RESEARCH PAPER

Development and Validation of an RP-HPLC Method for Simultaneous Estimation of Rosuvastatin and Ezetimibe in Solid Dosage Form Using Analytical Quality by Design (AQbD) Approach

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Abstract

Background: Hyperlipidemia is a major risk factor for cardiovascular disease, and the combination therapy of Rosuvastatin and Ezetimibe provides synergistic lipid-lowering benefits. Reliable analytical methods are required to ensure quality and stability of fixed-dose formulations. **Objective:** The present study aimed to develop and validate a simple, precise, and robust RP-HPLC method for simultaneous estimation of Rosuvastatin and Ezetimibe in solid dosage forms using an Analytical Quality by Design (AQbD) approach. **Methods:** Preliminary trials were performed to evaluate the effect of mobile phase composition, flow rate, and pH on chromatographic behavior. Critical method parameters were optimized using a Box–Behnken design, and the design space was established through ANOVA, 3D response surface plots, and overlay contour plots. The optimized chromatographic conditions consisted of acetonitrile:water (70:30, v/v) as mobile phase, a flow rate of 1.0 mL/min, and UV detection at 234 nm. Method validation was conducted according to ICH Q2(R1) guidelines. **Results:** The method exhibited excellent linearity over the range of 5–20 µg/mL with correlation coefficients above 0.999 for both drugs. Accuracy studies showed recoveries within 98–102%, while precision results demonstrated %RSD values less than 2.0% for repeatability and intermediate precision. Robustness studies confirmed the resilience of the method to minor variations, and LOD/LOQ values of 0.15/0.45 µg/mL (Ezetimibe) and 0.18/0.55 µg/mL (Rosuvastatin) confirmed high sensitivity. Forced degradation studies established the method as stability-indicating. **Conclusion:** The validated AQbD-based RP-HPLC method is reliable, accurate, and robust for routine quality control and stability testing of Rosuvastatin–Ezetimibe solid dosage formulations, fulfilling regulatory and industry requirements.

Keywords: Rosuvastatin, Ezetimibe, RP-HPLC, Analytical Quality by Design (AQbD), Method validation, Hyperlipidemia.

How to cite this article: Kumar S, Yedlapalli G, Latha DS, Guntupalli VK, Yamini G, Tripathi R, Gauri, Guntupalli R, Development and Validation of an RP-HPLC Method for Simultaneous Estimation of Rosuvastatin and Ezetimibe in Solid Dosage Form Using Analytical Quality by Design (AQbD) Approach. Int J Drug Deliv Technol. 2026;16(4s): 831-839; DOI: 10.25258/ijddt.16.4s.96

1. Introduction

Hyperlipidemia is one of the most significant metabolic disorders associated with an increased risk of cardiovascular morbidity and mortality worldwide [1]. Elevated levels of cholesterol, triglycerides, and low-density lipoprotein cholesterol (LDL-C), along with reduced levels of high-density lipoprotein cholesterol (HDL-C), contribute to the progression of atherosclerosis and subsequent cardiovascular diseases [2]. Among the various therapeutic options available,

statins remain the cornerstone for the treatment of hyperlipidemia because of their ability to inhibit the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, thereby reducing endogenous cholesterol synthesis [3]. Rosuvastatin, one of the most potent statins, is frequently prescribed due to its high efficacy in lowering LDL-C and its relatively favorable safety profile [4]. On the other hand, Ezetimibe works through a different mechanism by selectively inhibiting the absorption of cholesterol at the intestinal brush border. The combination of Rosuvastatin and Ezetimibe provides a dual therapeutic approach, offering synergistic benefits by addressing both endogenous cholesterol synthesis and dietary cholesterol absorption [5]. This combination is especially useful in patients who fail to achieve target lipid levels with statin monotherapy or who require more aggressive lipid-lowering therapy [6]. Consequently, fixed-dose combinations of Rosuvastatin and Ezetimibe have gained prominence in clinical practice for the effective management of dyslipidemia [7].

