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Research Article

STABILITY-INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ROSUVASTATIN CALCIUM AND CLOPIDOGREL BISULFATE IN PHARMACEUTICAL DOSAGE FORM BY REVERSE-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Objective: The present work was focused on the development and validation of reversed-phase high-performance liquid chromatography (RP-HPLC) method which is simple, rapid, precise, accurate, sensitive, economical, and stability-indicating for the quantification of rosuvastatin (RSV) calcium and clopidogrel bisulfate (CLO) in bulk and tablet formulation.

Methods: The separation was attained on RP Princeton (C18) column with dimensions (250 mm × 4.6 mm, 5 µ) employing buffer which is a mixture of water (pH 3.0, adjusted with orthophosphoric acid), and methanol in the ratio (20:80) v/v as mobile phase, at flow rate 1.0 ml/min and detection was carried out at wavelength 240 nm. The retention time under the optimized condition of RSV calcium and CLO was found to be 2.844 min and 4.388 min, respectively.

Results: The linearity of the method was demonstrated in the concentration range of 6–16 µg/ml and 45–120 µg/ml for RSV calcium and CLO with a correlation coefficient (r^2) of 0.9999 and 0.9996, respectively. The percentage relative standard deviation was <2% and percentage recovery was found to be 100.12–101.37% and 99.72–101.09% for RSV calcium and CLO, respectively. Assay of marketed tablet formulation was found to be 98.99% and 99.92%, respectively.

Conclusion: The developed RP-HPLC method was found to be simple, specific, sensitive, rapid, linear, accurate, precise, and economical and could be used for regular quality control of RSV calcium and CLO in bulk and tablet formulations.

Keywords: Rosuvastatin calcium, Clopidogrel bisulfate, Reversed-phase high-performance liquid chromatography, Validation, International conference on harmonization guidelines.

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INTRODUCTION

Rosuvastatin (RSV) calcium is chemically (3R,5R)-7-[4-(4-Fluorophenyl)-2-[methyl(methylsulfonyl)amino]-6-propan-2-ylpyrimidin-5-yl]-3,5-dihydroxyhept-6enoic acid (Fig. 1) [1]. It is an HMG-CoA reductase inhibitor used in the treatment of hypertension and abnormal lipid [2]. Clopidogrel bisulfate (CLO) is chemically Methyl 2-(2- Chlorophenyl) -2-(6, 7-dihydro thieno [3,2-C] Pyridine-5(4H)-yl) acetate sulfate (Fig. 2) [3]. It is an anti-platelet agent as an ADP receptor blocker mainly to treat patients with the acute coronary syndrome, myocardial infarction, peripheral vascular disease, and some stroke (ischemic type) patients [4]. An extensive literature survey revealed that several high-performance liquid chromatography (HPLC) should be methods were reported for the estimation of RSV calcium and CLO in bulk and tablet formulation [5-27]. The International Conference on Harmonization (ICH) guideline entitled "Stability testing of new drug substances and products" requires that stress testing be administered to elucidate the inherent stability characteristics of the active substance [28]. An ideal stability-indicating technique is one that resolves the drug, and its degradation products efficiently. Consequently, the implementation of an analytical methodology to work out RSV and CLO, in the presence of its degradation products is sort of a challenge for pharmaceutical analysts. Therefore, it was thought necessary to study the stability of RSV and CLO under acidic, alkaline, hydrolytic, oxidative, light, and thermal conditions. The reported methods have the drawbacks of long runtime and less economical with a high proportion of organic phase. Hence, an attempt was made to develop RP-HPLC method which is simple, rapid, accurate, precise, specific, economical, sensitive, and stability-indicating for the estimation of RSV calcium and CLO in bulk and tablet formulations.

METHODS

Chemicals and reagents

Pharmaceutical grade RSV calcium and CLO were procured as a gift sample from Cadila Pharmaceuticals Ltd., Ahmedabad (India), Rosmi CV, a tablet formulation, obtained commercially.

