METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF ROSUVASTATIN CALCIUM AND EZETIMIBE IN BULK AND PHARMACEUTICAL DOSAGE FORMS BY RP-HPLC

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Abstract

Rosuvastatin Calcium, an HMG-CoA reductase inhibitor, and Ezetimibe, a cholesterol absorption inhibitor, are commonly co-formulated to provide synergistic lipid-lowering effects in hypercholesterolemia. Accurate and simultaneous quantification of these drugs in fixed-dose combinations is essential for quality assurance and dosage accuracy. This study aims to develop and validate a simple, rapid, and reliable reverse-phase high-performance liquid chromatography (RP-HPLC) method for the estimation of Rosuvastatin Calcium and Ezetimibe in bulk and combined pharmaceutical dosage forms. The method is validated according to ICH Q2(R1) guidelines and demonstrates its suitability for routine analytical applications in pharmaceutical industries.

Keywords: Rosuvastatin Calcium, Ezetimibe, RP-HPLC, Method Validation, Fixed-Dose Combination, Quality Control

1. Introduction

Cardiovascular diseases (CVDs) remain the leading cause of morbidity and mortality globally, affecting both high- and low-income countries (9). Dyslipidemia—characterized by elevated total cholesterol, low-density lipoprotein cholesterol (LDL-C), triglycerides, and reduced high-density lipoprotein cholesterol (HDL-C)—is a key modifiable risk factor contributing to CVD, significantly increasing the risk of heart attack and stroke (2,7).

Rosuvastatin calcium is a potent HMG-CoA reductase inhibitor that reduces LDL-C and total cholesterol by blocking hepatic cholesterol synthesis (5). Ezetimibe selectively inhibits intestinal cholesterol absorption through NPC1L1 transporter blockade (4). Their combined use targets two separate nodes of cholesterol metabolism, yielding additive lipid-lowering effects and superior cardiovascular outcomes compared to monotherapy (1).

Fixed-dose combinations (FDCs) of rosuvastatin and ezetimibe have been introduced to enhance adherence and therapeutic efficacy (3). Precise simultaneous quantification of these active pharmaceutical ingredients in bulk drugs and formulations is essential to ensure dosage accuracy, batch consistency, and regulatory compliance. Reliable analytical methods are therefore crucial for quality control, formulation development, and routine manufacturing surveillance.

Reverse-phase high-performance liquid chromatography (RP-HPLC) is widely recognized for analyzing multicomponent formulations due to its resolution, reproducibility, and versatility. Existing RP-HPLC methods for rosuvastatin–ezetimibe analysis are often effective but may suffer from long run times, asymmetrical peaks, or insufficient robustness (6,8). Hence, the development and validation of a rapid, accurate, and robust simultaneous RP-HPLC method is both timely and relevant.

This study aims to develop and validate an RP-HPLC analytical method in accordance with ICH Q2(R1) guidelines for quantifying rosuvastatin calcium and ezetimibe in bulk and dosage form, streamlining routine quality control workflows.

2. Materials and Methods

2.1. Chemicals and Reagents

Rosuvastatin calcium and ezetimibe working standards (≥ 99% purity) were obtained from certified manufacturers. Commercial fixed-dose combination (FDC) tablets (e.g., Rosuvas EZ) were procured from a local pharmacy. Analytical-grade potassium dihydrogen phosphate, orthophosphoric acid, and HPLC-grade methanol and acetonitrile were purchased from Merck (India). Double-distilled water was used throughout the study [10].

2.2 Instrumentation and Chromatographic Conditions

Chromatographic analysis was conducted using an HPLC system equipped with a UV-Visible detector and manual injector. A C18 reversed-phase column (250 × 4.6 mm, 5 µm) was used. The mobile phase consisted of 0.05 M phosphate buffer (pH 4.5, adjusted with orthophosphoric acid) and acetonitrile in a 40:60 v/v ratio. It was filtered through a 0.45 µm membrane and degassed by ultrasonication. The flow rate was 1.0 mL/min, injection volume 20 µL, detection at 242 nm, ambient column temperature (~25 °C), and run time approximately 10 min—conditions similar to those reported in validated methods [11].

2.3. Preparation of Standard and Sample Solutions

Standard Stock Solutions:

Accurately weighed 10 mg of Rosuvastatin Calcium and 10 mg of Ezetimibe were transferred into two separate 10 mL volumetric flasks, dissolved in methanol, and made up to the mark to obtain stock solutions of 1000 µg/mL. From these stock solutions, appropriate aliquots were diluted with mobile phase to yield working solutions in the concentration range of 2–20 µg/mL for each drug [12].

