RESEARCH PAPER

Novel, Stability-Indicating RP-HPLC Method Development and Validation for Simultaneous Quantification of Rosuvastatin and Ezetimibe in Pharmaceutical Dosage Forms

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ABSTRACT

Background:
Rosuvastatin and Ezetimibe are widely used in combination therapy for effective management of hyperlipidemia. However, simultaneous estimation of these drugs remains analytically challenging due to differences in physicochemical properties and chromatographic behavior. Existing analytical methods often involve complex procedures and longer analysis times, limiting their routine applicability.

Methods:
A novel, rapid, and stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed using a C18 column. The optimized mobile phase consisted of acetonitrile and buffer in an appropriate ratio, delivered at a flow rate of 1.0 mL/min. Detection was performed at ~230 nm. The method was validated according to ICH Q2(R1) guidelines.

Results:
The developed method exhibited excellent linearity ($R^2 > 0.999$) over the selected concentration range. The retention times for Rosuvastatin and Ezetimibe were well-resolved within a short runtime. Precision studies showed %RSD < 2%, while recovery ranged from 98–102%, confirming accuracy. The method demonstrated robustness and sensitivity with low LOD and LOQ values.

Conclusion:
The validated RP-HPLC method is simple, precise, robust, and suitable for routine quality control analysis of Rosuvastatin and Ezetimibe in pharmaceutical dosage forms.

Keywords: *Rosuvastatin; Ezetimibe; RP-HPLC; Method Validation; Stability-Indicating Method; Pharmaceutical Analysis*
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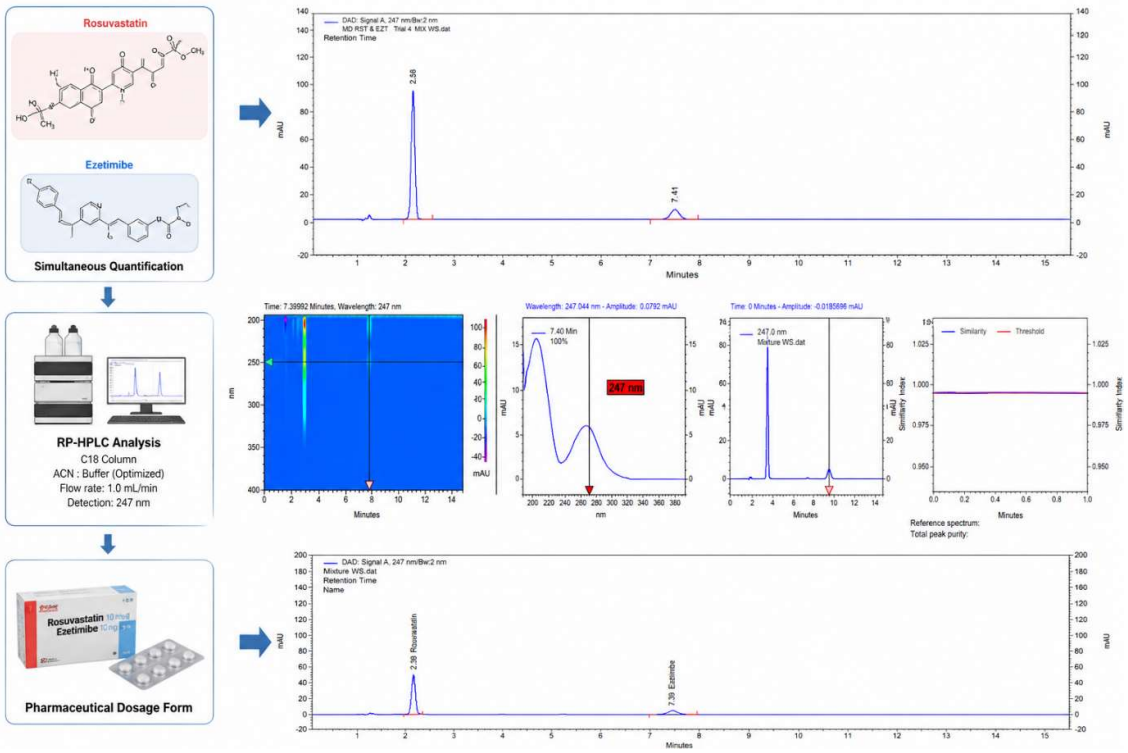
GRAPHICAL ABSTRACT



INTRODUCTION

Analytical chemistry serves as a cornerstone in pharmaceutical sciences by providing reliable tools for the identification, separation, and quantification of active pharmaceutical ingredients (APIs), excipients, and potential impurities present in drug formulations. It plays a critical role in ensuring the quality, safety, and therapeutic efficacy of pharmaceutical products. Through the application of validated analytical methodologies, regulatory compliance can be achieved in accordance with international guidelines, thereby safeguarding public health and maintaining consistency in drug performance [1].