The simultaneous presence of Rosuvastatin and Ezetimibe in a single solid dosage form, however, poses considerable analytical challenges. Both drugs differ significantly in their chemical structures, polarity, and physicochemical properties, which influence their retention behavior in chromatographic separation [8]. Rosuvastatin, being relatively hydrophilic with acidic functional groups, and Ezetimibe, which is more lipophilic, exhibit diverse solubility profiles and UV absorbance characteristics. Designing an analytical method capable of providing adequate resolution between these two drugs while maintaining acceptable sensitivity, linearity, and reproducibility is therefore complex [9]. Conventional spectrophotometric approaches often fail to achieve selective estimation due to overlapping absorption spectra. Similarly, non-optimized chromatographic methods may result in poor peak symmetry, co-elution, or unsatisfactory resolution [10]. These difficulties underscore the necessity for a robust, reliable, and reproducible chromatographic method capable of quantifying both analytes simultaneously in combined formulations. Traditional method development in liquid chromatography has largely relied on the trial-and-error approach, where variables such as mobile phase composition, flow rate, and detection wavelength are altered incrementally until acceptable separation is achieved [11]. While this empirical strategy has led to the development of numerous analytical methods, it is time-consuming, resource-intensive, and often lacks scientific justification for parameter selection. Furthermore, such methods are prone to variability when subjected to minor changes in laboratory conditions, equipment, or reagents. This limitation becomes especially critical when methods are intended for regulatory submission, routine quality control, or stability studies, where robustness and reproducibility are non-negotiable [12]. In addition, trial-and-error approaches do not provide insight into the interactions between multiple variables, thereby restricting the understanding of the method's performance space. The lack of systematic evaluation often results in methods that are not sufficiently resilient to minor perturbations, leading to potential failures during method transfer or scale-up [13]. These drawbacks highlight the pressing need for a more scientific and risk-based framework for analytical method development [14]. The concept of Quality by Design (QbD), originally introduced for pharmaceutical formulation and process development, has been increasingly extended to the analytical domain, leading to the evolution of Analytical Quality by Design (AQbD) [15]. AQbD emphasizes a structured and scientific approach to method development by integrating prior knowledge, risk assessment tools, and statistical experimental designs. Unlike conventional approaches, AQbD aims to define a method's "design space," which encompasses the multidimensional relationship between critical method parameters (CMPs) and critical quality attributes (CQAs). Within this design space, the method is expected to deliver consistent performance, thereby ensuring reliability during routine application and regulatory flexibility in the future [16]. Tools such as Ishikawa fishbone diagrams help identify potential factors affecting method performance, while Design of Experiments (DoE) enables systematic evaluation of variable interactions. Statistical models such as Response Surface Methodology (RSM) further aid in optimizing parameters with minimal experimental trials. AQbD not only improves method robustness but also reduces the need for repeated revalidation, as minor changes within the established design space are not considered regulatory variations. Thus, the application of AQbD in analytical method development represents a paradigm shift from empirical to knowledge-driven, risk-based methodologies. The present study is therefore directed towards the development and validation of an RP-HPLC method for the simultaneous estimation of

Rosuvastatin and Ezetimibe in solid dosage forms by employing an AQbD approach. The study involves systematic identification of critical method parameters using Ishikawa diagrams, optimization through experimental design, and establishment of a robust design space to ensure reliability. The developed method is subsequently validated in accordance with ICH Q2 (R1) guidelines, covering essential parameters such as linearity, accuracy, precision, robustness, specificity, limit of detection (LOD), and limit of quantification (LOQ). By adopting this approach, the research not only provides an effective analytical tool for routine quality control of Rosuvastatin–Ezetimibe formulations but also demonstrates the practical utility of AQbD in analytical method development. The outcomes are expected to contribute towards enhancing regulatory compliance, reducing long-term risks of method failure, and ensuring consistent therapeutic efficacy of combination formulations used in hyperlipidemia management.

2. Materials and Methods

2.1 Chemicals and Reagents

Rosuvastatin calcium and Ezetimibe pure APIs were obtained as gift samples from Sun Pharmaceutical Industries Ltd., India. Acetonitrile and methanol of HPLC grade were procured from Merck (India) Pvt. Ltd. Orthophosphoric acid and potassium dihydrogen phosphate (analytical grade) were supplied by Central Drug House (CDH), New Delhi. Milli-Q water was prepared in-house and used throughout the analysis. All other reagents and chemicals were of analytical grade and used without further purification.