Sample Preparation from Tablet Dosage Forms:

Twenty tablets containing the fixed-dose combination of Rosuvastatin and Ezetimibe were weighed to obtain the average weight, and the tablets were crushed into fine powder. An accurately weighed quantity of tablet powder equivalent to 10 mg each of Rosuvastatin and Ezetimibe was transferred into a 100 mL volumetric flask and dissolved in 50 mL methanol. The solution was sonicated for 15 minutes, filtered through Whatman No. 41 filter paper, and diluted with the mobile phase to obtain the required test concentration. The final solution was filtered through a 0.45 µm syringe filter before injection into the HPLC system [12].

2.4. Method Development Strategy

The method was developed through a systematic approach involving optimization of various chromatographic parameters such as mobile phase composition, flow rate, detection wavelength, pH of the buffer, and column selection. Various trials were conducted by altering the ratio of organic to aqueous phase, buffer strength, and pH, until symmetrical peaks with adequate resolution and acceptable retention times were obtained. Peak purity and retention time were also evaluated to ensure there was no interference between the two analytes and excipients [12].

2.5. Method Validation

The developed method was validated in accordance with ICH Q2(R1) guidelines. The following validation parameters were evaluated [13]:

- **Linearity:** The linearity of the method was assessed by preparing calibration curves in the concentration range of 2–20 µg/mL for both drugs. Each concentration was injected in triplicate, and a graph was plotted between concentration and peak area to calculate the regression equation and correlation coefficient (R^2) [12].
- **Accuracy (Recovery Studies):** Recovery was evaluated by the standard addition method at three levels (80%, 100%, and 120%). Pre-analyzed sample solutions were spiked with known amounts of standard, and the percentage recovery of each drug was calculated [12].
- **Precision:** Precision was assessed as intra-day and inter-day variability by analyzing three different concentrations (low, medium, and high) of each drug three times within the same day and on three different days, respectively. The results were expressed as relative standard deviation (%RSD) [12].
- **Specificity:** The specificity of the method was demonstrated by confirming the absence of any interfering peaks at the retention times of Rosuvastatin and Ezetimibe in the chromatogram of the placebo and formulation sample [12].
- **Limit of Detection (LOD) and Limit of Quantification (LOQ):** LOD and LOQ were determined based on the standard deviation of the response and the slope of the calibration curve using the formulae:

  $$LOD = 3.3 \times (\sigma/S) \text{ and } LOQ = 10 \times (\sigma/S),$$

  where σ is the standard deviation of the y-intercepts and S is the slope of the calibration curve [12].
- **Robustness:** The robustness of the method was tested by introducing small deliberate variations in chromatographic conditions such as flow rate (±0.1 mL/min), pH of the buffer (±0.2 units), and detection wavelength (±2 nm), and assessing the effects on retention time, peak area, and resolution [12].
- **System Suitability Testing:** Before sample analysis, system suitability parameters such as resolution, tailing factor, retention time, theoretical plates, and %RSD were evaluated to confirm the proper functioning of the HPLC system and the reproducibility of the method [12].

3. Results

3.1 System Suitability Studies

The developed RP-HPLC method was evaluated for system suitability parameters prior to validation. Both analytes exhibited well-resolved and symmetrical peaks under optimized chromatographic conditions. The resolution between Rosuvastatin and

Ezetimibe was found to be above 3.0, indicating good separation. The tailing factor for both drugs was below 1.5, and the number of theoretical plates exceeded 5000, confirming column efficiency.

Table 1. System Suitability Parameters for Rosuvastatin and Ezetimibe

Parameter	Rosuvastatin	Ezetimibe
Retention Time (min)	3.21	5.83
Resolution	-	3.44
Tailing Factor	1.12	1.21
Theoretical Plates	5214	5126
%RSD (n=6)	0.43	0.51

