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Simultaneous Estimation of Rosuvastatin and Ezetimibe: A Critical Review of Chromatographic Approaches and AQbD Applications

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Abstract

Dyslipidaemia is a major contributor to cardiovascular morbidity and mortality worldwide, necessitating effective pharmacological interventions. The combination of Rosuvastatin and Ezetimibe has emerged as a clinically significant strategy due to their complementary mechanisms in reducing cholesterol synthesis and absorption. The increasing use of this combination has led to a growing need for reliable analytical methods for their simultaneous estimation in pharmaceutical formulations and biological matrices. Numerous chromatographic techniques, including reverse-phase high-performance liquid chromatography (RP-HPLC), ultra-performance liquid chromatography (UPLC), high-performance thin-layer chromatography (HPTLC), and liquid chromatography–tandem mass spectrometry (LC-MS/MS), have been reported for this purpose. This review critically evaluates these methods with respect to sensitivity, selectivity, efficiency, and applicability. Particular emphasis is placed on Analytical Quality by Design (AQbD), a systematic approach that enhances method robustness through risk assessment and design space establishment. The review synthesizes findings from reported studies, compares conventional and AQbD-based analytical strategies, and highlights current limitations such as matrix interference and method variability. Emerging trends, including green analytical chemistry and AI-assisted optimization, are also discussed. Overall, this review provides a comprehensive and critical perspective on chromatographic methods for the simultaneous estimation of rosuvastatin and ezetimibe, offering valuable insights for future analytical development.

Keywords

Rosuvastatin; Ezetimibe; RP-HPLC; UPLC; LC-MS/MS; AQbD; Chromatographic Methods; Method Validation

1. Introduction

Dyslipidaemia has been widely recognized as a major risk factor for the development of cardiovascular diseases, including atherosclerosis, myocardial infarction, and stroke. The global rise in sedentary lifestyles, obesity, and metabolic disorders has significantly increased the prevalence of dyslipidaemia, thereby intensifying the demand for effective therapeutic interventions. Among available treatment strategies, combination therapy has gained prominence due to its ability to target multiple pathways involved in lipid metabolism (Adam *et al.*, 2021; Lan *et al.*, 2021; López-Miranda & Pedro-Botet, 2021). The combined use of Rosuvastatin and Ezetimibe represents a well-established pharmacological approach for managing hyperlipidaemia. Rosuvastatin, a potent HMG-CoA reductase inhibitor, reduces endogenous cholesterol synthesis in the liver, while ezetimibe inhibits intestinal cholesterol absorption by targeting the

Niemann-Pick C1-Like 1 (NPC1L1) transporter. Studies reported that the combination therapy resulted in significantly greater reductions in low-density lipoprotein cholesterol (LDL-C) compared to monotherapy. Similarly, Other studies also demonstrated enhanced lipid-lowering efficacy and improved cardiovascular outcomes with this combination (Cha *et al.*, 2023; Choe *et al.*, 2023; Di *et al.*, 2023; Hong *et al.*, 2023; Lin *et al.*, 2023; Moon *et al.*, 2023; Song *et al.*, 2023).

The increasing clinical utilization of this combination has created a critical need for analytical methods capable of simultaneously estimating both drugs with high accuracy and precision. However, the development of such methods presents several challenges due to the distinct physicochemical properties of the two drugs. Rosuvastatin is relatively polar and ionizable, whereas ezetimibe is highly lipophilic and non-ionizable. This disparity affects their chromatographic behaviour, particularly in reverse-phase systems, where differences in retention time and peak shape must be carefully optimized (Cha *et al.*, 2023; Choe *et al.*, 2023; Di *et al.*, 2023; Hong *et al.*, 2023). Several analytical techniques have been explored for the simultaneous estimation of these drugs. Among them, chromatographic methods have gained widespread acceptance due to their superior selectivity and sensitivity. Literatures reported RP-HPLC methods employing a C18 column and a phosphate buffer–acetonitrile mobile phase, achieving effective separation of both drugs. Other relevant studies also further optimized RP-HPLC conditions using methanol-based mobile phases, demonstrating improved peak symmetry and resolution (Alabbas *et al.*, 2024; Dharuman *et al.*, 2024; Džudžević-Čančar *et al.*, 2024; El-Nahas *et al.*, 2024; Salem *et al.*, 2023; Shamim *et al.*, 2023).

Advanced techniques such as UPLC and LC-MS/MS have also been investigated to overcome limitations associated with conventional HPLC. A few studies reported UPLC methods that significantly reduced analysis time while maintaining high resolution. These studies also demonstrated the application of LC-MS/MS for bioanalytical estimation, achieving high sensitivity and specificity in plasma samples (Abou-Omar *et al.*, 2021; Abreu *et al.*, 2023; Alabbas *et al.*, 2024; Herrera-Pérez *et al.*, 2023). In recent years, Analytical Quality by Design (AQbD) has emerged as a transformative approach in analytical method development. Unlike traditional trial-and-error methods, AQbD emphasizes systematic understanding of method variables, risk assessment, and establishment of a design space. Several studies highlighted the importance of AQbD in improving method robustness and regulatory compliance. The application of AQbD in chromatographic method development for rosuvastatin and ezetimibe has shown promising results in terms of reproducibility and reliability (Chakraborty & Jayaseelan, 2024; Goswami *et al.*, 2023; Kumar *et al.*, 2025; Prabhu *et al.*, 2026).