Methanol, orthophosphoric acid, hydrochloric acid, sodium hydroxide, and hydrogen peroxide 30% of analytical grade were used throughout the work.

Instrumentation

Shimadzu HPLC system and PDA detector with Lab Solution software were used.

Chromatographic conditions

Chromatographic separation was achieved on a reversed-phase (RP) Princeton (C18) column with dimensions (250 mm × 4.6 mm, 5 µ) at ambient temperature using a mobile phase consisting of a mixture of buffer (pH 3.0, adjusted with orthophosphoric acid), and methanol in the ratio of (20:80)v/v at a flow rate of 1.0 ml/min. Detection was carried out at 240 nm. The pH of the mobile phase was set at 3.0, and a column temperature of 30°C. The injection volume was 10 µl. The optimized chromatographic conditions are shown in Table 1.

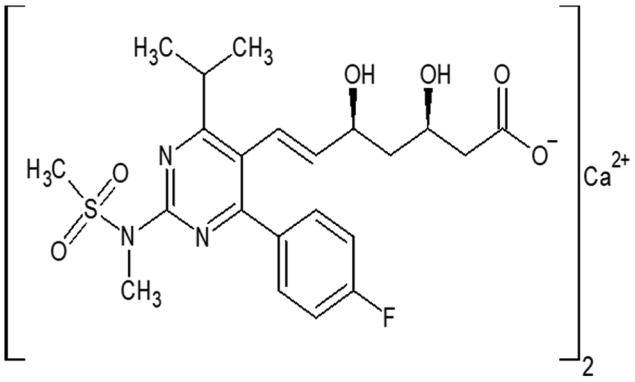

Fig. 1: Chemical structure of rosuvastatin calcium

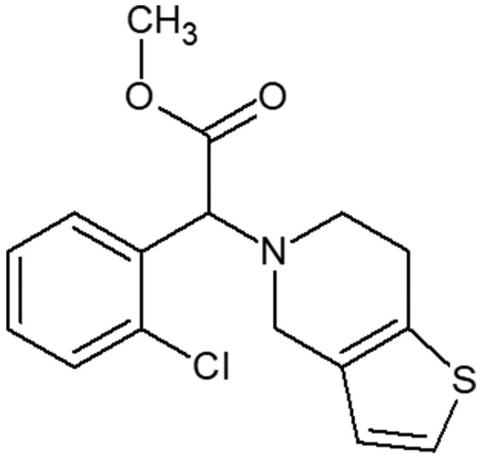

Fig. 2: Chemical structure of clopidogrel bisulfate

Table 1: Optimized chromatographic condition

Parameters	Chromatographic condition
Mobile phase	Water (pH adjusted to 3.0 with orthophosphoric acid):Methanol (20:80) v/v
Flow rate	1.0 ml/min
Column	Princeton C18 (250 mm × 4.6 mm, 5 µ)
Detector wavelength	240 nm
Column temperature	30°C
Injection volume	10 µl
Runtime	20 min
Diluent	Methanol
Retention time	About 2.844 min for rosuvastatin calcium peak and 4.388 min for clopidogrel bisulfate peak

Preparation of standard solution of RSV and CLO

For RSV, an accurately weighed 1.0 mg of RSV was transferred to a 10.0 ml volumetric flask and dissolved in 5.0 ml of methanol. The volume was completed to 10.0 ml with methanol. One milliliter of the resulting solution was pipetted in 10.0 ml volumetric flask and the volume was made up to 10.0 ml with methanol to furnish a solution of concentration 10 µg/ml of RSV. For CLO, an accurately weighed 7.5 mg of CLO was transferred to a 10.0 ml volumetric flask and dissolved in 5.0 ml of methanol. The volume was completed to 10.0 ml with methanol. One milliliter of the resulting solution was pipetted in 10.0 ml volumetric flask, and the volume was made up to 10.0 ml with methanol to furnish a solution of concentration 75 µg/ml of CLO. For the working mixed standard solution, an accurately weighed 1.0 mg of RSV and 7.5 mg of CLO were transferred to a 10.0 ml volumetric flask and dissolved in 5.0 ml of methanol. The volume was completed to 10.0 ml with methanol. One milliliter of the resulting solution was pipetted in 10.0 ml volumetric flask and the volume was made up to 10.0 ml with methanol to furnish a solution of concentration 10 µg/ml and 75 µg/ml of RSV and CLO, respectively.