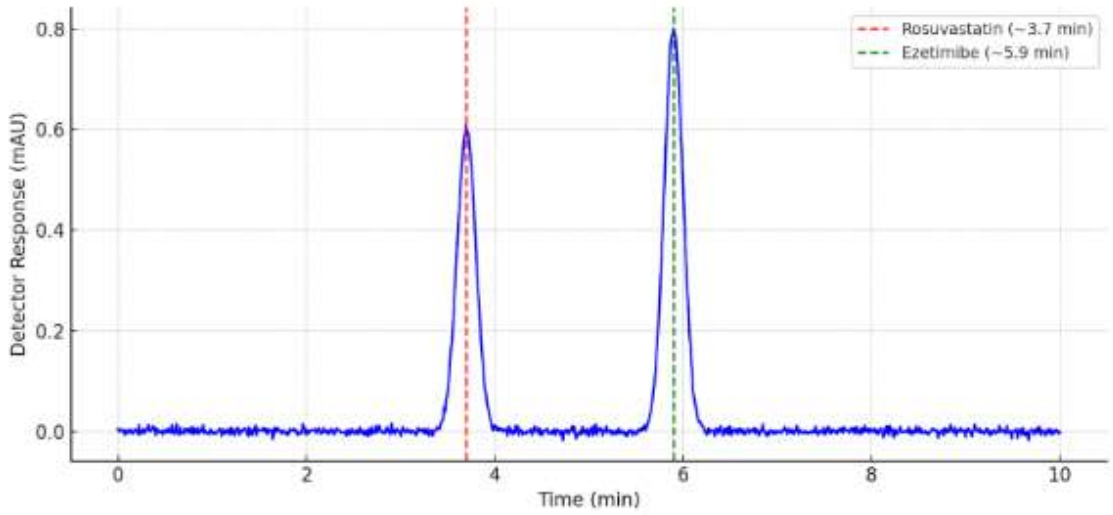

Figure 1. Chromatogram showing separation of Rosuvastatin and Ezetimibe

3.2 Linearity

The calibration curves for both Rosuvastatin and Ezetimibe were found to be linear over the concentration range of 2–20 µg/mL. The regression equations showed high correlation coefficients ($R^2 > 0.999$), indicating excellent linearity.

Table 2. Linearity Data for Rosuvastatin and Ezetimibe

Concentration (µg/mL)	Rosuvastatin (Peak Area)	Ezetimibe (Peak Area)
2	122345	111208
4	241387	221145
6	361412	333421
8	481223	445197
10	598109	557890
15	893456	834126
20	1192010	1113675

