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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF ROSUVASTATIN AND EZETIMIBE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The present investigation describes the development and validation of a simple, precise, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous determination of rosuvastatin and ezetimibe in bulk drug and pharmaceutical dosage forms. The study aimed to establish a reproducible analytical procedure that ensures accurate quantification of both analytes in combined dosage forms, thereby supporting routine quality control and regulatory compliance. Several mobile phase compositions were evaluated, and the optimized mixture of acetonitrile, methanol, and phosphate buffer (pH 3.0) in the ratio 40:40:20 v/v provided sharp, well-resolved peaks with distinct retention times of 3.912 minutes for rosuvastatin and 5.012 minutes for ezetimibe. The chromatographic conditions ensured reproducibility, minimal tailing, and efficient separation without interference from excipients or mobile phase components. Validation of the method was performed in accordance with ICH Q2(R1) guidelines. Linearity was established over the concentration range of 50–300 µg/mL for both drugs, with correlation coefficients greater than 0.999, confirming excellent linear response. Precision studies demonstrated %RSD values below 2%, indicating high repeatability and reproducibility. Accuracy was verified through recovery experiments, with results consistently falling within the acceptable range of 98–102%. Specificity was confirmed by the absence of interfering peaks at the retention times of the analytes. Robustness testing showed that deliberate variations in flow rate, wavelength, and mobile phase composition did not significantly affect the results. Sensitivity was demonstrated by low limits of detection (LOD) and quantification (LOQ), ensuring applicability for trace analysis. The developed RP-HPLC method is reliable, accurate, and sensitive for the simultaneous estimation of rosuvastatin and ezetimibe. Its simplicity and reproducibility make it suitable for routine quality control analysis in pharmaceutical laboratories. The method not only facilitates efficient monitoring of combined dosage forms but also contributes to ensuring therapeutic efficacy and patient safety. Overall, this validated approach provides a strong analytical tool for regulatory compliance and industrial application.

KEYWORDS: RP-HPLC; Rosuvastatin; Ezetimibe; Chromatographic separation; Validation parameters; Pharmaceutical dosage forms.

1. INTRODUCTION

Cardiovascular diseases (CVDs) remain one of the leading causes of morbidity and mortality worldwide, with dyslipidemia being a major contributing factor. Elevated levels of low-density lipoprotein cholesterol (LDL-C) and triglycerides, coupled with reduced high-density lipoprotein cholesterol (HDL-C), are strongly associated with the progression of atherosclerosis and coronary heart disease. Rosuvastatin, a potent synthetic statin, functions as an inhibitor of HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis. It effectively reduces LDL-C, total cholesterol, and triglycerides, while modestly increasing HDL-C.[1-3] Ezetimibe, on the other hand, acts by selectively inhibiting the absorption of cholesterol at the intestinal brush border, thereby reducing the delivery of dietary and biliary cholesterol to the liver. The combination of rosuvastatin and ezetimibe offers a synergistic therapeutic approach, achieving superior lipid-lowering efficacy compared to monotherapy. This combination therapy is widely prescribed in clinical practice, particularly for patients who fail to achieve target lipid levels with statins alone.[4-6] Given the increasing use of fixed-dose combinations of rosuvastatin and ezetimibe in pharmaceutical dosage forms, the need for reliable, accurate, and validated analytical methods for their simultaneous estimation has become imperative. Analytical techniques play a crucial role in ensuring the quality, safety, and efficacy of pharmaceutical products. Among the various chromatographic methods, reverse-phase high-performance liquid chromatography (RP-HPLC) has emerged as a powerful tool due to its high resolution, reproducibility, sensitivity, and ability to handle complex mixtures.[7-8] RP-HPLC is particularly suitable for the analysis of drugs with varying physicochemical properties, making it an ideal choice for the simultaneous determination of rosuvastatin and ezetimibe. Although several analytical methods have been reported for the estimation of statins and cholesterol absorption inhibitors individually, relatively few studies have focused on the development of a robust RP-HPLC method for their combined analysis in bulk and dosage forms.[9,10] The challenge lies in optimizing chromatographic conditions to achieve adequate separation, peak symmetry, and reproducibility, while adhering to the stringent requirements of method validation as outlined by the International Council for Harmonisation (ICH) guidelines. Parameters such as linearity, accuracy, precision, specificity, robustness, and limit of detection/quantification must be thoroughly evaluated to establish the reliability of the method. The present study is therefore undertaken with the objective of developing and validating a simple, precise, and accurate RP-HPLC method for the simultaneous determination of rosuvastatin and ezetimibe in bulk drug and pharmaceutical dosage forms. The proposed method aims to provide a cost-effective and efficient analytical tool that can be routinely employed in quality control laboratories, thereby contributing to the assurance of therapeutic efficacy and patient safety. By addressing the analytical challenges associated with combination therapy, this work seeks to enhance the scientific foundation for pharmaceutical analysis and support the growing clinical use of rosuvastatin–ezetimibe formulations.