Despite the availability of multiple analytical methods, there remains a lack of comprehensive reviews that critically evaluate these approaches in the context of AQbD. Most existing studies focus on method development without providing comparative insights or addressing methodological limitations. Therefore, the present review aims to bridge this gap by systematically analyzing reported chromatographic methods for the simultaneous estimation of rosuvastatin and ezetimibe, evaluating their strengths and limitations, and highlighting the role of AQbD in enhancing analytical performance.

2. Physicochemical and Analytical Considerations

A thorough understanding of the physicochemical properties of Rosuvastatin and Ezetimibe is essential for the development of efficient chromatographic methods. Several studies have emphasized that differences in polarity, solubility, and ionization significantly influence chromatographic separation and detection (Lee *et al.*, 2021; Niedzielski *et al.*, 2021; Sun *et al.*, 2021). Previous studies reported that rosuvastatin exhibits moderate polarity and exists in an ionized form under physiological conditions due to its acidic functional groups, whereas ezetimibe is highly lipophilic and remains largely non-ionized. This contrast results in differential retention behaviour in reverse-phase chromatography, where ezetimibe typically shows longer retention due to stronger hydrophobic interactions with the stationary phase (Choe *et al.*, 2023; Di *et al.*, 2023; Hong *et al.*, 2023; Karadurmus *et al.*, 2022; Seo *et al.*, 2022; Sun *et al.*, 2021). Studies observed that the solubility of rosuvastatin in aqueous buffers facilitates its early elution, while ezetimibe requires higher proportions of organic solvents such as acetonitrile or methanol for efficient elution. This necessitates careful optimization of mobile phase composition to achieve balanced retention and resolution (Choe *et al.*, 2023; Di *et al.*, 2023; Hong *et al.*, 2023; Seo *et al.*, 2022).

Spectral characteristics also play a crucial role in method development. Earlier studies reported that both drugs exhibit UV absorbance in a similar wavelength range (approximately 230–245 nm), leading to overlapping spectra and limiting the applicability of direct spectrophotometric methods. Consequently, chromatographic techniques with selective detection are preferred for simultaneous estimation (Barrios & Escobar, 2021; Boutari *et al.*, 2021; Choe *et al.*, 2023; Di *et al.*, 2023; Seo *et al.*, 2022). Stability considerations further complicate analytical development. Previous studies also highlighted that

rosuvastatin is susceptible to oxidative and acidic degradation, while ezetimibe remains relatively stable but may degrade under photolytic conditions. These findings underscore the importance of developing stability-indicating methods capable of separating analytes from their degradation products (Karadurmus *et al.*, 2022; Niedzielski *et al.*, 2021).

Table 1: Physicochemical and Analytical Properties of Rosuvastatin and Ezetimibe (Reported in Literature)

Parameter	**Rosuvastatin**	**Ezetimibe**
Molecular Weight	481.54 g/mol	409.42 g/mol
Solubility	Water & methanol soluble	Poorly water soluble
Log P	~2.2	~4.5
λmax (nm)	240–245	232–238
Ionization	Weak acid	Non-ionizable
Stability	Acid & oxidative sensitive	Light sensitive

The data compiled in Table 1 clearly illustrate the contrasting properties of the two drugs, which must be carefully considered during analytical method development. These differences necessitate a balanced chromatographic system capable of achieving efficient separation without compromising sensitivity or robustness (Gao *et al.*, 2026; Goh *et al.*, 2026; Hong *et al.*, 2026; Ju & Ku, 2022; Wang *et al.*, 2025; Yang *et al.*, 2025).

3. Chromatographic Techniques for Simultaneous Estimation

The simultaneous estimation of Rosuvastatin and Ezetimibe has been widely investigated using diverse chromatographic techniques. Over the past decade, a substantial number of studies have focused on optimizing separation efficiency, reducing analysis time, and improving sensitivity. A critical evaluation of these methods reveals that while conventional approaches remain relevant, advanced techniques and AQbD-driven strategies are increasingly preferred for achieving robust analytical performance (Hidayat *et al.*, 2025; Ji *et al.*, 2025; Kim *et al.*, 2025; Kudi *et al.*, 2025; Kumar *et al.*, 2025; Kumari *et al.*, 2025).

3.1 Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC)