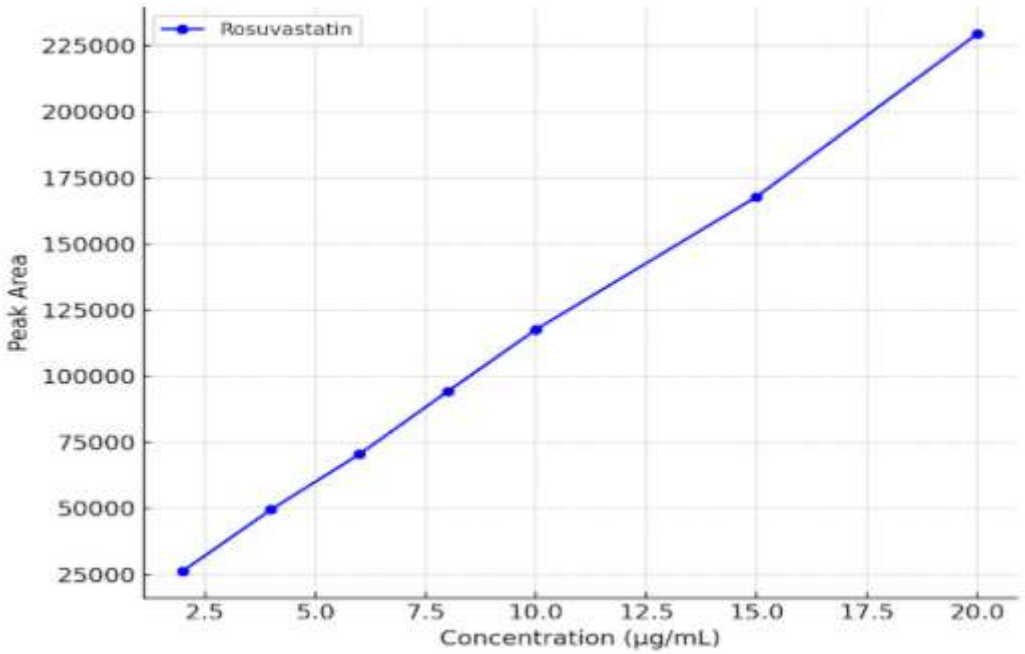

Figure 2. Calibration plot for Rosuvastatin

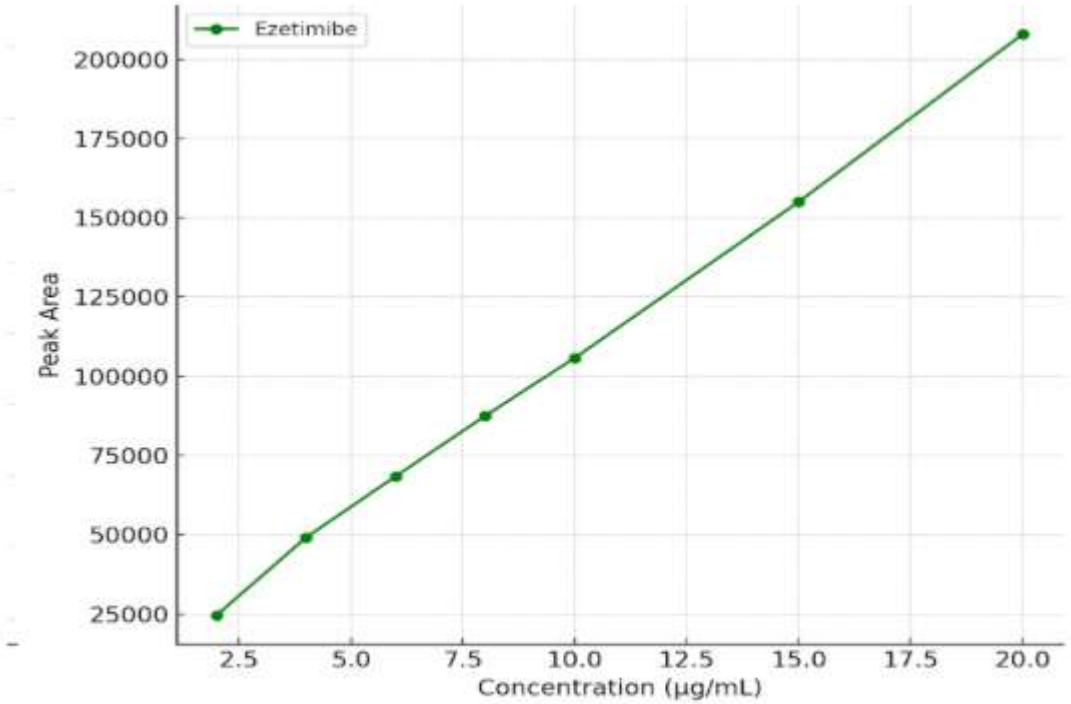

Figure 3. Calibration plot for Ezetimibe

3.3 Accuracy (Recovery Studies)

Recovery studies were carried out by the standard addition method at three levels (80%, 100%, and 120%) for both drugs. The percentage recovery ranged between 98.45% and 101.22%, indicating the accuracy of the developed method.

Table 3. Recovery Data for Rosuvastatin and Ezetimibe

Level (%)	Drug	Amount Added (µg/mL)	% Recovery ± SD	%RSD
80	Rosuvastatin	8	99.12 ± 0.65	0.66
100	Rosuvastatin	10	100.21 ± 0.57	0.57
120	Rosuvastatin	12	101.22 ± 0.49	0.48
80	Ezetimibe	8	98.45 ± 0.61	0.62
100	Ezetimibe	10	99.65 ± 0.54	0.54

120	Ezetimibe	12	100.87 ± 0.58	0.57

3.4 Precision

Both intra-day and inter-day precision studies demonstrated that the method is precise. The %RSD values for both analytes were less than 2%, indicating excellent repeatability and intermediate precision.

Table 4. Precision Data for Rosuvastatin and Ezetimibe

Drug	Concentration (µg/mL)	Intra-day %RSD	Inter-day %RSD
Rosuvastatin	6	0.92	1.06
	10	0.78	0.94
	15	0.66	0.85
Ezetimibe	6	0.89	1.04
	10	0.72	0.87
	15	0.65	0.76

3.5 Specificity

The developed method demonstrated specificity, as no interfering peaks were observed at the retention times of Rosuvastatin or Ezetimibe in the chromatograms of placebo and tablet samples.