Preparation of sample solution of RSV and CLO

Twenty tablets were weighed and finely powdered. An accurately weighed amount of powder equivalent to 1.0 mg of RSV and 7.5 mg of CLO was transferred into a 10.0 ml volumetric flask. Then, 5.0 ml of methanol was added in it. The flask contents were sonicated for 10 min to make the contents homogeneous. This solution was then diluted up to the mark with methanol. The resultant solution was filtered through Whatman Grade I filter paper. One milliliter of the filtrate was transferred to a 10 ml volumetric flask and then the volume was made up to the mark with methanol to furnish a sample solution containing 10 µg/ml of RSV and 75 µg/ml of CLO. Six replicate of tablet powder equivalent to 1.0 mg of RSV and 7.5 mg of CLO was transferred into six 10.0 ml volumetric flask, and homogeneous sample solutions were prepared similarly.

Method Validation

The developed method was validated following ICH guidelines (ICH Q2R1) for accuracy, precision, specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), and robustness [29].

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a standard true value or an accepted reference value, and therefore, the value found. It was computed at three different levels, that is., 80, 100, and 120% of the label claim. Standard addition and recovery experiments were conducted to determine the accuracy of RSV and CLO for the quantification of drugs in the samples.

Precision

The system precision was evaluated by measuring the area of six qualified working standards for RSV and CLO and calculating the percentage of relative standard deviation (RSD). The assay method precision was evaluated by conducting six independent assays of test samples of RSV and CLO against qualified working standards and calculating the percentage of RSD.

Linearity

Linearity test solutions of RSV and CLO were prepared at concentration levels of 6–16 µg/ml and 45–120 µg/ml, respectively. Linearity test solutions were prepared by diluting the stock solution to the required concentrations. Linearity was established by the least-squares linear regression analysis of the calibration data. Peak areas were plotted against the respective concentrations and linear regression analysis performed on the resulting curves. The linear curve of RSV calcium and CLO was shown in Figs. 3 and 4, respectively.

Specificity

The specificity of the developed method was established by comparing the chromatograph of the standard and sample. It was found that there was no interference due to excipients and impurities at the retention time of the drug.

LOD and LOQ

The LOD is the lowest analyte concentration that can be detected. LOQ is the lowest analyte concentration that can be quantified with acceptable accuracy and precision. The LOD and LOQ were calculated from the standard deviation of the response and the slope of the calibration plot. LOD and LOQ were established, under ICH definitions, by use of the equations LOD = 3.3 σ/S and LOQ = 10 σ/S, where σ is the standard deviation of the regression line and S is the slope of the calibration plot.

Robustness

To evaluate the robustness of the developed method, the chromatographic conditions were deliberately altered and the resolution between RSV and CLO was evaluated. To study the effect of wavelength on the estimation, the wavelength was altered by ± 2 nm, that is, 238 and 242 nm from the actual wavelength, 240 nm. To study the effect of flow rate on estimation, the flow rate was altered by ± 0.1 ml/min, that is, 0.9 and 1.1 ml/min from the actual flow rate, 1.0 ml/min.