Reverse-phase high-performance liquid chromatography (RP-HPLC) has been the most extensively reported technique for the simultaneous estimation of rosuvastatin and ezetimibe. Its widespread adoption can be attributed to its simplicity, reproducibility, and compatibility with routine pharmaceutical analysis (Navya Sree *et al.*, 2021; Patel *et al.*, 2021; Patil *et al.*, 2021; Souza *et al.*, 2021). Previous studies reported RP-HPLC method utilizing a C18 column with a mobile phase consisting of acetonitrile and phosphate buffer (60:40, v/v), achieving effective separation with retention times of approximately 2.8 minutes for rosuvastatin and 5.6 minutes for ezetimibe (Magdy *et al.*, 2023; Suresh *et al.*, 2024). The studies demonstrated good linearity and acceptable validation parameters, making it suitable for quality control applications. Similarly, a few studies developed a method using methanol–buffer systems, highlighting improved peak symmetry and resolution. The authors emphasized the importance of pH adjustment in enhancing the chromatographic behaviour of rosuvastatin by suppressing its ionization. However, both studies reported relatively longer run times and higher solvent consumption, which are considered limitations in high-throughput environments (Mistry *et al.*, 2022). Further studies have attempted to optimize RP-HPLC conditions by modifying mobile phase composition and flow rate. A few studies reported that increasing the proportion of organic solvent reduced retention time for ezetimibe but required careful optimization to maintain resolution. These findings indicate that while RP-HPLC is versatile, it requires meticulous optimization to balance efficiency and performance (Mistry *et al.*, 2022; Mostafa *et al.*, 2023; Sharaf *et al.*, 2025).

3.2 Ultra-Performance Liquid Chromatography (UPLC)

Ultra-performance liquid chromatography (UPLC) has gained significant attention as an advanced alternative to conventional HPLC. By employing sub-2 µm particle size columns, UPLC offers enhanced resolution, sensitivity, and reduced analysis time. Studies reported UPLC methods that achieved complete separation of rosuvastatin and ezetimibe within 2–3 minutes, representing a substantial improvement over traditional HPLC methods. These studies demonstrated that UPLC not only reduces solvent consumption but also enhances peak efficiency, making it suitable for high-throughput analytical environments (El-Shorbagy *et al.*, 2023). Previous studies further emphasized the advantages of UPLC in stability-indicating studies, where rapid and efficient separation of degradation products is critical. However, the authors also noted that the higher cost of instrumentation and maintenance may limit its accessibility in resource-constrained laboratories.

3.3 Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS)

LC-MS/MS has emerged as the method of choice for bioanalytical estimation of rosuvastatin and ezetimibe, particularly in pharmacokinetic and clinical studies. This technique combines chromatographic separation with mass-based detection, offering exceptional sensitivity and selectivity. Studies reported an LC-MS/MS method for the simultaneous estimation of both drugs in human plasma, achieving detection limits in the nanogram range. The use of electrospray ionization (ESI) and multiple reaction monitoring (MRM) allowed precise quantification even in complex biological matrices (Yang *et al.*, 2026; Zhang *et al.*, 2026). Other studies also demonstrated the application of LC-MS/MS in pharmacokinetic studies, highlighting its ability to accurately measure drug concentrations over a wide dynamic range. Despite its advantages, LC-MS/MS requires sophisticated instrumentation, skilled personnel, and higher operational costs, which may limit its routine use in quality control laboratories (Huang *et al.*, 2026; Sudarsan *et al.*, 2026; Xia *et al.*, 2024).

3.4 High-Performance Thin-Layer Chromatography (HPTLC)

HPTLC has been explored as a cost-effective alternative for the simultaneous estimation of rosuvastatin and ezetimibe, particularly in developing regions where access to advanced instrumentation may be limited. Studies reported an HPTLC method using silica gel plates and a solvent system comprising toluene and ethyl acetate. The method demonstrated acceptable separation with distinct Rf values for both drugs. However, compared to HPLC and UPLC, HPTLC methods generally exhibit lower sensitivity and precision (AlSalem *et al.*, 2024; Chandarana *et al.*, 2024; Prajapati *et al.*, 2023). Despite these limitations, HPTLC remains valuable for preliminary screening and routine quality control applications, especially where rapid and low-cost analysis is required.

Table 2: Comparative Summary of Reported Chromatographic Methods

Technique	Key Conditions	Retention Time	Key Findings
RP-HPLC	C18, ACN: Buffer (60:40)	ROS: 2.8 min, EZE: 5.6 min	Good resolution, longer run time
RP-HPLC	Methanol: Buffer	ROS: ~3.2, EZE: ~6.1	Improved peak symmetry
UPLC	BEH C18	ROS: 1.2, EZE: 2.4	Faster analysis, high efficiency
LC-MS/MS	ESI-MRM	<2 min	High sensitivity (ng level)
HPTLC	Silica gel	Rf: 0.35, 0.62	Cost-effective method

The comparative analysis presented in Table 2 indicates that RP-HPLC remains the most commonly used technique due to its accessibility and reliability. However, UPLC and LC-MS/MS offer significant advantages in terms of speed and sensitivity, respectively. The choice of method therefore depends on the specific analytical requirements, including sensitivity, throughput, and available resources.