2.2 Method Validation

The developed RP-HPLC method was validated according to the International Conference on Harmonization (ICH Q2 R1) guidelines to ensure its suitability for the intended purpose. The validation parameters included linearity, accuracy, precision, robustness, specificity, and determination of limits of detection (LOD) and quantification (LOQ). Each parameter was evaluated systematically using Rosuvastatin and Ezetimibe in solid dosage forms, and the acceptance criteria were predefined to maintain the reliability of the method [17].

a. Linearity :

Linearity of the method was established by preparing calibration curves across a concentration range of 50–150% of the target assay level. A series of standard solutions of Rosuvastatin and Ezetimibe were injected in duplicate, and peak area versus concentration plots were constructed. The regression analysis yielded the slope, intercept, and correlation coefficient. A correlation coefficient value greater than 0.999 for both analytes confirmed excellent linearity across the tested range, with residuals within acceptable limits, demonstrating the ability of the method to provide test results proportional to analyte concentration [18].

b. Accuracy :

Accuracy was evaluated through recovery studies performed by spiking known amounts of pure standards of Rosuvastatin and Ezetimibe into placebo formulations at three concentration levels, namely 80%, 100%, and 120% of the nominal test concentration. Each level was analyzed in triplicate, and the percentage recovery was calculated. The recoveries for both drugs were found to be within the range of 98–102%, with %RSD less than 2.0%, confirming that the method was accurate and free from interference by excipients. These findings demonstrated that the method could reliably quantify the analytes in commercial formulations [19].

c. Precision :

Precision was assessed at two levels, repeatability and intermediate precision. Repeatability (intra-day precision) was determined by preparing six independent sample solutions at 100% of the target assay concentration and analyzing them under the same experimental conditions. The %RSD of the assay values was below 2.0% for both drugs, confirming satisfactory repeatability. Intermediate precision was studied by conducting the assay on different days, with a different analyst and using a separate HPLC system. The pooled %RSD of assay values across analysts and instruments also remained below 2.0%, indicating that the method was precise and reproducible under varied conditions [20].

d. Robustness :

Robustness of the method was evaluated by introducing small but deliberate changes in the chromatographic conditions, such as slight variations in flow rate (±0.1 mL/min), detection wavelength (±2 nm), organic composition of the mobile phase (±2%), and column temperature (±2 °C). These changes did not significantly affect the retention times, resolution, or assay values of Rosuvastatin and Ezetimibe. System suitability parameters including resolution, tailing factor, and theoretical plates remained within the acceptance criteria, confirming that the method was robust and reliable even under slightly altered operating conditions [21].

e. Specificity :

Specificity was demonstrated by analyzing blank, placebo, standard, and sample solutions to confirm the absence of interference at the retention times of Rosuvastatin and Ezetimibe. Further, forced degradation studies were carried out by subjecting the drugs to stress conditions such as acid and base hydrolysis, oxidation, thermal stress, and photolysis. Degradation peaks were well resolved from the analyte peaks, and peak purity analysis using PDA confirmed that Rosuvastatin and Ezetimibe peaks were spectrally pure. This confirmed the method's specificity and stability-indicating nature [22].

f. limits of detection (LOD) and quantification (LOQ):

limits of detection (LOD) and quantification (LOQ) were determined based on the standard deviation of the response and the slope of the calibration curve, using the equations:

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$.

The LOD and LOQ values demonstrated that the method was sensitive enough to detect and quantify Rosuvastatin and Ezetimibe at very low concentrations. At the LOQ level, precision and accuracy were verified, with %RSD values less than 10% and recoveries within the acceptable range [23,24].

3. Results and Discussion

3.1 Preliminary Trials

In the initial stage of method development, several chromatographic trials were carried out to evaluate the effect of mobile phase composition, flow rate, and pH on the simultaneous separation of Rosuvastatin (RSV) and Ezetimibe (EZE). These parameters were systematically varied to study their influence on retention time, peak symmetry, and resolution. When the organic content of the mobile phase was increased to 80% acetonitrile, both drugs eluted rapidly with retention times of 1.82 min for Ezetimibe and 2.65 min for Rosuvastatin. Although the peaks were sharp, the resolution between the two analytes was poor (Rs = 1.1). Reducing the acetonitrile ratio to 60% prolonged retention (EZE at 4.10 min and RSV at 6.25 min), improving the resolution to 2.5, but causing peak broadening and longer run time. An intermediate composition of 70:30 (acetonitrile:water) produced balanced results, with Ezetimibe eluting at 3.05 min and Rosuvastatin at 4.68 min, giving a resolution of 2.1 and symmetrical peaks. The effect of flow rate was also significant. At 0.8 mL/min, retention times increased (4.25 min for EZE and 6.42 min for RSV) with good resolution (Rs = 2.6) but at the cost of prolonged analysis time and slight broadening. Increasing the flow rate to 1.2 mL/min reduced retention to 2.35 min for EZE and 3.75 min for RSV, but resolution dropped to 1.4 with increased tailing. A flow rate of 1.0 mL/min provided the optimum compromise with sharp peaks and acceptable analysis time. Similarly, the pH of the aqueous phase influenced chromatographic behavior. At low pH (3.5), Rosuvastatin showed significant tailing (tailing factor ~1.9) due to partial ionization of its acidic groups. At higher pH (6.5), both drugs eluted faster but with slight fronting and baseline instability. The best results were obtained at pH 5.5, where both analytes produced sharp, symmetrical peaks with resolution above 2.0 and stable baseline. Preliminary trials demonstrated that a mobile phase composition of acetonitrile:water (70:30, v/v), flow rate of 1.0 mL/min, and aqueous phase pH 5.5 gave the most reliable and reproducible chromatographic performance. These conditions were considered suitable for further AQbD-based optimization and validation.