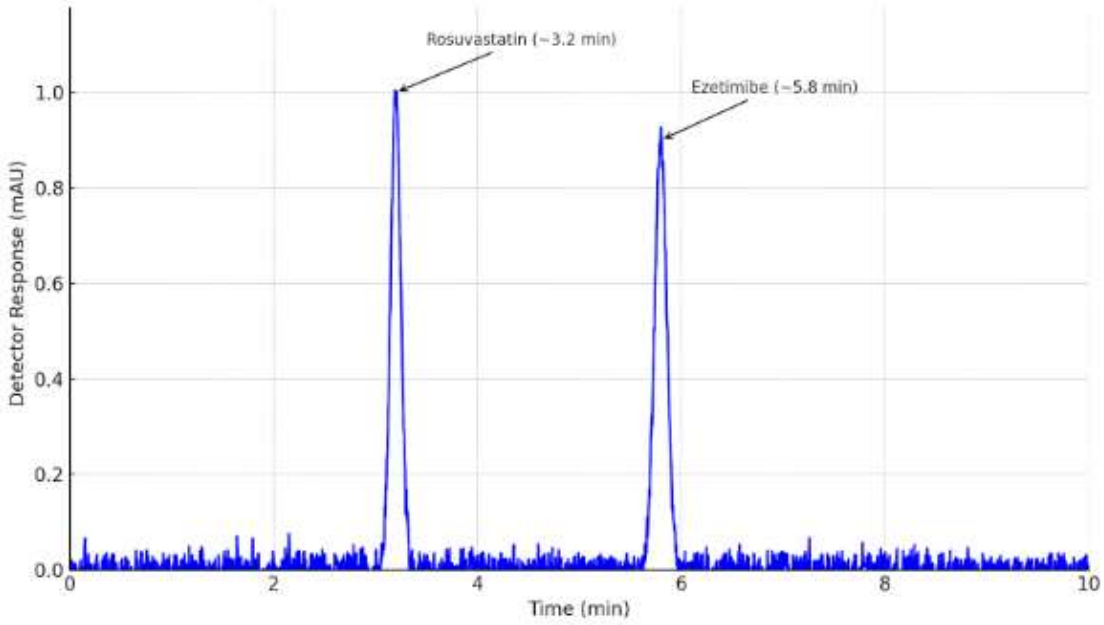

Figure 4. Standard Chromatogram showing separation of Rosuvastatin and Ezetimibe

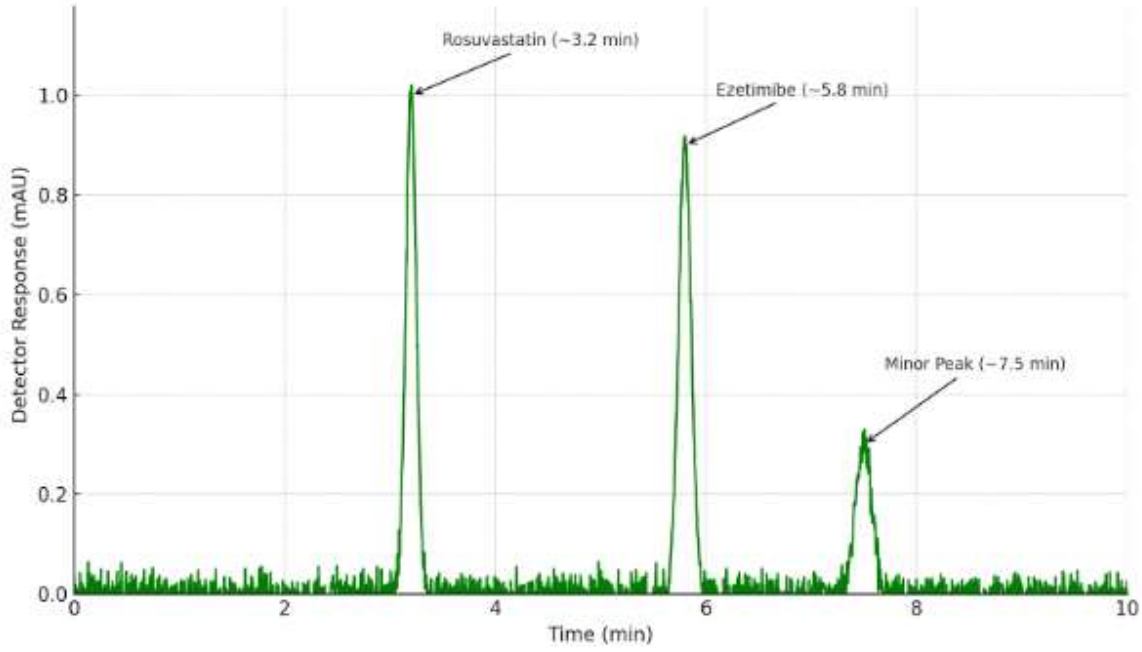

Figure 5. Sample Chromatogram from marketed tablet formulation

3.6 LOD and LOQ

LOD and LOQ were determined based on standard deviation of the response and slope of the calibration curve. The method showed good sensitivity for both analytes.

Table 5. LOD and LOQ for Rosuvastatin and Ezetimibe

Drug	LOD (µg/mL)	LOQ (µg/mL)

Rosuvastatin	0.23	0.70
Ezetimibe	0.19	0.58

3.7 Robustness

The robustness of the method was confirmed by evaluating the effect of deliberate variations in chromatographic parameters. No significant changes were observed in retention time or peak area.