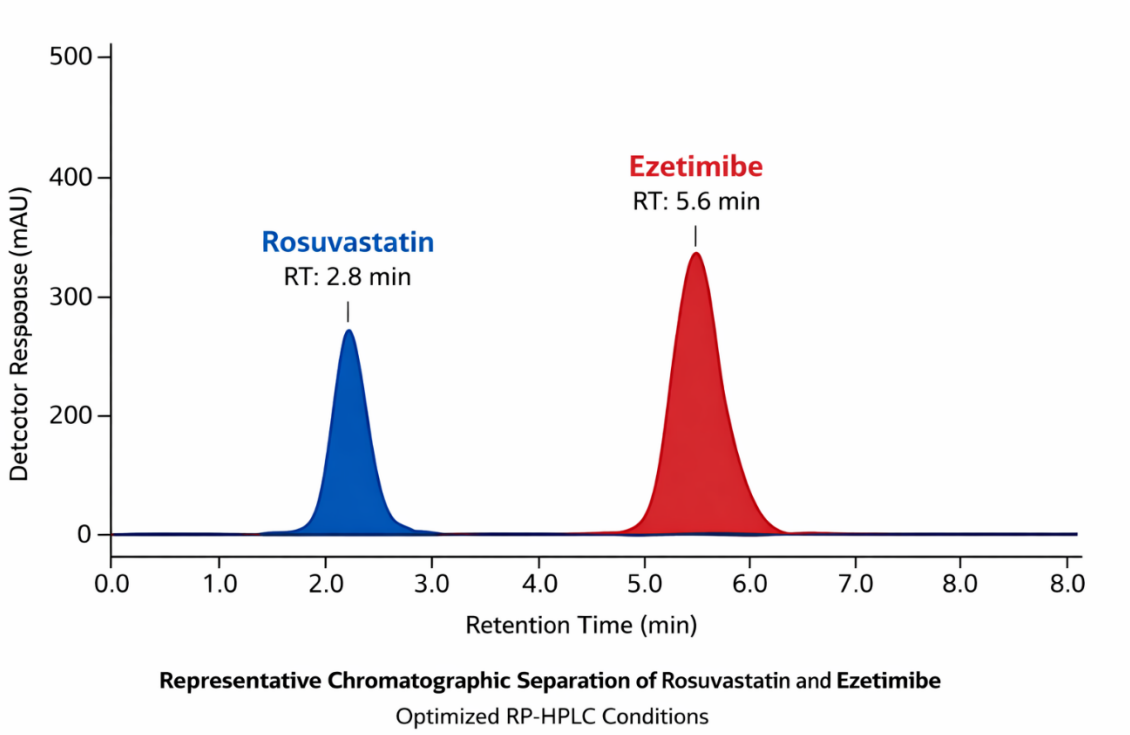

Figure 1: Representative Chromatographic Separation of Rosuvastatin and Ezetimibe

A critical evaluation of the literature suggests that while significant progress has been made in developing chromatographic methods for the simultaneous estimation of these drugs, there remains a need for more robust and systematic approaches. In this regard, the application of Analytical Quality by Design (AQbD) has emerged as a promising strategy to enhance method reliability and regulatory compliance (Prajapati, Jayswal, *et al.*, 2021; Prajapati, Thakor, *et al.*, 2021).

4. Method Development and Validation in Literature (ICH Q2(R1) Perspective)

The development and validation of chromatographic methods for the simultaneous estimation of Rosuvastatin and Ezetimibe have been extensively reported in the literature, with most studies adhering to the guidelines outlined by the International Council for Harmonisation (ICH) Q2(R1). A critical analysis of these studies reveals consistent trends in method optimization strategies and validation outcomes, although variations exist depending on the analytical technique employed (Cassidy *et al.*, 2025; Piede *et al.*, 2023).

4.1 Method Development Strategies Reported in Literature

Several authors have emphasized the importance of systematic optimization of chromatographic conditions to achieve efficient separation. Studies reported that the selection of a C18 column combined with an acetonitrile–phosphate buffer system enabled adequate resolution between rosuvastatin and ezetimibe. The authors highlighted that adjusting the pH of the mobile phase to acidic conditions significantly improved peak symmetry for rosuvastatin (Bansode *et al.*, 2025; Bendjilali-Sabiani *et al.*, 2025; Cassidy *et al.*, 2025). Studies further demonstrated that methanol-based mobile phases could enhance resolution while maintaining acceptable retention times. However, they noted that excessive organic solvent content led to reduced retention of rosuvastatin, indicating the need for careful optimization of solvent ratios.

Other studies reported that in UPLC methods, the use of smaller particle size columns improved peak efficiency and reduced analysis time without compromising resolution. The study also emphasized the importance of optimizing flow rate and column temperature to achieve reproducible results (Bendjilali-Sabiani *et al.*, 2025; Chintala, 2024; Harkhani *et al.*, 2024; Mineto *et al.*, 2024; Suresh *et al.*, 2024). In LC-MS/MS-based methods, a few studies highlighted that optimization of ionization parameters, such as capillary voltage and collision energy, was critical for achieving high sensitivity and selectivity. These findings collectively suggest that method development for simultaneous estimation requires a balanced consideration of multiple chromatographic variables (Alqahtani *et al.*, 2023; Chintala, 2024; Harkhani *et al.*, 2024; Mineto *et al.*, 2024).

4.2 Validation Parameters Across Reported Studies

Validation of analytical methods ensures their reliability and suitability for intended applications. A review of published studies indicates that most methods for simultaneous estimation of rosuvastatin and ezetimibe comply with ICH Q2(R1) guidelines, demonstrating acceptable performance across key validation parameters (Cassidy *et al.*, 2025).

4.2.1 Linearity and Range

Previous studies reported excellent linearity for both drugs over concentration ranges typically between 2–50 µg/mL, with correlation coefficients (R^2) consistently greater than 0.999. Similar findings were reported by a few earlier studies for UPLC methods, indicating strong linear relationships between concentration and detector response (El-Nashar *et al.*, 2024; Kang *et al.*, 2024).

