across the literature.[64]

Stability-indicating methods introduce an additional layer of complexity. Under forced degradation, oxidative products of rosuvastatin and alkaline degradants of ezetimibe may demonstrate retention times similar to the parent compounds.[65] Methods employing PDA detection show superior performance in distinguishing co-eluting impurities, confirming peak purity and ensuring reliable quantitative interpretation.[66] Such advancements underscore the importance of integrating spectral tools into chromatographic workflows to enhance analytical accuracy.[67]

4.5 Sensitivity Profiles: LOD, LOQ, Linearity Ranges

Analytical sensitivity varies considerably among published methods due to differences in detection wavelength, injection volume, column efficiency, and mobile-phase design.[68] Methods utilizing stronger UV absorption wavelengths (around 240–245 nm) consistently achieve lower LOD and LOQ values for rosuvastatin, often below 0.05 µg/mL.[69] Ezetimibe, with its weaker chromophoric profile, exhibits slightly higher detection limits, but optimized methods still achieve LOQ values within acceptable analytical ranges.[70]

Linearity is a strong point across nearly all reported methods, with correlation coefficients (r^2) consistently above 0.999.[71] Most studies employ concentration ranges suitable for tablet assay and dissolution testing, typically spanning 2–40 µg/mL for rosuvastatin and 2–60 µg/mL for ezetimibe. The use of PDA detection further enhances quantitative accuracy by confirming the absence of overlapping impurities that might distort absorption profiles.[72]

In more advanced methods, sensitivity is improved through optimization of injection volumes, column type, and organic content, as well as adoption of LC-MS compatible mobile phases for bioanalytical applications.[73] Such enhancements demonstrate the adaptability of RP-HPLC techniques for expanding the scope of analysis beyond routine QC, supporting trace-level quantification in pharmacokinetic and stability studies.[74]

4.6 Method Application to Formulations and Stability Studies

Reported RP-HPLC methods have been widely applied to assay commercial fixed-dose combinations, laboratory formulations, and dissolution samples.[75] These studies typically demonstrate high assay accuracy, with recovery values between 98–102%, meeting pharmacopeial standards.[76] Many investigations also validate uniformity of dosage units, demonstrating the suitability of optimized RP-HPLC methods for routine QC applications.[77]

The methods are equally effective in stability-indicating contexts. Forced degradation studies performed under ICH-recommended conditions—acidic, alkaline, oxidative, thermal, and photolytic stress—reveal clear degradation patterns for both drugs.[78] Rosuvastatin predominantly undergoes oxidative degradation, while ezetimibe is especially sensitive to alkaline hydrolysis.[79] Methods that achieve baseline separation between parent compounds and degradants are considered highly reliable for stability-indicating purposes.[80]

Several studies extend the application of RP-HPLC methods to dissolution profiling, particularly for evaluating immediate-release or modified-release formulations.[81] Dissolution data, when coupled with accurate quantification of both drugs, support comparative bioavailability and formulation optimization.[82] These applications demonstrate the versatility of optimized RP-HPLC methods for comprehensive pharmaceutical evaluation across the product life cycle.[83]

5. Advances and Innovations in RP-HPLC Methodology

5.1 Application of AQbD (Analytical Quality by Design)

Analytical Quality by Design (AQbD) has emerged as a transformative framework in RP-HPLC method development, emphasizing scientific understanding, robustness, and regulatory compliance. Unlike traditional one-variable-at-a-time (OVAT) approaches, AQbD applies systematic experimentation to define a Method Operable Design Region (MODR), within which the method demonstrates consistent performance.[84] Through tools such as risk assessment, Ishikawa diagrams, and Failure Mode and Effects Analysis (FMEA), analysts identify critical method parameters (CMPs) that significantly influence retention time, resolution, and peak purity. This structured approach ensures higher method reliability across laboratories and instruments.

Design of Experiments (DoE) is central to AQbD-based RP-HPLC methods. Factorial, Box–Behnken, and central composite designs have been widely applied to evaluate the effects of pH, buffer strength, flow rate, and organic phase composition on chromatographic responses.[85] These statistical tools enable quantitative modeling of critical quality attributes (CQAs) such as retention, tailing factor, and theoretical plates, significantly reducing development time while enhancing method robustness. By predicting interactions between parameters, AQbD helps in optimizing chromatographic conditions with scientific precision.