In recent decades, instrumental analytical techniques have significantly advanced, with high-performance liquid chromatography (HPLC) emerging as one of the most widely utilized methods in pharmaceutical analysis. HPLC offers remarkable advantages, including high sensitivity, excellent resolution, reproducibility, and the ability to analyze complex mixtures with precision. Its adaptability to various detection systems further enhances its applicability in routine quality control, impurity profiling, and stability studies of pharmaceutical formulations [2-3].

Rosuvastatin is a synthetic lipid-lowering agent belonging to the statin class of drugs. It functions as a competitive inhibitor of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is the rate-limiting enzyme in cholesterol biosynthesis. By inhibiting this enzyme, Rosuvastatin effectively reduces endogenous cholesterol production and promotes upregulation of hepatic low-density lipoprotein (LDL) receptors, thereby enhancing the clearance of LDL cholesterol from the bloodstream. Beyond its primary lipid-lowering action, Rosuvastatin also exhibits pleiotropic effects, including anti-inflammatory activity, improvement of endothelial function, and stabilization of atherosclerotic plaques, which contribute to its cardiovascular protective properties [4–6].

Ezetimibe, on the other hand, represents a distinct class of lipid-lowering agents that act by selectively inhibiting the absorption of cholesterol in the small intestine. It targets the Niemann-Pick C1-like 1 (NPC1L1) transporter protein located on the brush border of enterocytes, thereby reducing the uptake of dietary and biliary cholesterol. This mechanism complements that of statins, as it decreases the overall cholesterol pool available in the body without affecting triglyceride absorption or fat-soluble vitamin uptake. Consequently, Ezetimibe is frequently used in

combination therapy to achieve enhanced lipid-lowering effects [7-8].

The combined administration of Rosuvastatin and Ezetimibe offers a synergistic therapeutic approach for the management of hyperlipidemia and associated cardiovascular disorders. This combination targets both endogenous cholesterol synthesis and intestinal absorption pathways, resulting in a more comprehensive reduction in plasma lipid levels. Such combination therapies are increasingly preferred in clinical practice due to their improved efficacy and patient outcomes [9].

Despite their therapeutic advantages, the simultaneous estimation of Rosuvastatin and Ezetimibe in pharmaceutical formulations poses significant analytical challenges. These challenges arise primarily due to differences in their physicochemical properties, including polarity, solubility, and chromatographic behavior. Variations in retention characteristics and interactions with stationary phases can complicate the development of a unified analytical method capable of accurately quantifying both drugs within a single run [10].

Although several analytical methods have been reported for the estimation of these drugs, many of them suffer from limitations such as complex mobile phase compositions, prolonged analysis times, and inadequate sensitivity or specificity. These drawbacks restrict their applicability in high-throughput quality control environments, where simplicity, speed, and reliability are essential [11].

In light of these challenges, there is a clear need for the development of an improved analytical method that is simple, rapid, sensitive, and capable of providing accurate and reproducible results. Therefore, the present study aims to develop and validate a novel reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of Rosuvastatin and Ezetimibe in pharmaceutical dosage forms. The method is designed to comply with International Conference on Harmonisation (ICH) guidelines, ensuring its suitability for routine quality control as well as stability-indicating applications [12].

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Rosuvastatin and Ezetimibe were obtained as gift samples from Aadhaar Life Sciences Pvt. Ltd., Solapur, India. Methanol of HPLC grade was procured from Qualigens, Thomas Fisher Scientific India Pvt. Ltd., while LiChrosolv® grade water was supplied by Merck Specialities Pvt. Ltd., Mumbai, India. Disodium hydrogen phosphate of analytical grade was sourced from Thomas Baker Chemicals Pvt. Ltd. All chemicals and reagents used in the study were of high purity and suitable for analytical applications. Purified water was utilized throughout the experimental work. Prior to chromatographic analysis, all prepared solutions were filtered using 0.45 µm membrane filters to remove particulate impurities and to maintain system suitability and analytical accuracy.

2.2 Instrumentation

Chromatographic analysis was performed using an HPLC system equipped with a UV detector and autosampler. Data acquisition and processing were performed using validated chromatography software.