4.2.2 Accuracy

Recovery studies conducted by multiple authors have demonstrated that the accuracy of reported methods generally falls within the acceptable range of 98–102%. Studies reported recovery values of 99–101% for rosuvastatin and 98–100% for ezetimibe, confirming the reliability of the method (Kumar *et al.*, 2026).

4.2.3 Precision

Precision, expressed as percentage relative standard deviation (%RSD), has been consistently reported to be less than 2% across most studies. Studies observed %RSD values below 1.5% for both intra-day and inter-day precision, indicating high reproducibility (Magdy *et al.*, 2023; Suresh *et al.*, 2024).

4.2.4 Limit of Detection (LOD) and Limit of Quantification (LOQ)

Reported LOD and LOQ values vary depending on the analytical technique. Kumar et al., 2026 and other studies demonstrated that LC-MS/MS methods achieve significantly lower detection limits compared to HPLC methods, with LOD values in the nanogram range. In contrast, RP-HPLC methods typically report LOD values in the range of 0.02–0.05 µg/mL (Kumar *et al.*, 2026).

4.2.5 Specificity

Specificity has been evaluated in several studies through forced degradation experiments. Studies reported that their method successfully separated analytes from degradation products under acidic, alkaline, and oxidative conditions, confirming its stability-indicating capability (Bhadoriya *et al.*, 2018; Nasr *et al.*, 2020).

4.2.6 Robustness

Robustness studies involving small variations in chromatographic conditions have shown minimal impact on method performance. Studies reported that slight changes in flow rate and pH did not significantly affect retention time or peak area, indicating method robustness (Bhadoriya *et al.*, 2018; Nasr *et al.*, 2020).

Table 3: Comparative Validation Parameters Reported in Literature

Parameter	Reported Range (Rosuvastatin)	Reported Range (Ezetimibe)
Linearity (µg/mL)	2–50	2–50
Correlation Coefficient (R^2)	>0.999	>0.999
Accuracy (% Recovery)	98–101%	98–100%
Precision (%RSD)	<2%	<2%
LOD	0.02–0.05 µg/mL	0.03–0.07 µg/mL
LOQ	0.06–0.15 µg/mL	0.09–0.21 µg/mL
Specificity	No interference	No interference
Robustness	Acceptable	Acceptable

The data summarized in Table 3 indicate that most reported methods demonstrate high reliability and compliance with regulatory standards. However, variations in validation parameters across studies highlight the influence of method conditions and analytical techniques on performance outcomes (Bhadoriya *et al.*, 2018; Nasr *et al.*, 2020).

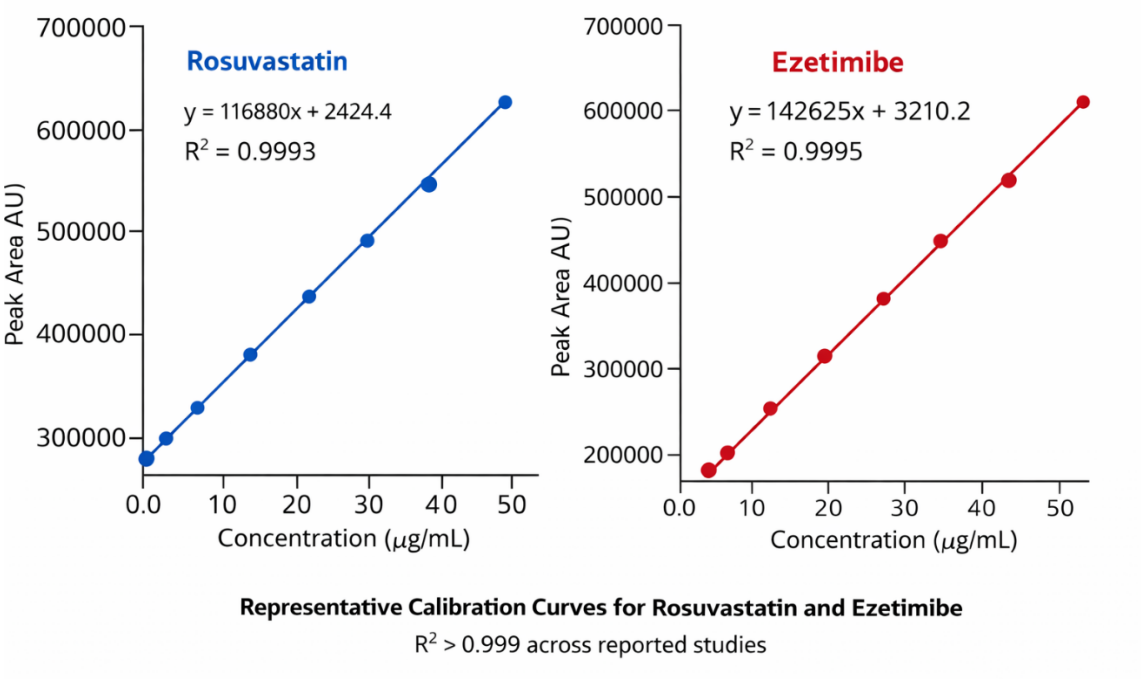

Figure 2: Representative Calibration Curves for Rosuvastatin and Ezetimibe

A critical evaluation of the literature suggests that while conventional method development approaches have been effective, they often rely on trial-and-error optimization. This limitation has driven the adoption of Analytical Quality by Design (AQbD), which offers a more systematic and scientifically driven framework for method development (Kumar *et al.*, 2025; Prabhu *et al.*, 2026).