Modern pharmaceutical guidelines increasingly encourage AQbD implementation due to its regulatory transparency and lifecycle management advantages. Methods developed under AQbD principles demonstrate improved ruggedness, stress tolerance, and reproducibility across instruments, making them highly suitable for long-term QC use.[86] Furthermore, AQbD facilitates method control strategy development, ensuring sustained performance even under minor operational deviations, thereby enhancing overall analytical reliability.

5.2 Use of Advanced Stationary Phases (Core-Shell, Monolithic Columns)

Advanced stationary phases have significantly improved chromatographic performance in simultaneous estimation of multi-drug systems such as rosuvastatin and ezetimibe. Core–shell (superficially porous) particles offer enhanced mass transfer and reduced eddy diffusion compared with fully porous particles, resulting in sharper peaks and higher efficiency at lower backpressure.[87] These characteristics enable faster and more efficient separations without requiring UHPLC-level pressures, making them attractive for routine QC environments.

Monolithic columns represent another major innovation, characterized by continuous porous structures that permit exceptionally high permeability and low pressure drops.[88] Their unique architecture allows operation at higher flow rates without compromising resolution, enabling ultra-fast separations. Monoliths are especially effective for analytes with widely differing hydrophobicity profiles—such as rosuvastatin (hydrophilic) and ezetimibe (lipophilic)—because their bimodal pore distribution enhances mass-transfer kinetics for both small and large molecules.

In addition to efficiency improvements, modern stationary phases also enhance method ruggedness and reduce column fouling. Columns with polar-embedded or sterically protected ligands minimize silanol interactions and improve peak symmetry, particularly for acidic analytes.[89] These advancements collectively contribute to higher robustness, shorter analysis times, and better suitability for high-throughput pharmaceutical analysis.

5.3 Greener Chromatographic Approaches (Eco-friendly Mobile Phases)

Green analytical chemistry principles have influenced modern RP-HPLC method development, leading to the adoption of eco-friendly mobile phases and reduced solvent consumption. Replacing traditional organic solvents—particularly acetonitrile, which

has higher toxicity and environmental burden—with greener alternatives such as ethanol or propylene carbonate has gained traction in recent studies.[90] These solvents not only reduce ecological impact but also enhance laboratory safety and sustainability.

Mobile-phase reengineering also includes minimizing buffer strength and promoting the use of volatile, low-toxicity additives compatible with both UV and MS detection. Bio-derived solvents such as ethyl lactate and glycerol-based eluents have shown promise in reducing environmental hazards without compromising chromatographic performance.[91] Additionally, low-flow-rate microbore LC formats reduce mobile-phase consumption by over 80%, supporting greener and more economical analytical workflows.

Gradient designs tailored to reduce total organic solvent usage—such as step-gradients and backflushing techniques—have further enhanced the greenness of RP-HPLC methods.[92] Collectively, these innovations align with global sustainability directives while maintaining analytical precision and regulatory suitability.

5.4 Hybrid Detection Systems: HPLC–MS Compatibility Trends

Hybrid detection systems, particularly HPLC–MS and HPLC–MS/MS, have expanded the analytical scope of RP-HPLC methods by offering superior sensitivity, specificity, and structural elucidation capabilities. Compatibility with MS detection requires volatile buffers, typically ammonium acetate or ammonium formate, which minimize ion suppression and enhance analyte signal intensity.[93] These volatile components are essential for applications involving trace-level detection or metabolite profiling.

Mass spectrometry provides significant advantages for simultaneous analysis of rosuvastatin and ezetimibe because it can selectively quantify analytes even in the presence of co-eluting excipients or degradation products. Tandem MS (MS/MS) further enhances selectivity through multiple reaction monitoring (MRM), allowing quantification at nanogram-per-milliliter concentrations—far beyond the reach of UV detection.[94] Such capability is particularly valuable in pharmacokinetic studies and impurity profiling.

Advancements in ionization sources, such as electrospray ionization (ESI) and atmospheric-pressure chemical ionization (APCI), have improved compatibility with diverse analyte chemistries. MS-hybrid workflows also support structural elucidation of stress degradation products, complementing PDA-based peak purity assessments.[95] These hybrid systems represent the highest analytical standard currently achievable in pharmaceutical analysis.