2.3 Chromatographic Conditions

Parameter	Condition
Column	Phenomenex Kinetex XB-C18 (150 × 4.6 mm, 5 µm)
Mobile Phase	Buffer: Methanol (42:58 % v/v)
Flow Rate	1.0 mL/min
Detection Wavelength	247 nm
Injection Volume	10 µL
Run Time	15 minutes
Mode	Isocratic

2.4 Preparation of Standard Solutions

A primary stock solution of Rosuvastatin was prepared by accurately weighing 40 mg of the drug and dissolving it in an appropriate volume of diluent, followed by dilution to obtain a final concentration of 400 µg/mL.

Similarly, a stock solution of Ezetimibe was prepared by dissolving 10 mg of the drug in diluent and making up the volume to achieve a concentration of 100 µg/mL.

Further dilutions of these stock solutions were carried out to prepare a mixed working standard containing Rosuvastatin at 40 µg/mL and Ezetimibe at 10 µg/mL, which was used for chromatographic analysis [13].

2.5 Sample Preparation

The pharmaceutical tablet formulation containing both drugs was accurately weighed and finely powdered. A quantity equivalent to the required dose was transferred into a volumetric flask, dissolved in the diluent, and subjected to sonication to ensure complete extraction of the active components. The resulting solution was filtered to remove insoluble excipients and further diluted appropriately to obtain the desired concentration for analysis [14].

3. Method Validation

The developed reverse-phase high-performance liquid chromatographic (RP-HPLC) method for the simultaneous determination of Rosuvastatin and Ezetimibe was validated in accordance with the International Council for

Harmonisation (ICH) guideline Q2(R1). The validation protocol encompassed specificity, system suitability, linearity and range, accuracy, precision, sensitivity (LOD and LOQ), robustness, and intermediate precision [15].

3.1 Specificity

The specificity of the method was assessed to ensure unequivocal separation and identification of Rosuvastatin and Ezetimibe in the presence of potential interferences such as excipients and diluent components. Blank, individual standard solutions, and mixed working standard solutions were injected and analyzed under optimized chromatographic conditions.

The obtained chromatograms demonstrated well-resolved peaks corresponding to both analytes without any co-eluting peaks or baseline interference. The retention times remained consistent, confirming that the method is highly specific and suitable for analysis of combined pharmaceutical dosage forms [16].

3.2 System Suitability

System suitability testing was performed prior to sample analysis to confirm the adequacy of the chromatographic system. Six replicate injections of the mixed working standard solution were evaluated.

The parameters examined included retention time, theoretical plate count (N), tailing factor, and resolution between peaks. Theoretical plates were consistently greater than 2000, while the tailing factor remained below 2.0, indicating efficient column performance and symmetrical peak shapes. Additionally, the resolution between Rosuvastatin and Ezetimibe exceeded 2.0, ensuring adequate peak separation. The % relative standard deviation (%RSD) for retention time was found to be negligible, confirming system precision [17].

3.3 Linearity and Range

Linearity was evaluated by analyzing standard solutions at five concentration levels ranging from 80% to 120% of the target concentration. The concentration ranges investigated were 32–48 µg/mL for Rosuvastatin and 8–12 µg/mL for Ezetimibe.

Calibration curves were constructed by plotting peak area against corresponding concentrations. The method exhibited excellent linearity within the studied range, with correlation coefficients (R^2) of 0.9994 for Rosuvastatin and 0.999 for Ezetimibe. These results confirm a strong linear relationship between analyte concentration and detector response [18].

3.4 Accuracy

Accuracy of the method was determined through recovery studies using the standard addition technique at three concentration levels (80%, 100%, and 120%). Known quantities of each analyte were spiked into the pre-analyzed sample matrix and analyzed in triplicate.

The percentage recovery values for Rosuvastatin ranged from 99.75% to 100.16%, while those for Ezetimibe ranged from 98.75% to 101.85%. The low %RSD values observed across all levels indicate high accuracy and reliability of the method for quantitative analysis [19].

3.5 Precision

Precision was evaluated in terms of repeatability (intra-day precision) and intermediate precision (inter-day precision).

3.5.1 Repeatability

Six replicate injections of the working standard solution were analyzed under identical conditions within a single day. The %RSD values obtained were 0.51% for Rosuvastatin and 0.44% for Ezetimibe, indicating excellent repeatability.

3.5.2 Intermediate Precision

Intermediate precision was assessed by performing the analysis on different days and at different time intervals. The % assay values remained consistent, and %RSD values were found to be below 2%, demonstrating the reproducibility of the method under varied conditions [20].