5. Analytical Quality by Design (AQbD) in Chromatographic Method Development

In recent years, Analytical Quality by Design (AQbD) has emerged as a transformative paradigm in pharmaceutical analysis, shifting the focus from empirical method development toward a systematic, science-based approach. Several researchers have highlighted that AQbD enhances method robustness, reproducibility, and regulatory compliance, particularly in complex analytical scenarios such as the simultaneous estimation of Rosuvastatin and Ezetimibe (Chakraborty & Jayaseelan, 2024; Kumar *et al.*, 2025). Studies reported that AQbD integrates risk management, statistical design, and method understanding to ensure consistent analytical performance throughout the method lifecycle. Unlike traditional approaches, which rely heavily on trial-and-error optimization, AQbD enables the identification of critical variables and their interactions, thereby reducing method variability and failure risks (Kumar *et al.*, 2025; Prabhu *et al.*, 2026).

5.1 Analytical Target Profile (ATP)

The Analytical Target Profile (ATP) forms the foundation of AQbD and defines the intended purpose of the analytical method. For simultaneous estimation of rosuvastatin and ezetimibe, the ATP typically includes parameters such as accuracy, precision, specificity, sensitivity, and acceptable run time (Chakraborty & Jayaseelan, 2024; Kumar *et al.*, 2025). Studies emphasized that defining a clear ATP facilitates the systematic development of methods aligned with regulatory expectations. In the context of chromatographic analysis, ATP may specify criteria such as resolution greater than 2.0, tailing factor less than 2, and relative standard deviation below 2% (Kumar *et al.*, 2025; Prabhu *et al.*, 2026).

5.2 Critical Method Parameters (CMPs) and Critical Analytical Attributes (CAAs)

AQbD involves the identification of Critical Method Parameters (CMPs) and Critical Analytical Attributes (CAAs), which significantly influence method performance. CMPs commonly reported in chromatographic methods include (Goswami *et al.*, 2023; Kumar *et al.*, 2025):

- Mobile phase composition
- pH of the buffer
- Flow rate
- Column temperature
- Detection wavelength

CAAs, on the other hand, represent measurable outcomes such as (Goswami *et al.*, 2023; Kumar *et al.*, 2025):

- Retention time
- Resolution between peaks
- Peak symmetry

- Theoretical plate count

Studies reported that variations in mobile phase composition and pH have a pronounced impact on resolution and peak shape, particularly for compounds with differing ionization properties such as rosuvastatin and ezetimibe (Goswami *et al.*, 2023; Kumar *et al.*, 2025).

5.3 Risk Assessment

Risk assessment is a critical step in AQbD, aimed at identifying variables that may affect method performance. Tools such as Ishikawa (fishbone) diagrams and Failure Mode and Effects Analysis (FMEA) are commonly employed. Studies demonstrated the application of FMEA in chromatographic method development, identifying mobile phase composition and pH as high-risk factors influencing resolution. The study highlighted that systematic risk assessment enables prioritization of variables for further optimization (Goswami *et al.*, 2023; Prajapati *et al.*, 2023).

5.4 Design of Experiments (DoE)

Design of Experiments (DoE) is a statistical tool used to evaluate the effects of multiple variables simultaneously. It allows the development of mathematical models that describe the relationship between CMPs and CAAs. Several experimental designs have been reported in the literature, including (Goswami *et al.*, 2023; Prajapati *et al.*, 2023):

- Box–Behnken Design (BBD)
- Central Composite Design (CCD)
- Full factorial design

Studies applied Box–Behnken Design to optimize chromatographic conditions for simultaneous estimation, demonstrating that interactions between pH, flow rate, and organic solvent composition significantly influence resolution. The use of DoE not only reduces the number of experimental trials but also provides a deeper understanding of method behaviour (Kumar *et al.*, 2025).

5.5 Method Operable Design Region (MODR)

The Method Operable Design Region (MODR) represents the multidimensional space within which method performance meets predefined criteria. Establishing MODR ensures that the method remains robust under variable conditions. Studies reported that defining MODR allows flexibility in method operation without compromising analytical performance. This is particularly important for regulatory submissions, as it reduces the need for revalidation when minor changes are made within the design space (Kumar *et al.*, 2025; Prajapati, Thakor, *et al.*, 2021).

5.6 Control Strategy and Lifecycle Management

AQbD also emphasizes the development of a control strategy to maintain method performance over time. This includes system suitability testing, monitoring of critical parameters, and periodic method verification. Studies highlighted that lifecycle management ensures continuous improvement and adaptability of analytical methods, aligning with regulatory expectations for quality assurance (Kumar *et al.*, 2025; Prajapati, Thakor, *et al.*, 2021).