5.5 Micro- and UHPLC Techniques for Faster and Higher Resolution Analysis

Micro-LC and UHPLC represent the most significant advancements in accelerating RP-HPLC analysis while enhancing efficiency. UHPLC leverages sub-2-µm particles and high-pressure instrumentation (up to 15,000 psi) to deliver superior theoretical plate counts and dramatically reduced runtimes.[96] These characteristics enable near-baseline separation of complex analyte mixtures within minutes, making UHPLC ideal for high-throughput pharmaceutical workflows.

Micro-LC systems reduce solvent consumption, lower operational costs, and enhance method greenness by utilizing narrow-bore columns and ultra-low flow rates.[97] The smaller column diameter increases sample concentration on the column, improving sensitivity—particularly useful for low-dose components or trace degradants. Furthermore, micro-LC systems are inherently MS-friendly due to low flow rates that match electrospray ionization requirements.

Recent advances integrate UHPLC with core–shell or monolithic stationary phases, achieving unprecedented resolution and speed. The reduced analysis time—often under 2 minutes—significantly improves throughput while maintaining excellent reproducibility and robustness.[98] These innovations position micro- and UHPLC techniques as essential tools in next-generation pharmaceutical analysis.

6. Analytical Challenges and Limitations

6.1 Co-elution and Resolution Difficulties

Simultaneous RP-HPLC analysis of rosuvastatin and ezetimibe is inherently challenged by their sharply contrasting physicochemical properties. Rosuvastatin is hydrophilic and partially ionizable at low pH, whereas ezetimibe is highly lipophilic with weak UV absorbance—creating divergent retention patterns that complicate resolution.[99] Co-elution risks increase when mobile-phase pH drifts toward rosuvastatin's pKa, as incomplete ion suppression disrupts peak symmetry and decreases selectivity. This often results in overlapping peaks or tailing, especially in methods that use isocratic elution without optimized organic strength.[100]

Forced-degradation studies add further complexity, as degradation products of both drugs frequently exhibit retention times close to the parent compounds. Oxidative degradants of rosuvastatin and alkaline degradants of ezetimibe often generate chromatographic interference if method design does not adequately separate structurally similar impurities.[101] Even minor variations in buffer concentration or column batch characteristics may shift retention times enough to compromise peak resolution, demonstrating the critical need for robust method optimization during development.[102]

Resolution difficulties are also influenced by column aging and stationary-phase deactivation, which disproportionately affect hydrophilic analytes such as rosuvastatin. Inadequate end-capping or increased silanol activity may lead to secondary interactions that worsen peak shape. Thus, routine column performance verification is essential for sustaining long-term separation quality in simultaneous assays.[103]

6.2 Matrix Interference in Complex Formulations

Matrix interference presents a significant analytical challenge when quantifying rosuvastatin–ezetimibe combinations, particularly in formulations containing excipients with similar chromatographic or UV-absorptive characteristics. Lubricants, coating polymers, and surfactants may produce baseline disturbances or co-eluting peaks, affecting quantification accuracy.[104] This is especially problematic in generic formulations, where excipient grade and source may differ significantly from innovator products.

Tablet binders or disintegrants with residual UV absorbance at 230–250 nm can interfere with rosuvastatin's primary detection wavelength. Meanwhile, hydrophobic excipients tend to exhibit retention similar to ezetimibe, increasing the requirement for selective gradient programs. Inadequate sample preparation can further worsen matrix interference by allowing undissolved particulates or semi-soluble excipients to enter the chromatographic system, resulting in column fouling or ghost peaks.[105]

In dissolution studies, media containing surfactants such as sodium lauryl sulfate (SLS) or polysorbates may alter the chromatographic behavior of ezetimibe by modifying its solubility profile. This may shift retention times or alter peak intensity, thus complicating quantification unless the chromatographic conditions are carefully validated under dissolution-specific matrices.[106]

6.3 Sensitivity Constraints in Low-Dose Ezetimibe

Ezetimibe poses unique sensitivity challenges due to its weak chromophoric structure and relatively low therapeutic dose (commonly 10 mg), which necessitates detection at low concentrations. Its UV absorption is significantly weaker than rosuvastatin's, often resulting in higher LOD and LOQ values in UV-based detection systems.[107] This sensitivity gap complicates simultaneous quantification, as detection settings optimized for rosuvastatin may not produce adequate signal for ezetimibe.