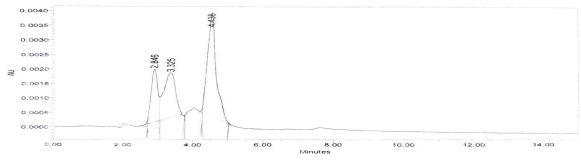

Fig.1Representative RP-HPLC Chromatogram of Standard Mixture Showing Separation of Rosuvastatin and Ezetimibe with Distinct Retention Times

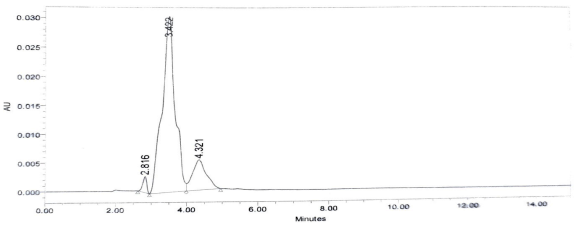

Fig. 2 RP-HPLC Chromatogram of Standard Mixture Showing Well-Resolved Peaks of Rosuvastatin and Ezetimibe

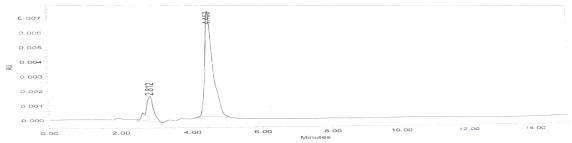

Fig. 3 RP-HPLC Chromatogram of Standard Mixture Depicting Distinct Peaks of Rosuvastatin and Ezetimibe

Table 1: Effect of Mobile Phase Composition, Flow Rate and pH on Chromatographic Performance

Parameter Varied	Condition	Retention Time	Retention Time	Resolution (Rs)	Tailing

		(EZE, min)	(RSV, min)		Factor
Mobile phase composition (ACN: Water)	80:20	1.82	2.65	1.1	1.2–1.3
	70:30	3.05	4.68	2.1	1.1–1.2
	60:40	4.10	6.25	2.5	1.2–1.3
Flow rate (mL/min)	0.8	4.25	6.42	2.6	1.3–1.4
	1.0	3.05	4.68	2.1	1.1–1.2
	1.2	2.35	3.75	1.4	1.5 +
pH of aqueous phase	3.5	3.90	5.95	1.9	1.8–1.9
	5.5	3.05	4.68	2.2	1.1–1.2
	6.5	2.85	4.10	2.0	1.4–1.5

3.2 AQbD Optimization

The development of the RP-HPLC method for simultaneous estimation of Rosuvastatin (RSV) and Ezetimibe (EZE) was further refined by adopting the Analytical Quality by Design (AQbD) approach. Critical method parameters (CMPs), namely mobile phase composition (percentage of acetonitrile), flow rate, and detection wavelength, were identified using the Ishikawa diagram. A three-factor, three-level Box–Behnken Design (BBD) was employed to systematically study their effect on critical quality attributes (CQAs), including retention time (RT) of both analytes, resolution, and peak symmetry.

The experimental matrix generated 15 runs, and the observed data were subjected to regression analysis and ANOVA. The models for retention time and resolution were found to be statistically significant ($p < 0.05$) with lack-of-fit values non-significant ($p > 0.1$), indicating good model fit. High coefficients of determination ($R^2 = 0.994$ for Ezetimibe RT, 0.991 for Rosuvastatin RT, and 0.988 for resolution) confirmed the predictive ability of the models. The adjusted R^2 values were also >0.98, suggesting excellent agreement between predicted and experimental responses.