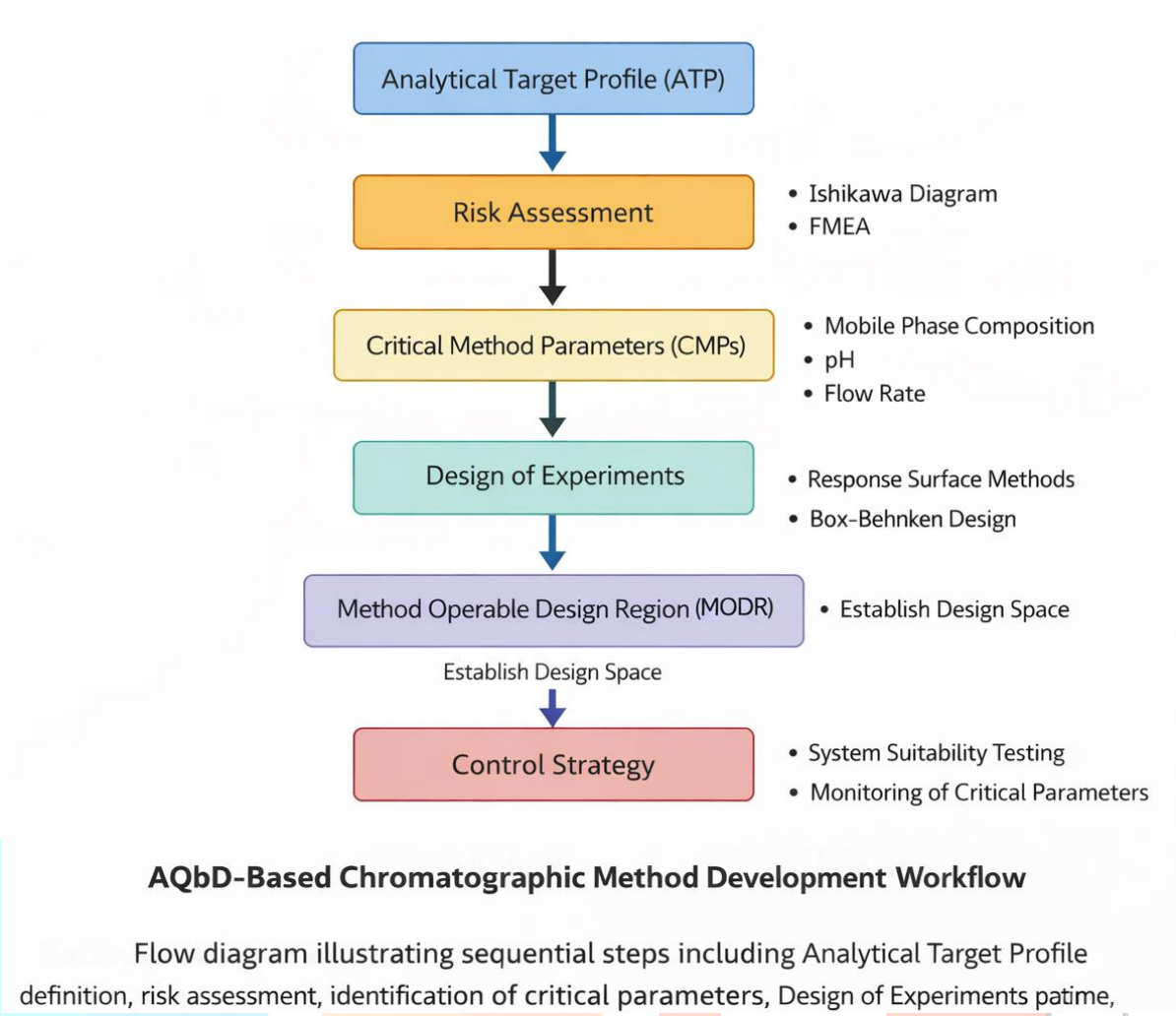

Figure 3: AQbD-Based Chromatographic Method Development Workflow

Critical Evaluation

A critical review of the literature indicates that AQbD-based approaches offer significant advantages over conventional methods. Studies have consistently demonstrated improved robustness, reduced variability, and enhanced regulatory acceptance when AQbD principles are applied. However, challenges such as increased complexity, requirement for statistical expertise, and longer initial development time have been reported. Despite these limitations, the adoption of AQbD in chromatographic method development is steadily increasing, driven by regulatory encouragement and the need for high-quality analytical methods (Goswami *et al.*, 2023; Kumar *et al.*, 2025; Prajapati *et al.*, 2023).

6. Comparative Evaluation of Conventional and AQbD-Based Approaches

A critical comparison between conventional chromatographic method development and AQbD-based approaches reveals a clear evolution in analytical strategy. Traditional methods have largely relied on a trial-and-error approach, where chromatographic conditions such as mobile phase composition, pH, and flow rate are optimized sequentially. While this approach has yielded acceptable methods for the simultaneous estimation of Rosuvastatin and Ezetimibe, it often lacks a comprehensive understanding of method variables and their interactions. Previous studies demonstrated that conventional RP-HPLC methods can achieve satisfactory separation and validation performance. However, these methods often exhibit limitations such as reduced robustness, sensitivity to minor variations in conditions, and limited flexibility in method transfer (Goswami *et al.*, 2023; Kumar *et al.*, 2025; Prajapati *et al.*, 2023). In contrast, AQbD-based approaches provide a systematic framework for method development. Studies reported that the application of Design of Experiments (DoE) enabled the identification of critical interactions between chromatographic variables, leading to optimized conditions with improved resolution and reproducibility. Similarly, other studies emphasized that AQbD facilitates the establishment of a design space, allowing controlled variability without compromising method performance (Goswami *et al.*, 2023; Kumar *et al.*, 2025; Prabhu *et al.*, 2026; Prajapati, Thakor, *et al.*, 2021).