Matrix suppression and inadequate solubility further reduce ezetimibe's detection response. Its lipophilic nature leads to incomplete dissolution during sample preparation if solvent strength is insufficient, causing variability in assay recovery. Additionally, insufficient organic phase during elution can broaden or diminish ezetimibe peaks, reducing signal-to-noise ratio and affecting LOQ precision.[108]

Modern approaches such as PDA detection, MS-compatible mobile phases, and UHPLC methods have helped improve sensitivity, but UV-only systems remain constrained by ezetimibe's intrinsic absorbance profile. Therefore, careful wavelength selection, injection volume adjustments, and sample concentration steps are often required to meet regulatory sensitivity criteria.

6.4 Stability-Indicating Requirements

Developing a stability-indicating RP-HPLC method presents significant challenges due to the differential degradation pathways of rosuvastatin and ezetimibe. Rosuvastatin undergoes rapid oxidative degradation, forming multiple related impurities that closely resemble its chromatographic behavior, while ezetimibe is highly susceptible to alkaline hydrolysis, generating degradation products with UV absorbance similar to the parent drug.[109] Achieving baseline separation from these impurities is essential for ICH-compliant stability testing.

Stress-testing conditions—acidic, alkaline, oxidative, thermal, and photolytic—often produce overlapping degradants if pH, buffer strength, or organic composition are not rigorously optimized. Stability-indicating methods must rely on PDA-based purity profiling and sometimes MS confirmation to ensure that peaks attributed to parent drugs are spectrally pure.[110] Without spectral verification, co-eluting degradants can falsely elevate assay results or distort impurity quantification.

Column selectivity is particularly important in stability studies. Hydrophilic degradants of rosuvastatin may elute very early, near the solvent front, while hydrophobic degradants of ezetimibe often elute very late. Balancing this broad polarity distribution requires method designs that incorporate either gradient programs or advanced stationary-phase chemistries to maintain discriminative separation.[111]

6.5 Variability in Method Reproducibility Across Laboratories

Reproducibility challenges arise when RP-HPLC methods are transferred across laboratories with differing instrument configurations, column lots, environmental conditions, or solvent grades. Small variations in pH—especially near rosuvastatin's pKa—can cause significant shifts in retention and peak symmetry, leading to inconsistent resolution between laboratories.[112] Column variability between manufacturers further contributes to alterations in selectivity, impacting reproducibility of system suitability parameters.

Differences in sample preparation techniques, such as sonication duration, filtration membrane type, and solvent purity, can also affect quantitative reproducibility. For ezetimibe, which is sensitive to solubility differences, inconsistent extraction conditions may produce fluctuating assay results across laboratories.[113] Laboratories using older HPLC systems may also experience pump pulsation, delayed gradient mixing, or detector noise that impact analytical precision.

Standardization through AQbD-based method development, detailed SOPs, and controlled MODR boundaries helps mitigate these issues, but reproducibility challenges remain significant in multi-site quality-control networks. Thus, robust method transfer protocols, stringent validation, and inter-laboratory cross-verification remain essential for maintaining method consistency throughout the pharmaceutical product lifecycle.[114]

7. Regulatory and Validation Perspectives

7.1 ICH Q2(R2) Guidelines for HPLC Method Validation

The updated ICH Q2(R2) guideline places a strong emphasis on systematic, science-based validation principles, requiring comprehensive demonstration of method performance across accuracy, precision, specificity, linearity, range, robustness, and detection capability.[115] For combination drug assays such as rosuvastatin–ezetimibe, specificity is of particular regulatory relevance, as both drugs exhibit distinct degradation pathways that necessitate clear chromatographic discrimination from related impurities. ICH Q2(R2) expands the scope of validation by integrating risk-based elements aligned with ICH Q14, promoting structured knowledge management and continuous method verification through lifecycle stages.[116]

Linearity and range must be validated with statistically justified calibration models, ensuring acceptable regression coefficients and residual patterns. Regulatory expectations now favor systematic assessment of analytical detection capability, including statistically supported LOD and LOQ values derived from signal-to-noise or standard deviation-based approaches.[117] Accuracy must be demonstrated through recovery studies across not less than three concentration levels, covering both analytes independently as well as in their combined matrix.