Response surface plots revealed that resolution increased as the proportion of acetonitrile decreased up to 70%, but further reduction caused peak broadening. Similarly, flow rate showed an inverse relationship with resolution: lower flow rates improved resolution but prolonged analysis time. The detection wavelength between 230–236 nm did not significantly affect retention but slightly influenced peak area response, with 234 nm yielding maximum absorbance for both analytes. Overall, the model demonstrated strong interaction effects between mobile phase composition and flow rate, making them the most critical factors to control.

Numerical optimization using the desirability function identified the optimal chromatographic conditions as acetonitrile:water (70:30, v/v) at a flow rate of 1.0 mL/min and detection wavelength of 234 nm. Under these conditions, the predicted retention times were 3.05 min for Ezetimibe and 4.68 min for Rosuvastatin, with a resolution of 2.2 and tailing factors close to 1.1 for both analytes. Confirmation experiments performed in triplicate at these optimized conditions closely matched the predicted values, with percentage prediction error below 5%, thereby validating the robustness of the AQbD-based model.

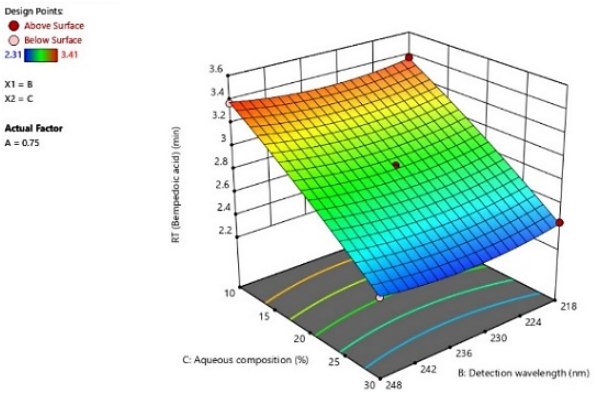

Fig. 3 3D Response Surface Plot Showing the Effect of Flow Rate and Detection Wavelength on Retention Time of Rosuvastatin

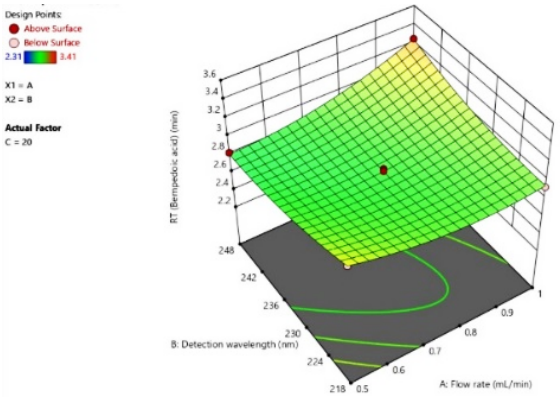

Fig. 4 3D Response Surface Plot Showing the Effect of Aqueous Composition and Detection Wavelength on Retention Time of Rosuvastatin

Table 2: Optimized Chromatographic Conditions from AQbD Design

Parameter	Tested Range	Optimized Value	Response Achieved
Mobile phase composition (ACN:Water, v/v)	60:40 – 80:20	70:30	Balanced retention, Rs = 2.2
Flow rate (mL/min)	0.8 – 1.2	1.0	Sharp peaks, reduced run time
Detection wavelength (nm)	230 – 236	234	Maximum sensitivity
Retention time (EZE)	2.35 – 4.25 min	3.05 min	Symmetrical peak (TF = 1.12)
Retention time (RSV)	3.75 – 6.42 min	4.68 min	Symmetrical peak (TF = 1.15)
Resolution (Rs)	1.1 – 2.6	2.2	Meets system suitability

3.3 Method Validation Results

The developed RP-HPLC method for simultaneous estimation of Rosuvastatin (RSV) and Ezetimibe (EZE) was validated in accordance with ICH Q2(R1) guidelines. The validation parameters included linearity, accuracy, precision, robustness, and sensitivity (LOD and LOQ), and the results confirmed that the method is suitable for routine analysis of solid dosage forms.

Linearity was established over the concentration range of 5–20 µg/mL for both RSV and EZE. Calibration curves constructed from triplicate injections at each level showed excellent correlation, with regression coefficients (r^2) of 0.9992 for Ezetimibe and 0.9991 for Rosuvastatin. The regression equations were found to be $y = 12456x + 2134$ for EZE and $y = 11328x + 1987$ for RSV, indicating a strong linear relationship between concentration and peak area. The residuals across the calibration range were within ±2%, confirming proportionality.