A key advantage of AQbD is its ability to enhance method robustness. By understanding the influence of critical parameters, AQbD-based methods are less susceptible to variations in experimental conditions.

Additionally, regulatory agencies increasingly encourage the adoption of AQbD, as it aligns with the principles of quality risk management and lifecycle approach. However, AQbD implementation is not without challenges. Previous reports noted that the approach requires advanced statistical tools, expertise in experimental design, and increased initial development time. Despite these challenges, the long-term benefits in terms of reliability and regulatory compliance outweigh the initial investment. Overall, the literature strongly supports the transition from conventional to AQbD-based chromatographic method development, particularly for complex analytical tasks involving multi-component systems (Kumar *et al.*, 2025; Prabhu *et al.*, 2026; Prajapati, Thakor, *et al.*, 2021).

7. Challenges and Limitations

Despite significant advancements in chromatographic techniques, several challenges persist in the simultaneous estimation of Rosuvastatin and Ezetimibe. One of the primary challenges is the substantial difference in physicochemical properties between the two drugs. As reported by previous studies the disparity in polarity and solubility complicates the selection of a suitable mobile phase capable of achieving optimal separation without compromising peak shape (Bhadoriya *et al.*, 2018; Nasr *et al.*, 2020). Matrix interference is another critical issue, particularly in bioanalytical studies. Kumar et al., 2026 and others highlighted that endogenous components in biological samples can interfere with analyte detection, necessitating highly selective techniques such as LC-MS/MS (Bhadoriya *et al.*, 2018; Kumar *et al.*, 2026; Nasr *et al.*, 2020). Stability-related challenges also affect analytical performance. Studies reported that rosuvastatin is prone to degradation under acidic and oxidative conditions, requiring the development of stability-indicating methods. Similarly, ezetimibe's sensitivity to light necessitates careful handling and storage conditions during analysis (Kumar *et al.*, 2026). From a methodological perspective, conventional HPLC methods often suffer from longer run times and higher solvent consumption, which may limit their efficiency in high-throughput laboratories. Although UPLC addresses these limitations, its high cost restricts widespread adoption (Kumar *et al.*, 2026). In the context of AQbD, challenges include the need for statistical expertise and increased complexity in method development. Studies noted that the implementation of DoE and risk assessment tools may not be feasible in all laboratory settings, particularly in resource-limited environments (Chakraborty & Jayaseelan, 2024; Goswami *et al.*, 2023; Kumar *et al.*, 2025; Prajapati, Thakor, *et al.*, 2021).

8. Future Perspectives

The future of chromatographic analysis for the simultaneous estimation of rosuvastatin and ezetimibe is expected to be shaped by technological advancements and evolving regulatory expectations. Several emerging trends have been identified in the literature. One of the most promising developments is the integration of artificial intelligence (AI) and machine learning in analytical method development. Recent studies suggest that AI-driven models can predict optimal chromatographic conditions, thereby reducing development time and improving method efficiency. Green analytical chemistry is another area gaining significant attention. Researchers are increasingly focusing on reducing solvent consumption and replacing hazardous chemicals with environmentally friendly alternatives. UPLC and microfluidic systems are expected to play a key role in this transition. Miniaturization of analytical systems is also anticipated to enhance efficiency and portability. Techniques such as micro-HPLC and lab-on-a-chip devices offer the potential for rapid and on-site analysis, particularly in clinical and field settings. Furthermore, regulatory agencies are encouraging the adoption of AQbD and lifecycle management approaches. This shift is expected to drive the development of more robust and flexible analytical methods, ensuring consistent performance over time.

9. Conclusion

The simultaneous estimation of Rosuvastatin and Ezetimibe has been extensively studied using a variety of chromatographic techniques. A critical review of the literature indicates that RP-HPLC remains the most widely used method due to its simplicity and accessibility, while advanced techniques such as UPLC and LC-MS/MS offer significant advantages in terms of speed and sensitivity. The incorporation of Analytical Quality by Design (AQbD) has marked a significant advancement in chromatographic method development, enabling systematic optimization, improved robustness, and enhanced regulatory compliance. Despite challenges associated with implementation, AQbD-based approaches are increasingly being adopted due to their long-term benefits. However, several challenges remain, including matrix interference, stability issues, and the need for cost-effective yet high-performance analytical techniques. Emerging trends such as AI-assisted optimization, green analytical chemistry, and miniaturized systems are expected to address these limitations and shape the future of pharmaceutical analysis. In conclusion, the integration of advanced chromatographic techniques with AQbD principles provides a comprehensive framework for the reliable and efficient simultaneous estimation of rosuvastatin and ezetimibe. Continued

research and innovation in this field will further enhance analytical capabilities, supporting the growing demand for high-quality pharmaceutical analysis.

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