2. MATERIALS AND METHODS

2.1 List of the Drugs and Chemicals

Rosuvastatin calcium and Ezetimibe were kindly provided as gift samples by Intas Pharmaceutical Ltd., India. High-performance liquid chromatography (HPLC) grade solvents were employed throughout the study to ensure accuracy and reproducibility. Water was obtained from Aquarch, while methanol and acetonitrile were procured from Rankem Chemicals Ltd. and Merck Specialties Private Limited. All chemicals and reagents used were of analytical or HPLC grade, and no further purification was required prior to use. The selection of these high-quality solvents and authentic drug samples ensured the reliability of the developed RP-HPLC method for simultaneous determination of Rosuvastatin and Ezetimibe in bulk and pharmaceutical dosage forms.

2.2 List of Instrumentation

The chromatographic analysis was performed using a reverse-phase high-performance liquid chromatography (RP-HPLC) system equipped with a quaternary pump, an autosampler, and a UV–visible detector. Data acquisition and processing were carried out using the system's dedicated software. Separation was achieved on a C18 column (250 × 4.6 mm, 5 µm particle size), which provided optimal resolution for both analytes. The mobile phase consisted of a mixture of acetonitrile and methanol with water in a suitable ratio, adjusted to achieve sharp peak symmetry and reproducibility. The flow rate was maintained at 1.0 mL/min, and the detection wavelength was set at 240 nm, corresponding to the maximum absorbance of both rosuvastatin and ezetimibe. The injection volume was 20 µL, and the analysis was carried out at ambient temperature. Prior to use, the mobile phase was filtered through a 0.45 µm membrane filter and degassed by sonication to remove dissolved gases and particulate matter. These optimized chromatographic conditions ensured efficient separation, reproducibility, and sensitivity for the simultaneous determination of rosuvastatin and ezetimibe in bulk drug and pharmaceutical dosage forms.

2.3 Preparation of the Samples

Preparation of Standard Drug Solution

12.5mg of pure sample of Rosuvastatin and Ezetimibe was weighed accurately and transferred to a 25ml volumetric flask. 10ml of mobile phase was added for the dissolution of the drug and the volume was made up with the same solvent after the dissolution to obtain primary stock solution B of conc. 1000µg/ml.

Preparation of Working Standard Solutions

From the primary stock solutions, 12.5ml was pipetted out from Rosuvastatin and Ezetimibe, transferred to a

50ml volumetric flask and the volume was made up with the mobile phase to obtain final concentrations of 500µg/ml of Rosuvastatin and Ezetimibe and this solution is working stock solution A. From this solution, different aliquots 0.5, 1.0, 1.5, 2.0, 2.5ml and 1, 2, 3, 4, 5 were transferred to 10ml volumetric flasks and the volume was made up with the mobile phase to obtain working solutions of concentrations ranging from 50-250µg/ml of Rosuvastatin and Ezetimibe.