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

The sensitivity of the method was evaluated by determining the limit of detection (LOD) and limit of quantification (LOQ) using the standard deviation of the response and the slope of the calibration curve.

The LOD values were found to be 1.94 µg/mL for Rosuvastatin and 0.62 µg/mL for Ezetimibe, whereas the LOQ values were 5.89 µg/mL and 1.87 µg/mL, respectively. These results demonstrate the high sensitivity of the developed method for detecting and quantifying low concentrations of both analytes [21].

3.7 Robustness

Robustness of the method was evaluated by introducing deliberate, minor variations in chromatographic conditions, including column oven temperature (±2°C) and detection wavelength (±2 nm). Each modified condition was analyzed in triplicate.

The results indicated no significant changes in retention time, peak area, or system suitability parameters, with %RSD values remaining within acceptable limits (<2%). This confirms that the method is robust and capable of producing reliable results under slight variations in analytical conditions [22].

4. RESULTS AND DISCUSSION

4.1 HPLC Method Development and Optimization

A systematic approach was employed to develop an efficient RP-HPLC method for the simultaneous estimation of Rosuvastatin and Ezetimibe as shown in Table 1. Initially, methanol and water (50:50, % v/v) were selected as the diluent, ensuring adequate solubility and stability of both analytes.

Chromatographic separation was carried out using a Phenomenex Kinetex XB-C18 column (150 × 4.6 mm, 5 µm), which provided appropriate retention behavior and peak symmetry. Various mobile phase compositions consisting of methanol and disodium hydrogen phosphate

buffer were investigated to achieve optimal chromatographic performance.

During method optimization, several trials were performed by varying mobile phase ratios and detection wavelengths. In the initial trial (50:50, buffer:methanol at 250 nm), Rosuvastatin eluted at approximately 7.44 minutes, while Ezetimibe did not produce a detectable peak. Upon increasing the organic phase content (40:60), Rosuvastatin eluted earlier (~2.31 min), and Ezetimibe appeared at ~6.19 minutes with acceptable resolution.

Further refinement of chromatographic conditions led to the selection of a mobile phase ratio of 42:58 (buffer:methanol) at a detection wavelength of 247 nm as shown in Figure 1. Under these optimized conditions, Rosuvastatin and Ezetimibe were eluted at retention times of 2.58 minutes and 7.41 minutes, respectively, with well-defined peak shapes, acceptable asymmetry (<2), theoretical plates (>2000), and resolution greater than 2. This composition was therefore selected as the final optimized method.

Table 1: Optimization trials of mobile phase composition and detection wavelength for RP-HPLC method development showing chromatographic parameters of Rosuvastatin and Ezetimibe

Trial No.	Mobile Phase	Ratio	Wavelength	Rosuvastatin				Ezetimibe			
				RT	TP	ASY	RES	RT	TP	ASY	RES
1	Buffer: Methanol	50-50	250	7.44	6152	0.96	0.00	No peak observed			
2	Buffer: Methanol	50-50	250	-	-	-	-	No peak observed			
3	Buffer: Methanol	40-60	250	2.31	5248	1.04	0.00	6.19	7070	1.01	18.40
4	Buffer: Methanol	42-58	247	2.58	5364	0.99	0.00	7.41	6830	0.97	19.33