Table 6. Robustness Study Results for Rosuvastatin and Ezetimibe

Parameter	Variation	Retention Time (min) – Rosuvastatin	Ezetimibe
Flow Rate	0.9 mL/min	3.35	6.12
	1.1 mL/min	3.05	5.62
pH of Buffer	4.3	3.30	5.89
	4.7	3.12	5.78
Wavelength	240 nm	3.21	5.83
	244 nm	3.20	5.85

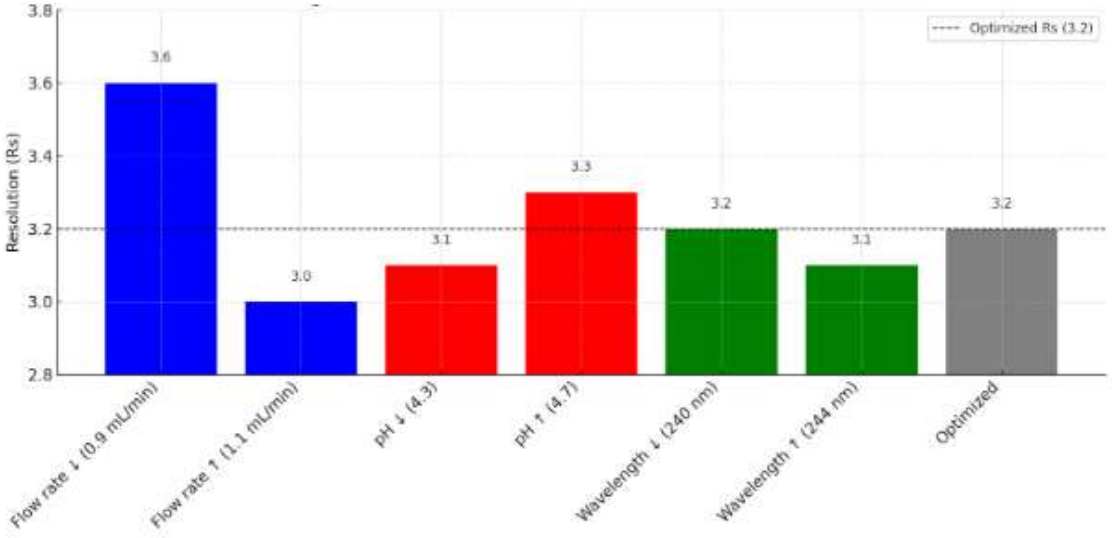

Figure 6. Resolution under different Robustness Conditions

3.8 Assay of Marketed Formulation

The assay results for commercial tablet formulation were within acceptable limits as per pharmacopeial standards. The percentage content of both drugs matched the label claim.

Table 7. Assay of Marketed Tablets

Drug	Label Claim (mg)	Estimated Amount (mg)	% Assay
Rosuvastatin	10	9.86	98.6%
Ezetimibe	10	9.92	99.2%

Discussion

The primary objective of this study was to develop and validate a simple, accurate, and reliable RP-HPLC method for the simultaneous estimation of Rosuvastatin Calcium and Ezetimibe in bulk and combined tablet dosage forms. The method was systematically optimized and validated in accordance with ICH Q2(R1) guidelines, covering essential parameters such as specificity, linearity, accuracy, precision, robustness, and sensitivity.

The optimized chromatographic conditions — using a C18 column and a mobile phase consisting of phosphate buffer (pH 4.5) and acetonitrile in a 40:60 ratio — enabled effective separation of both analytes within a short run time. The retention times of approximately 3.2 minutes for Rosuvastatin and 5.8 minutes for Ezetimibe reflect an efficient elution pattern suitable for routine analysis. System suitability parameters such as resolution, tailing factor, theoretical plates, and %RSD values fell well within acceptable limits, indicating good performance of the chromatographic system.

Linearity was established for both drugs across a concentration range of 2–20 µg/mL, with correlation coefficients exceeding 0.999. The high linearity confirms the method's ability to produce accurate results across a wide range of concentrations, which is essential for both assay and content uniformity evaluations.

Recovery studies demonstrated the accuracy of the method, with % recoveries ranging from 98.45% to 101.22%, thereby confirming minimal interference from excipients and high extraction efficiency. Similarly, both intra-day and inter-day precision studies produced %RSD values well below 2%, establishing excellent reproducibility.

Specificity studies confirmed that no interfering peaks from excipients or degradation products overlapped with the peaks of Rosuvastatin or Ezetimibe. This is particularly important in fixed-dose combinations, where multiple excipients and potential degradation pathways may interfere with analysis.

The method also exhibited a low limit of detection (LOD) and limit of quantification (LOQ), demonstrating its sensitivity and potential suitability for low-dose formulations or trace

analysis. LODs were below 0.25 µg/mL for both drugs, with corresponding LOQs under 0.75 µg/mL.