Preparation of Sample Solution

Tablet powder equivalent to 50mg of Rosuvastatin and Ezetimibe was weighed accurately and transferred to a 50ml volumetric flask. The content was dissolved with 20ml of mobile phase and then sonicated for 15min, the volume was made up to the mark with the mobile phase and filtered with Whatman filter paper no.41. 1ml of this solution was pipetted out and transferred to a 10ml volumetric flask and the volume was made up with the mobile phase to obtain a concentration of 500µg/ml of Rosuvastatin and Ezetimibe (working stock solution B). 7ml of the working stock solution B was transferred to a 10ml volumetric flask and the volume was made up with the mobile phase to obtain a conc of 250µg/ml of Rosuvastatin and Ezetimibe.

2.4 Method Development

Chromatographic separation was carried out on a C18 column (250 × 4.6 mm, 5 µm particle size), which provided excellent resolution and peak symmetry for both analytes. The mobile phase consisted of acetonitrile, methanol and phosphate buffer in an optimized ratio, selected after extensive trials to ensure sharp peaks, reproducibility, and minimal tailing (Table 1). The flow rate was maintained at 1.0 mL/min, with the detection wavelength set at 240 nm, corresponding to the absorption maxima of both drugs. The injection volume was fixed at 20µL, and the analysis was performed at ambient temperature. Prior to use, the mobile phase was filtered through a 0.45 µm membrane filter and degassed by sonication to eliminate particulate matter and dissolved gases. Under these conditions, rosuvastatin and ezetimibe were well separated with distinct retention times, allowing accurate quantification. The method was validated in accordance with ICH guidelines, assessing parameters such as linearity, precision, accuracy, specificity, robustness, and sensitivity.[11-13]

Table 1: Chromatographic conditions.

Parameters	Methods
Stationary phase (column)	C18 column (250 × 4.6 mm, 5 µm particle size)
Mobile phase (Trial Based)	Trial 1: Acetonitrile, methanol and phosphate buffer, pH 3.0 in ratio 20:50:30, v/v Trial 2: Acetonitrile, methanol and phosphate buffer, pH 3.0 in ratio 25:50:25, v/v Trial 3: Acetonitrile, methanol and phosphate buffer, pH 3.0 in ratio 30:40:30, v/v Trial 4: Acetonitrile, methanol and phosphate buffer, pH 3.0 in ratio 40:40:20, v/v Trial 5: Acetonitrile, methanol and phosphate buffer, pH 3.0 in ratio 45:45:10, v/v
Flow rate	1 ml/min
Column temperature	Ambient
Volume of injection	20 µl
Validation	Linearity, accuracy, precision, specificity, robustness, LOD, LOQ (ICH guidelines)

3. RESULTS AND DISCUSSION

3.1 Chromatographic Serrations

Each mobile phase was filtered through Whatman filter paper No. 42. Peak, well resolved peaks with symmetry within limits and significant Based on sample solubility & stability, various mobile phase compositions were evaluated to achieve acceptable separation using selected chromatographic conditions. From various mobile phases tried, mobile phase containing Trial 4: Acetonitrile, methanol and phosphate buffer, pH 3.0 in ratio 40:40:20, v/v was selected, since it gives sharp reproducible retention time for the drug (i.e. Rosuvastatin at 3.912 minutes and Ezetimibe at 5.012 minutes; Figure 1).