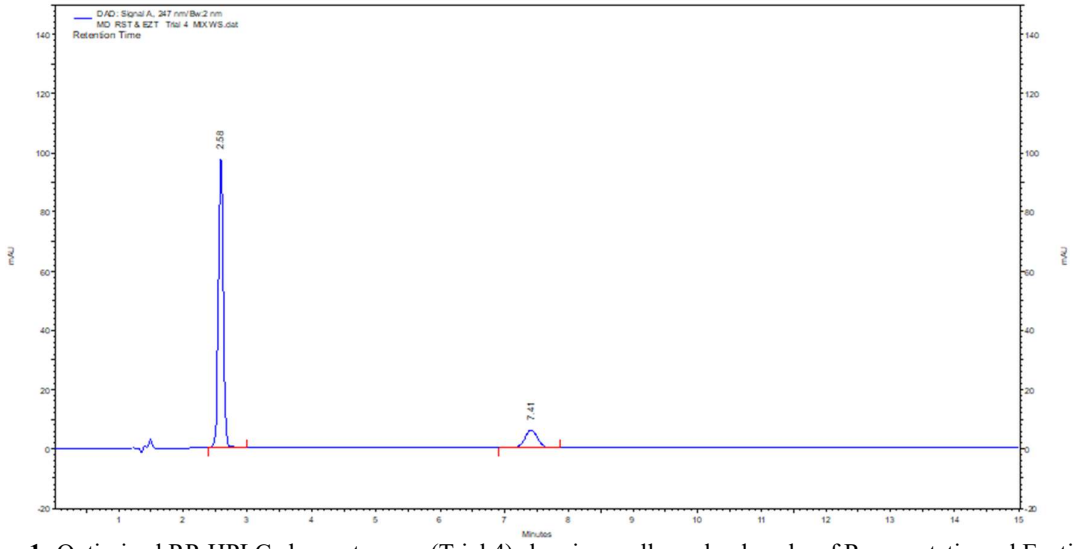


Figure 1: Optimized RP-HPLC chromatogram (Trial 4) showing well-resolved peaks of Rosuvastatin and Ezetimibe using buffer:methanol (42:58 v/v) at 247 nm under isocratic conditions

4.2 Selection of Detection Wavelength

The UV spectra of both analytes were recorded using a PDA detector over the range of 200–400 nm. Based on the overlay spectra, 247 nm was selected as the optimal detection wavelength, as it provided adequate sensitivity and peak response for both Rosuvastatin and Ezetimibe without compromising selectivity as shown in Figure 2.

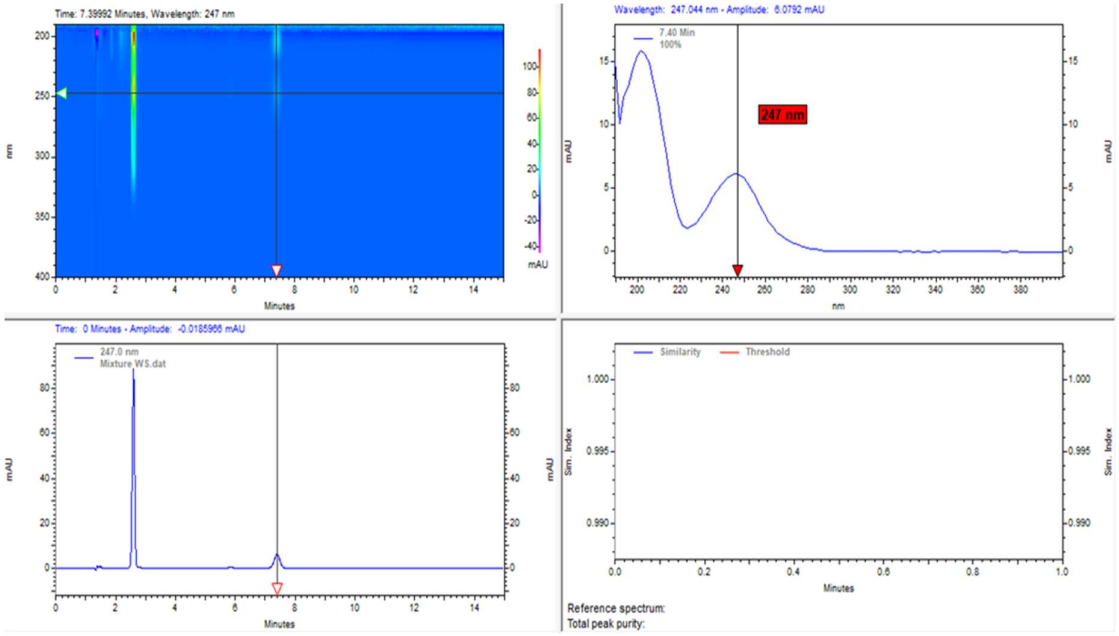


Figure 2: DAD spectrum illustrating wavelength selection at 247 nm for simultaneous detection of Rosuvastatin and Ezetimibe based on optimal peak intensity and response

4.3 Method Validation Results

4.3.1 Specificity

Specificity studies demonstrated that there was no interference from blank or excipient components at the retention times of Rosuvastatin and Ezetimibe. The retention times were found to be approximately 2.59 minutes for Rosuvastatin and 7.39 minutes for Ezetimibe.

The chromatograms of individual standards, mixed standards, and drug product confirmed that both analytes were well resolved, with no overlapping peaks or matrix interference. This establishes the selectivity of the developed method.