Accuracy of the method was determined by recovery studies using placebo spiked with known quantities of the analytes at 80%, 100%, and 120% of the test concentration. The mean recovery for Ezetimibe was 99.4%, 100.2%, and 100.5% across the three levels, while Rosuvastatin showed recoveries of 98.9%, 99.8%, and 100.6%. The overall recovery values fell within the acceptance range of 98–102%, with %RSD values less than 1.5%, demonstrating that the method is accurate and free from interference of excipients.

Precision was evaluated through repeatability and intermediate precision studies. For repeatability, six replicate sample preparations at 100% concentration gave %RSD values of 0.82% for Ezetimibe and 0.95% for Rosuvastatin, confirming intra-day precision. Intermediate precision, performed on a different day with a second analyst and a different instrument, showed %RSD values of 1.12% for Ezetimibe and 1.25% for Rosuvastatin. Both results were well below the 2% limit, indicating excellent reproducibility of the method.

Robustness was examined by introducing small deliberate variations in chromatographic conditions, including flow rate (±0.1 mL/min), organic content (±2% acetonitrile), and detection wavelength (±2 nm). None of these changes caused significant shifts in retention time, peak area, or resolution. For example, resolution values under varied conditions remained above 2.0, and assay results deviated by less than 1.5% from the nominal value. These findings confirm that the method is robust and capable of withstanding minor variations without compromising performance.

LOD and LOQ were calculated based on the standard deviation of response and the slope of the calibration curve. The LOD was found to be 0.15 µg/mL for Ezetimibe and 0.18 µg/mL for Rosuvastatin, while the LOQ values were 0.45 µg/mL and 0.55 µg/mL, respectively. Precision and accuracy at the LOQ levels were confirmed, with recoveries between 98–102%

and %RSD less than 5%, confirming the sensitivity of the method.

Collectively, the validation results demonstrate that the developed RP-HPLC method is linear, accurate, precise, robust, and sensitive, making it suitable for simultaneous estimation of Rosuvastatin and Ezetimibe in solid dosage forms for routine quality control and stability testing.

Table 3: Summary of Method Validation Results

Parameter	Ezetimibe (EZE)	Rosuvastatin (RSV)	Acceptance Criteria
Linearity range	5–20 µg/mL, r^2 = 0.9992	5–20 µg/mL, r^2 = 0.9991	$r^2 \geq 0.999$
Accuracy (% recovery)	99.4–100.5% (RSD < 1.5%)	98.9–100.6% (RSD < 1.5%)	98–102%, RSD ≤ 2%
Repeatability (%RSD)	0.82%	0.95%	≤ 2%
Intermediate precision (%RSD)	1.12%	1.25%	≤ 2%
Robustness (Resolution)	Rs > 2.0 under variations	Rs > 2.0 under variations	Rs ≥ 2.0
LOD (µg/mL)	0.15	0.18	Low, method-sensitive
LOQ (µg/mL)	0.45	0.55	Verified, accurate

4.Conclusion

The present study successfully developed and validated a simple, precise, and robust RP-HPLC method for the simultaneous estimation of Rosuvastatin and Ezetimibe in solid dosage forms, employing the principles of Analytical Quality by Design (AQbD). Preliminary trials highlighted the critical influence of mobile phase composition, flow rate, and pH on chromatographic behavior, which were systematically optimized using a Box–Behnken design. The statistical models and response surface analyses confirmed significant interactions among method parameters, while the overlay contour plot defined a reliable design space ensuring consistent method performance. Validation studies carried out in accordance with ICH Q2(R1) guidelines demonstrated excellent linearity (r^2 > 0.999), accuracy (recoveries within 98–102%), and precision (%RSD < 2%). The method was further proven to be robust against minor deliberate variations, while low LOD and LOQ values confirmed its sensitivity for detecting trace levels of both analytes. The specificity studies, including forced degradation experiments, established the stability-indicating capability of the method. The developed AQbD-driven method not only meets the regulatory expectations for reliability and reproducibility but also provides flexibility for routine quality control and stability testing of fixed-dose Rosuvastatin–Ezetimibe formulations. By shifting from conventional trial-and-error approaches to a systematic, risk-based framework, this work demonstrates the advantages of AQbD in building scientifically sound and regulatory-compliant analytical methods that ensure consistent therapeutic efficacy of lipid-lowering drug combinations.

5. Acknowledgements

The authors sincerely thank each other for their collective contributions, dedication, and teamwork throughout the course of this research. Every author played an important role in study design, experimentation, data analysis, and manuscript preparation.

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