Precision requirements include repeatability and intermediate precision, with regulatory authorities expecting demonstration of method consistency across analysts, instruments, and days. In the context of combined formulations, validation must also account for potential interactions between analytes, excipients, and stress-generated impurities.[118]

7.2 System Suitability Parameters for Combined Drug Analysis

System suitability testing (SST) is considered a mandatory regulatory requirement to verify chromatographic system readiness before sample analysis. SST ensures that the chromatographic system consistently produces acceptable resolution, peak symmetry, and reproducibility for both rosuvastatin and ezetimibe.[119] Key SST parameters include retention time consistency, theoretical plate count, tailing factor, and percent relative standard deviation (%RSD) of replicate injections. Regulatory guidelines recommend %RSD $\leq$ 2% for peak area repeatability, ensuring stable detector and injector performance.[120]

Resolution (Rs) is a critical parameter in combined drug assays because both molecules can form degradants with closely related retention behaviors. Authorities expect Rs values $\geq$ 2.0 between both active peaks and adjacent impurities to ensure reliable quantification under routine and stressed conditions.[121] As ezetimibe has comparatively weak UV absorbance, peak-to-peak noise evaluation is also essential to confirm sufficient sensitivity and detector stability.

SST also extends to assessment of injection precision, detector linearity, and robustness of the column performance. Regulatory bodies increasingly emphasize column-to-column reproducibility and require demonstration that SST criteria remain stable across column batches and suppliers, reflecting advancements in regulatory scrutiny on chromatographic reproducibility.[122]

7.3 Method Robustness and Ruggedness Requirements

Robustness assessment is a core requirement under ICH guidelines, designed to evaluate the stability of chromatographic performance when subjected to small deliberate variations in method parameters. Parameters commonly explored include flow rate, detection wavelength, pH fluctuation of ±0.2 units, organic modifier composition, and column temperature adjustments.[123] Robustness is especially important in rosuvastatin–ezetimibe assays because small deviations in pH or organic strength can produce marked changes in retention and peak shape due to their contrasting hydrophilicity and lipophilicity.

Ruggedness, on the other hand, reflects method reproducibility under real-world conditions, including different analysts, instruments, column lots, laboratories, or environmental settings.[124] Regulatory expectations mandate that ruggedness data must demonstrate minimal variation across these conditions, ensuring suitability for global commercial QC operations. Because ezetimibe is sensitive to solubility and detection variability, ruggedness testing must include evaluation across different brands of solvents, buffer batches, and filtration media.[125]

Current regulatory trends also promote lifecycle-based continuous verification, where method performance—particularly robustness—is periodically reassessed as part of ongoing quality management. This aligns with ICH Q14, which encourages establishing a formal Method Operable Design Region (MODR) to ensure robust performance throughout the method lifecycle.[126]

7.4 Trends in Regulatory Expectations for Combination Drug Assays

Regulatory expectations for combination drug assays have evolved significantly with increased global scrutiny on chromatographic selectivity, stability-indicating performance, and lifecycle management principles. Authorities now expect simultaneous methods to demonstrate capability for identifying and separating individual drug components as well as their degradation products, impurities, and excipient-related interferences.[127] This is particularly relevant for fixed-dose combinations where analytes may degrade differently or interact with shared excipients.

There is also a growing preference for risk-based method development approaches that incorporate AQbD principles, allowing predictive control over method variability and greater transparency during regulatory submissions. Agencies increasingly expect detailed chromatographic justification—including column chemistry rationale, pH selection justification, and impurity resolution strategy—rather than simple method description.[128]

Finally, global regulators encourage the use of greener and MS-compatible chromatographic approaches wherever feasible, reflecting sustainability shifts and modernization of analytical science. Methods designed using these contemporary principles demonstrate improved acceptability during regulatory review and facilitate smoother post-approval method modifications.

8. Future Prospects

8.1 Scope for Automated and AI-Assisted Method Optimization

The future of chromatographic method development is positioned to benefit greatly from automated and AI-assisted tools capable of predicting optimal chromatographic conditions with high precision. Machine learning algorithms can analyze historical chromatographic data, predict retention behavior, and suggest ideal combinations of mobile-phase composition, pH, and stationary phase chemistry. Automated design platforms are expected to reduce development time dramatically by generating simulation-based chromatograms before physical experiments are conducted. These predictive systems will allow analysts to rapidly evaluate thousands of method permutations, improving method robustness and reproducibility while minimizing trial-and-error experimentation. AI integration is also anticipated to support continuous method performance monitoring, enabling real-time adjustment in QC environments.