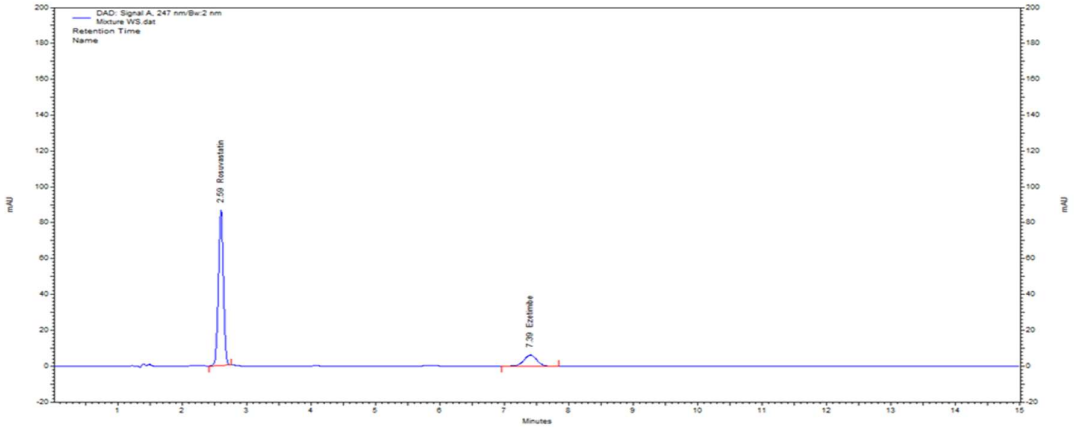


Figure 3: HPLC chromatogram demonstrating specificity, showing well-resolved peaks of Rosuvastatin (RT: 2.59 min) and Ezetimibe (RT: 7.39 min)

4.3.2 Assay of Pharmaceutical Formulation

The developed method was successfully applied to the analysis of tablet dosage form. The percentage assay values obtained were:

- Rosuvastatin: 100.18%
- Ezetimibe: 99.83%

These values fall within acceptable limits, confirming the suitability of the method for routine quality control analysis as shown in Table 2.

Table 2: Results for Specificity and Assay

Sample	Rosuvastatin			Ezetimibe		
	RT	**Area**	**% Assay**	**RT**	**Area**	**% Assay**
Rosuvastatin	2.59	980026	-	-	-	-
Ezetimibe	-	-	-	7.39	175045	-
MIX WS	2.59	972193	-	7.39	179856	-
Drug Product	2.60	973972	100.18	7.41	179546	99.83
Sample	**Rosuvastatin**			**Ezetimibe**		
	RT	**Area**	**% Assay**	**RT**	**Area**	**% Assay**
Rosuvastatin	2.59	980026	-	-	-	-
Ezetimibe	-	-	-	7.39	175045	-
MIX WS	2.59	972193	-	7.39	179856	-
Drug Product	2.60	973972	100.18	7.41	179546	99.83

4.3.3 System Suitability

System suitability parameters were evaluated using six replicate injections. The retention times were highly consistent with %RSD of 0.00, indicating excellent system precision.

Theoretical plate counts for both drugs were well above 2000, confirming good column efficiency. The tailing factor for both analytes remained below 2, indicating symmetrical peak shapes. The resolution between Rosuvastatin and Ezetimibe was found to be greater than 18, demonstrating excellent separation (Table 3). All system suitability parameters complied with ICH acceptance criteria.

Table 3: System Suitability for Rosuvastatin and Ezetimibe

	Rosuvastatin				Ezetimibe			
Reps	**RT**	**ASY**	**TP**	**RES**	**RT**	**ASY**	**TP**	**RES**
Rep 1	2.59	0.97	5297	0.00	7.39	0.97	6446	18.79
Rep 2	2.59	0.99	5194	0.00	7.39	0.98	6305	18.81
Rep 3	2.59	0.96	5366	0.00	7.39	0.96	6766	18.75
Rep 4	2.59	0.97	5510	0.00	7.39	0.98	6162	18.73
Rep 5	2.59	0.98	5286	0.00	7.39	0.97	6662	18.74
Rep 6	2.59	0.99	5434	0.00	7.39	0.97	6524	18.78
Avg	2.59				7.39			
STDEV	0.00				0.00			
RSD	0.00				0.00			

RT - Retention Time; ASY – Asymmetry; TP – Theoretical Plates; RES - Resolution

4.3.4 Precision

Repeatability

The repeatability study showed %RSD values of 0.51% for Rosuvastatin and 0.44% for Ezetimibe based on peak areas from six replicate injections. These low values indicate excellent precision of the method.

Intermediate Precision

The method exhibited consistent % assay values when analyzed on different days and at different time intervals. The %RSD values remained below 2%, confirming the reproducibility and reliability of the method under varied conditions (Figure 4).