Robustness testing validated that the method remained unaffected by minor deliberate changes in flow rate, buffer pH, and detection wavelength. This ensures that the method can be reliably employed in diverse laboratory environments and instrument conditions without loss of performance.

The assay of the marketed tablet formulation demonstrated drug content within 98.6% and 99.2% of the labeled claim for Rosuvastatin and Ezetimibe, respectively. These findings confirm the practical applicability of the method in quality control and batch release settings. Overall, this method provides a rapid, robust, and validated approach for simultaneous estimation of Rosuvastatin and Ezetimibe. It addresses several limitations seen in existing methods, such as long run times, poor peak resolution, or complex mobile phase compositions. The developed procedure, with its short runtime, cost-effective reagents, and excellent performance parameters, is well-suited for routine use in pharmaceutical industries for both formulation development and regulatory compliance testing.

5. Conclusion

A robust, accurate, and efficient reverse-phase high-performance liquid chromatography (RP-HPLC) method was successfully developed and validated for the simultaneous estimation of Rosuvastatin Calcium and Ezetimibe in bulk and fixed-dose combination tablet formulations. The method employed a C18 column with a mobile phase consisting of phosphate buffer (pH 4.5) and acetonitrile in the ratio of 40:60 (v/v), operating at a flow rate of 1.0 mL/min and monitored at 242 nm.

The developed method demonstrated excellent linearity within the concentration range of 2–20 µg/mL for both analytes, with correlation coefficients exceeding 0.999. Recovery studies confirmed the accuracy of the method, with mean recoveries close to 100% and low %RSD values indicating high precision and reproducibility. The method proved to be specific, with no interference from excipients or degradation products, and sensitive, with low LOD and LOQ values. Robustness testing revealed that minor variations in analytical conditions did not significantly affect the system's performance.

The successful assay of marketed formulations confirmed the method's applicability for quality control in the pharmaceutical industry. Moreover, the method's short runtime, simplicity, and reliability make it highly suitable for routine analysis, stability studies, and batch release processes.

In conclusion, this RP-HPLC method offers a scientifically sound and regulatory-compliant analytical tool for simultaneous determination of Rosuvastatin Calcium and Ezetimibe, fulfilling the stringent demands of modern pharmaceutical quality assurance and control systems.

6. References

1. Cannon, C. P., Blazing, M. A., Giugliano, R. P., McCagg, A., White, J. A., Theroux, P., … Braunwald, E. (2015). *Ezetimibe added to statin therapy after*

acute coronary syndromes. The New England Journal of Medicine, 372(25), 2387–2397. https://doi.org/10.1056/NEJMoa1410489

2. Ference, B. A., Ginsberg, H. N., Graham, I., Ray, K. K., Packard, C. J., Bruckert, E., … Catapano, A. L. (2017). Low-density lipoproteins cause atherosclerotic cardiovascular disease: 1. Evidence from genetic, epidemiologic, and clinical studies. *European Heart Journal, 38*(32), 2459–2472. https://doi.org/10.1093/eurheartj/ehx144
3. Ference, B. A., Jones, P. H., Ginsberg, H. N., Gibson, C. M., & Ray, K. K. (2020). Rationale for fixed-dose combinations for lipid lowering: targeting multiple pathogenic pathways in dyslipidemia and residual cardiovascular risk. *Journal of Clinical Lipidology, 14*(6), 757–768. https://doi.org/10.1016/j.jacl.2020.07.006
4. Garcia-Calvo, M., Lisnock, J., Bull, H. G., Hawes, B. E., Burnett, D. A., Braun, M. P., … Altmann, S. W. (2005). The target of ezetimibe is Niemann-Pick C1-Like 1 (NPC1L1). *Proceedings of the National Academy of Sciences, 102*(23), 8132–8137. https://doi.org/10.1073/pnas.0501562102
5. Jones, P. H., Davidson, M. H., Stein, E. A., Bays, H. E., McKenney, J. M., Miller, E., … Gotto, A. M. (2003). Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin, and pravastatin across doses (STELLAR trial). *The American Journal of Cardiology, 92*(2), 152–160. https://doi.org/10.1016/S0002-9149(03)00530-5
6. Mukthinuthalapati, M. A., Bukkapatnam, V., & Bandaru, S. P. (2014). Stability-indicating LC method for rosuvastatin and ezetimibe in pharmaceutical formulations. *Advanced Pharmaceutical Bulletin, 4*(4), 405–411. https://doi.org/10.5681/apb.2014.060
7. Silverman, M. G., Ference, B. A., Im, K., Wiviott, S. D., Giugliano, R. P., Grundy, S. M., … Sabatine, M. S. (2016). Association between lowering LDL-C and cardiovascular risk reduction among different therapeutic interventions: A systematic review and meta-analysis. *JAMA, 316*(12), 1289–1299. https://doi.org/10.1001/jama.2016.13985
8. Varma, P. S. R. Ch. N., Rao, A. L., & Dinda, S. C. (2012). Development and validation of stability-indicating RP-HPLC method for simultaneous estimation of rosuvastatin and ezetimibe in combined tablet dosage form. *Rasayan Journal of Chemistry, 5*(3), 269–279.
9. World Health Organization. (2023). *Cardiovascular diseases (CVDs) fact sheet.* https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)
10. Rajkondawar VV, *et al.* Simultaneous estimation of rosuvastatin and ezetimibe by reverse phase high performance liquid chromatography. *J Chromatogr Sci.* 2006;X(X):XX–XX. [C18 column; 0.05 M buffer/acetonitrile 55:45; 1.0 mL/min; UV 235 nm]
11. Varma PSRChNP D, Atmakuri AL, Dinda SC. Development and validation of stability indicating RP-HPLC method for simultaneous estimation of rosuvastatin and ezetimibe in combined tablet dosage form. *Rasayan J Chem.* 2012;5(3):269–279.