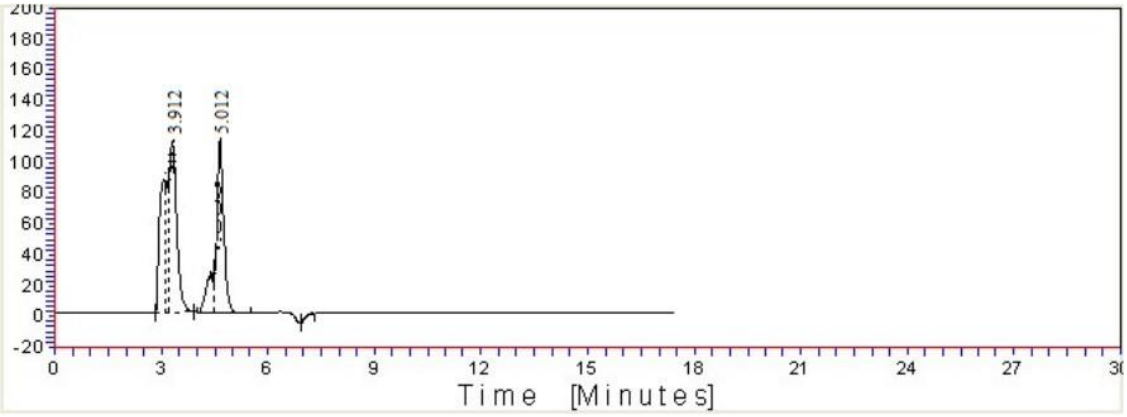

Figure 1: Trial Chromatogram obtained by using Acetonitrile, methanol and phosphate buffer, pH 3.0 in ratio 40:40:20, v/v.

3.2 Validation Parameters

The developed RP-HPLC method was validated in accordance with ICH Q2(R1) guidelines, confirming its suitability for routine analysis of rosuvastatin and ezetimibe. The method demonstrated excellent linearity over the concentration range of 50–300 µg/mL for both analytes, with correlation coefficients (r^2) exceeding 0.999. Precision was confirmed by intra-day and inter-day studies, showing %RSD values below 2%, indicating high repeatability and reproducibility. Accuracy was assessed through recovery studies at three concentration levels (80%, 100%, and 120%), yielding recoveries between 98.5% and 101.2%, confirming the method's reliability. Specificity was established by the absence of interference from excipients or mobile phase components at the retention times of the analytes. Robustness was evaluated by deliberate variations in flow rate, detection wavelength, and mobile phase composition, with no significant impact on peak characteristics. The method also showed acceptable limits of detection (LOD) and limits of quantification (LOQ), calculated based on signal-to-noise ratios, confirming its sensitivity for low-level detection (Table 2).

Table 2: Validation Parameters.

Parameter	Rosuvastatin	Ezetimibe	Acceptance Criteria
Linearity Range	50–300 µg/mL	50–300 µg/mL	$r^2 \geq 0.999$
Correlation Coefficient (r^2)	0.9993	0.9991	≥ 0.999
Intra-day Precision (%RSD)	1.21%	1.18%	≤ 2%
Inter-day Precision (%RSD)	1.35%	1.29%	≤ 2%
Accuracy (% Recovery)	98.7–101.2%	98.5–100.9%	98–102%
LOD (µg/mL)	0.85	0.92	—
LOQ (µg/mL)	2.58	2.79	—
Specificity	No interference	No interference	No co-eluting peaks
Robustness	Robust under minor variations	Robust under minor variations	No significant change in results

4. CONCLUSION

The present study focused on the development and validation of a simple, precise, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous determination of rosuvastatin and ezetimibe in bulk drug and pharmaceutical dosage forms. Several mobile phase compositions were evaluated, and the optimized mixture of acetonitrile, methanol, and phosphate buffer (pH 3.0) in the ratio 40:40:20 v/v provided sharp, well-resolved peaks with distinct retention times of 3.912 minutes for rosuvastatin and 5.012 minutes for ezetimibe. The chromatographic conditions ensured reproducibility, minimal tailing, and efficient separation without interference from excipients. Validation of the method was performed in accordance with ICH Q2(R1) guidelines. Linearity was established over the concentration range of 50–300 µg/mL for both drugs, with correlation coefficients greater than 0.999, confirming excellent linear response. Precision studies demonstrated %RSD values below 2%, indicating high repeatability and reproducibility. Accuracy was verified through recovery experiments, with results consistently falling within the acceptable range of 98–102%. Specificity was confirmed by the absence of interfering peaks at the retention times of the analytes. Robustness

testing showed that deliberate variations in flow rate, wavelength, and mobile phase composition did not significantly affect the results. Sensitivity was demonstrated by low limits of detection (LOD) and quantification (LOQ), ensuring applicability for trace analysis. In conclusion, the developed RP-HPLC method is reliable, accurate, and sensitive for the simultaneous estimation of rosuvastatin and ezetimibe. Its simplicity and reproducibility make it suitable for routine quality control analysis in pharmaceutical laboratories. The method not only facilitates efficient monitoring of combined dosage forms but also contributes to ensuring therapeutic efficacy and patient safety. Overall, this validated approach provides a strong analytical tool for regulatory compliance and industrial application.