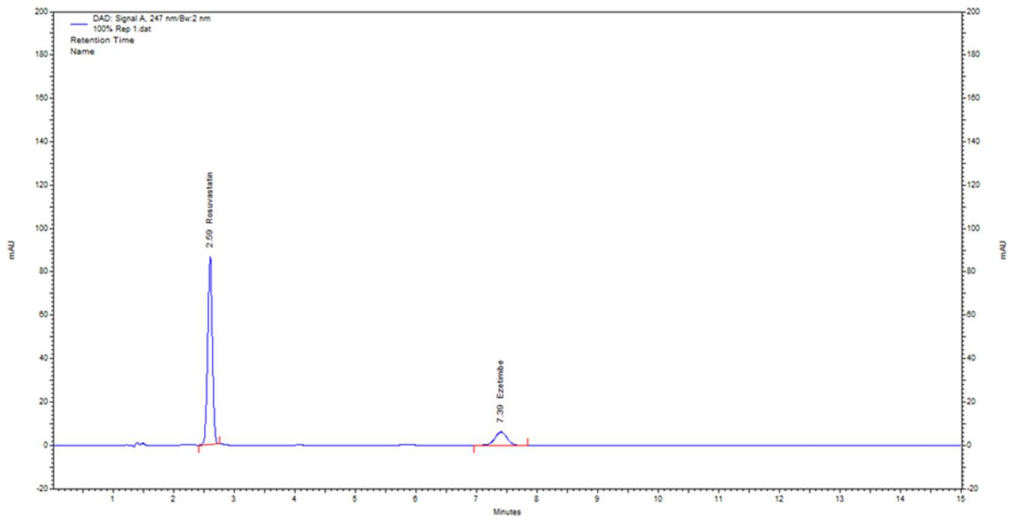


Figure 4: HPLC chromatogram for precision study (repeatability), showing consistent and well-resolved peaks of Rosuvastatin and Ezetimibe with low %RSD, indicating excellent method precision and reproducibility.

4.3.5 Linearity and Range

The calibration curves constructed for both analytes demonstrated excellent linearity over the concentration range of:

- Rosuvastatin: 32–48 µg/mL
- Ezetimibe: 8–12 µg/mL

The corresponding peak areas increased proportionally with concentration. The regression equations were:

- Rosuvastatin: $y = 24163x + 4226.4$
- Ezetimibe: $y = 19509x - 14531$

The correlation coefficients (R^2) were found to be 0.9994 and 0.999 for Rosuvastatin and Ezetimibe, respectively, indicating excellent linear relationships (Table 4, Table 5, Figure 5 & Figure 6).

Table 4: Linearity for Rosuvastatin

Rosuvastatin		
% Level	**Conc (ug/ml)**	**Area**
80	32	776730
90	36	876237
100	40	972193
110	44	1061080
120	48	1167577

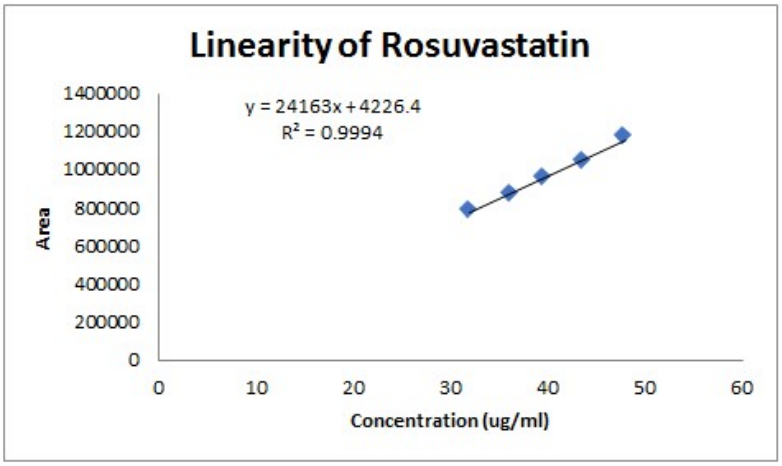


Figure 5: Linearity Graph for Rosuvastatin

Table 5: Linearity data for Ezetimibe

Ezetimibe		
% Level	**Conc (ug/ml)**	**Area**
80	8	140777
90	9	162191
100	10	179856
110	11	201085
120	12	218874