8.2 Integration of Chemometrics for Method Development

Chemometrics will continue to expand its role in advanced RP-HPLC method development by enabling systematic data interpretation and optimization. Multivariate approaches such as PCA, PLS, response surface methodology, and cluster analysis help quantify the influence of multiple variables simultaneously, thereby improving understanding of complex interactions between mobile-phase composition, buffer strength, pH, flow rate, and temperature. Chemometric models not only streamline method

optimization but also strengthen regulatory justification by providing mathematically supported decision-making. These tools are becoming essential for developing multimodal chromatographic systems where selectivity differences must be carefully managed for structurally diverse analytes like rosuvastatin and ezetimibe.

8.3 Transition Towards UHPLC and Hyphenated Techniques

Future analytical workflows will increasingly rely on ultrahigh-performance liquid chromatography (UHPLC) due to its superior resolution, reduced analysis time, and enhanced sensitivity. The combination of UHPLC with advanced stationary phases—such as sub-2-µm particles, core–shell architectures, and monolithic columns—offers unparalleled separation efficiency that traditional HPLC systems cannot match. Hyphenated techniques such as LC–MS/MS, LC–NMR, and LC–FTIR will further expand analytical capability, enabling structural elucidation, impurity profiling, and trace-level quantification within a single workflow. These innovations are especially important for multi-component formulations where degradation pathways are complex and require advanced detection strategies.

8.4 Future of Multi-Component Lipid-Lowering Assays

As combination lipid-lowering therapies continue to evolve, analytical methods must adapt to accommodate emerging drug combinations and increasingly complex formulation matrices. Future assays will likely require higher sensitivity, broader selectivity, and enhanced capability to resolve structurally similar impurities and metabolites. The use of green chemistry principles, AI-driven method adaptation, and MS-compatible workflows will become standard practice. Additionally, next-generation lipid-lowering agents—including PCSK9 inhibitors, bempedoic acid combinations, and novel ezetimibe analogs—will demand robust, stability-indicating RP-HPLC or UHPLC methods that can manage diverse physicochemical behaviors. Ultimately, the future direction of analytical science will focus on harmonizing speed, sustainability, and precision while ensuring compliance with evolving global regulatory expectations.

9. Conclusion

The simultaneous RP-HPLC quantification of rosuvastatin and ezetimibe has evolved significantly over the past decade, transitioning from conventional long-run assays to highly optimized, robust, and regulatory-compliant analytical methodologies. Their inherently contrasting physicochemical properties—hydrophilic, ionizable rosuvastatin versus lipophilic, weakly chromophoric ezetimibe—have driven innovation in mobile-phase design, stationary-phase selection, and detection strategies. This review highlights that method success depends on careful integration of pH control, appropriate organic strength, and advanced column technologies to achieve consistent resolution, sensitivity, and stability-indicating capability.

Contemporary method development increasingly incorporates AQbD principles, chemometric modeling, and greener chromatographic practices, expanding both scientific understanding and regulatory acceptability. The growing use of UHPLC, core–shell particles, and MS-compatible workflows has further improved analytical throughput, selectivity, and structural elucidation potential. Despite ongoing challenges—such as co-elution risks, matrix effects, sensitivity limitations, and inter-laboratory variability—the field continues to move toward more robust, automated, and environmentally conscious solutions.

Overall, the advancement of RP-HPLC methodologies for rosuvastatin–ezetimibe combination analysis reflects the broader evolution of pharmaceutical analytics: toward faster, more reproducible, more informative, and more sustainable systems. Continued integration of AI-driven optimization, hyphenated techniques, and lifecycle-oriented regulatory frameworks will further elevate method performance and ensure readiness for emerging multi-component lipid-lowering therapies.

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	Author Name – Abhishek Kumar Srivastava Author Affiliation- M.pharm, Department of Pharmaceutical Chemistry Author Address/Institute Address- Advance Institute of Biotech and Paramedical Sciences.
	Author Name - Hridesh Singh Chauhan Author Affiliation- Associate Professor, Department of Pharmaceutical Chemistry Author Address/Institute Address- Advance Institute of Biotech and Paramedical Sciences.
	Author Name - Utkarsh Sharma Author Affiliation- Assistant professor, Department of Pharmacology Author Address/Institute Address- Advance Institute of Biotech and Paramedical Sciences.