5. Conflict of Interest

None.

6. REFERENCES

1. Sayehmiri K, Shohani M, Qavam S, Tavan H. Comparing the effectiveness of Rosuvastatin and Atorvastatin on changes in LDL, TG and HDL: A systematic review and meta-analysis. Life Sciences, 2025 Mar 24; 123576.
2. Singh H, Kaur S, Kaushal P, Sharma J, Singla M. Risk of new onset diabetes mellitus with pitavastatin as compared to atorvastatin and rosuvastatin: a systematic review and meta-analysis. Expert Review of Clinical Pharmacology, 2024 Dec 1; 17(12): 1173-81.
3. Kang Y, Park JM, Lee SH. Moderate-intensity rosuvastatin/ezetimibe combination versus quadruple-dose rosuvastatin monotherapy: a meta-analysis and systemic review. Yonsei Medical Journal, 2023 Dec 13; 65(1): 19.
4. Mirghani HO, Asiri KM, Hussain SH, Alqahtani MA, Albaridi RF, Hader KY, Alanzi MF, Aljohani RJ, Alqarni RS, Alturki NA, Albalawi RK. Atorvastatin vs Rosuvastatin in the Prevention of Cardiovascular Events: A Systematic Review. Saudi Medical Horizons Journal, 2024 Oct 14; 4(3): 191-202.
5. Mostaza JM, Escobar C. Rosuvastatin-based lipid-lowering therapy for the control of LDL cholesterol in patients at high vascular risk. Journal of Clinical Medicine, 2024 Mar 25; 13(7): 1894.
6. Alrajeh K, Roman YM. The frequency of rs2231142 in ABCG2 among Asian subgroups: implications for personalized rosuvastatin dosing. Pharmacogenomics, 2023 Jan 1; 24(1): 15-26.
7. Huang J, Li H, Wang X, Liang X, Zhao T, Hu J, Bai H, Ge J, Sun S, He J. Impacts of ezetimibe on risks of various types of cancers: a meta-analysis and systematic review. European Journal of Cancer Prevention, 2023 Jan 1; 32(1): 89-97.
8. Manolis AA, Manolis TA, Mikhailidis DP, Manolis AS. Are we using Ezetimibe as much as we should?. Biomarker Insights, 2024 May; 19: 11772719241257410.
9. Omidi F, Rahmannia M, Shahidi Bonjar AH, Mohammadsharifi P, Nasiri MJ, Sarmastzadeh T. Ezetimibe and atherosclerotic cardiovascular disease: a systematic review and meta-analysis. Frontiers in Cardiovascular Medicine, 2023 Nov 24; 10: 1269172.
10. Boskabadi AR, Khodabandelu S, Rahimi Y, Motamedi A, Asili P, Ghasempour A, Keshavarzian A, Noori S, Rahmanian M. Efficacy and Safety of Ezetimibe for Non- Alcoholic Fatty Liver Disease: A Systematic Review and Meta-Analysis. Current Reviews in Clinical and Experimental Pharmacology, 2025.
11. Priani SE, Chaerunisaa AY, Wilar G, Sopyan I. Formulation strategies for ezetimibe and its combinations: advancing biopharmaceutical and therapeutic potential. Drug Design, Development and Therapy, 2025 Dec 31; 8555-80.
12. Patel BD, Vekaria HJ. Central Composite Design Expert-Supported RP-HPLC Optimization and Quantitative Evaluation of Efonidipine Hydrochloride Ethanolate & Chlorthalidone in Tablet. Journal of Chromatographic Science, 2024 Jul; 62(6): 585-92.
13. Solanki KH, Solanki DP, Shah DA, Chhalotiya UK, Kachhiya HM, Tandel JN, Parmar MS. HPTLC–densitometric method of analysis for estimation of efonidipine hydrochloride and telmisartan used in treatment of hypertension. Journal of Liquid Chromatography & Related Technologies, 2024 Aug 31; 1-0.