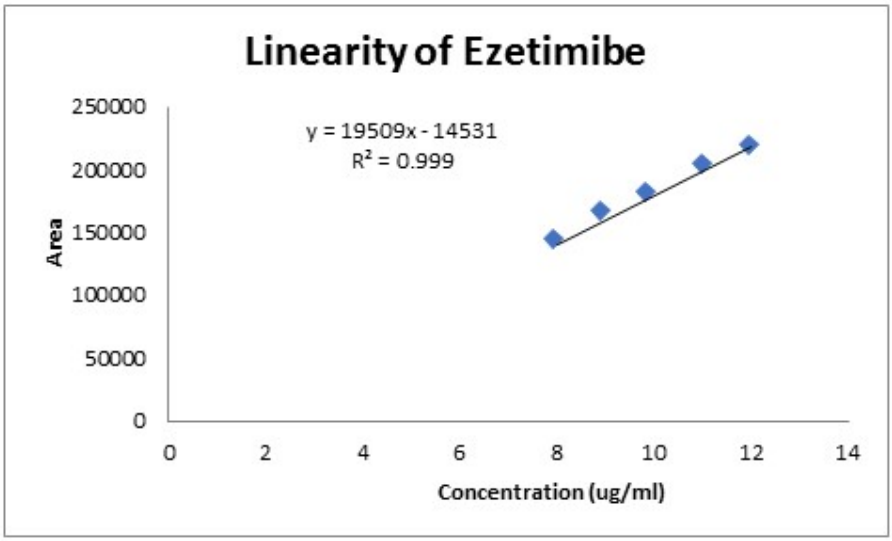


Figure 6: Linearity Graph for Ezetimibe

4.3.6 Accuracy

Recovery studies conducted at 80%, 100%, and 120% levels demonstrated high accuracy of the method. The percentage recoveries obtained were given in Table 6 below:

Table 6: Accuracy and Recovery Results of Rosuvastatin and Ezetimibe at Different Levels

Level	Rosuvastatin (%)	Ezetimibe (%)
80%	99.75	98.75
100%	99.87	99.95
120%	100.16	101.85

All recovery values were within the acceptable range (98–102%) with %RSD less than 2%, confirming the accuracy of the method.

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

The LOD and LOQ values obtained were:

- Rosuvastatin: LOD = 1.94 µg/mL; LOQ = 5.89 µg/mL
- Ezetimibe: LOD = 0.62 µg/mL; LOQ = 1.87 µg/mL

These results indicate that the developed method possesses high sensitivity and is capable of detecting and quantifying low concentrations of both analytes.

4.3.8 Robustness

Robustness studies were performed by introducing small deliberate changes in chromatographic conditions, including variation in column temperature (±2°C) and detection wavelength (±2 nm). The results showed no significant changes in retention time, peak area, or system suitability parameters, with %RSD values remaining within acceptable limits. This confirms that the method is robust and reliable under slight variations in analytical conditions.

5. CONCLUSION

The present study successfully established a reliable and efficient RP-HPLC method for the simultaneous estimation of Rosuvastatin and Ezetimibe in pharmaceutical formulations. The method demonstrated excellent separation with well-defined peaks and minimal interference, ensuring its specificity. Validation results confirmed strong linearity, high accuracy, and consistent precision within acceptable limits. The low detection and quantification limits indicated good sensitivity, while robustness studies proved the method's stability under slight variations in conditions. Overall, the developed method is simple, rapid, and reproducible, making it highly suitable for routine quality control and stability analysis. Its compliance with regulatory guidelines further

supports its applicability in pharmaceutical industries for reliable and consistent drug analysis.

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7. FINANCIAL ASSISTANCE
Nil

8. CONFLICT OF INTEREST
The authors declare no conflict of interest

9. AUTHOR CONTRIBUTION
Nikhil S. Shrisunder contributed to conceptualization, methodology design, experimental investigation, data acquisition, and original draft preparation; Saili S. Madur assisted in method development, experimental execution, and data validation; Smeeta N. Patil performed formal data analysis, interpretation, and manuscript editing; Shital M. Kurhade carried out validation studies, statistical analysis, and documentation; Asalm R. Tamboli contributed to literature review, data curation, and manuscript preparation; and Sachin N. Kothawade provided supervision, critical review, scientific guidance, and final approval of the manuscript. All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

10. ABBREVIATIONS
- HPLC – High Performance Liquid Chromatography
- RP-HPLC – Reverse Phase High Performance Liquid Chromatography
- LOD – Limit of Detection
- LOQ – Limit of Quantification
- %RSD – Percent Relative Standard Deviation
- ICH – International Conference on Harmonisation
- API – Active Pharmaceutical Ingredient

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