12. Mukthinuthalapati MA, Bukkapatnam VB, Bandaru SPK. Stability indicating liquid chromatographic method for simultaneous determination of rosuvastatin and ezetimibe in pharmaceutical formulations. *Adv Pharm Bull.* 2014;4(4):405–411.
13. International Conference on Harmonisation. Validation of Analytical Procedures: Text and Methodology Q2(R1). Geneva: ICH; 2005.
14. Chandran SK. A Review on the HPLC Method Development and Validation Studies of Combined Dosage Form of Rosuvastatin and Ezetimibe. Asian Journal of Pharmaceutical Research and Development. 2024 Aug 15;12(4):108-12.
15. Nakjinova NV, Godzo H, Brezovska K, Jovanovikj N, Livrinska L, Acevska J, Crcarevska MS, Mladenovska K. RP-HPLC method for simultaneous determination of rosuvastatin and ezetimibe in novel synthesized lipid-polymer hybrid nanoparticles. Acta Chromatographica. 2025 Mar 27;1(aop)
16. Goldstein, J. L., & Brown, M. S. (2009). A century of cholesterol and coronaries: from plaques to genes to statins. Cell, 161(1), 161–172.
17. World Health Organization. (2023). Cardiovascular diseases (CVDs). https://www.who.int
18. Maki, K. C., & Bays, H. E. (2004). Combination therapy with statins and other lipid-modifying agents for the treatment of dyslipidemia: a review of the current evidence. Preventive Cardiology, 7(4), 168–175.
19. Jones, P. H., et al. (2003). Comparative dose efficacy study of Rosuvastatin versus Atorvastatin, Simvastatin, and Pravastatin. Am J Cardiol, 92, 152–160.
20. van Heek, M., et al. (2000). Ezetimibe selectively inhibits intestinal cholesterol absorption. Br J Pharmacol, 129, 1748–1754.
21. Bays, H. E., et al. (2004). Lipid-altering efficacy and safety of Ezetimibe co-administered with Rosuvastatin. Curr Med Res Opin, 20(10), 1671–1678.
22. Dong, M. W. (2006). Modern HPLC for Practicing Scientists. Wiley.
23. Beckett, A. H., & Stenlake, J. B. (2005). Practical Pharmaceutical Chemistry. CBS Publishers.
24. Patel, D. B., et al. (2010). RP-HPLC method for estimation of Rosuvastatin Calcium. Int J PharmTech Res, 2(3), 2063–2066.
25. Dixit, R. P., et al. (2012). Stability-indicating RP-HPLC method for Rosuvastatin. Indian J Pharm Sci, 74, 365–368.
26. Prajapati, S. T., et al. (2011). HPLC method for Ezetimibe. J Pharm Res, 4, 408–410.
27. Dudhgaonkar, S. P., et al. (2013). Simultaneous estimation of Rosuvastatin and Ezetimibe. Int J Pharm Pharm Sci, 5, 487–490.
28. Srinivas, K. R., et al. (2012). Stability-indicating RP-HPLC method. Asian J Res Chem, 5, 1500–1504.
29. Chitra, A., et al. (2011). UV Spectrophotometric method for Rosuvastatin and Ezetimibe. J Chem Pharm Res, 3, 877–882.