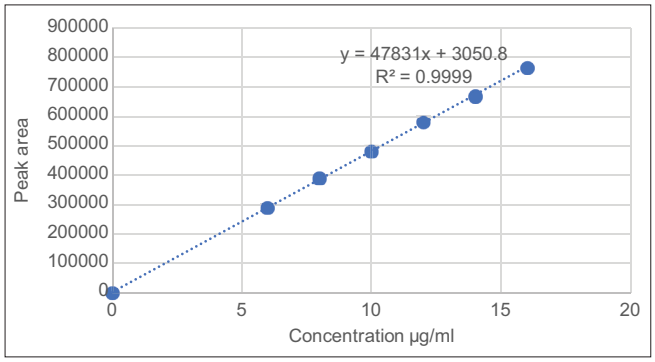

Fig. 3: Linear curve of rosuvastatin calcium

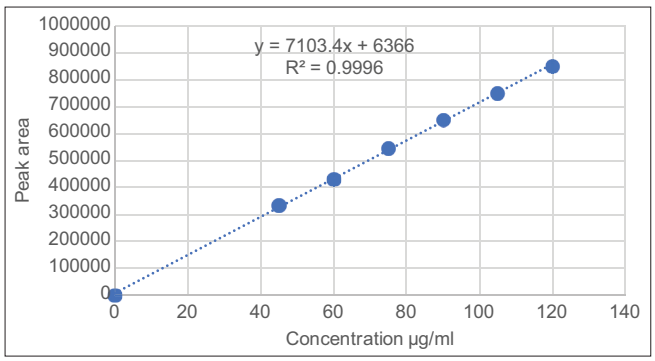

Fig. 4: Linear curve of clopidogrel bisulfate

Stability of the solution

The stability of both the standard and sample solution was checked up to 24 h at room temperature.

Stability-indicating assay

The drug was subjected to acidic (0.1 N HCl), alkaline (0.1 N NaOH), oxidative (0.3% H_2O_2), photo (light), thermal (sand bath at 50°C), and hydrolytic (water) condition and the percentage degradation was calculated.

RESULTS AND DISCUSSION

HPLC method development and optimization

Initially, pure drugs solution was chromatographed using a mobile phase consisting of a mixture of buffer (pH 3.0, adjusted with orthophosphoric acid), and methanol in the ratio of (20:80) v/v at a flow rate of 1.0 ml/min gives well-resolved peaks of drugs as well. Detection was carried out at 240 nm. The retention time under the optimized condition of RSV calcium and CLO was found to be 2.844 min and 4.388 min, respectively. The total run time of the chromatogram was about 20 min. A typical chromatograph of a mixture of standard and sample of RSV calcium and CLO was shown in Figs. 5 and 6, respectively.

Validation of the method

System suitability

The suitability of the system was demonstrated by assessing various parameters. It was established by injecting six replicate injections of the standard solution. Theoretical plates were found to be 2816 and 3811, tailing factor of 1.60 and 1.54, and %RSD of peak area was 0.9 and

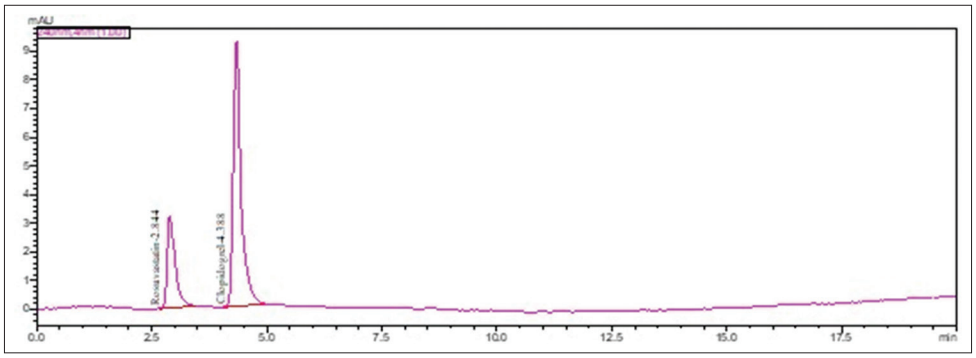

Fig. 5: Chromatogram of mixture of standard RSV and CLO. Rosuvastatin calcium (RSV) and clopidogrel bisulfate (CLO) with Rt of 2.844 min and 4.388 min, respectively

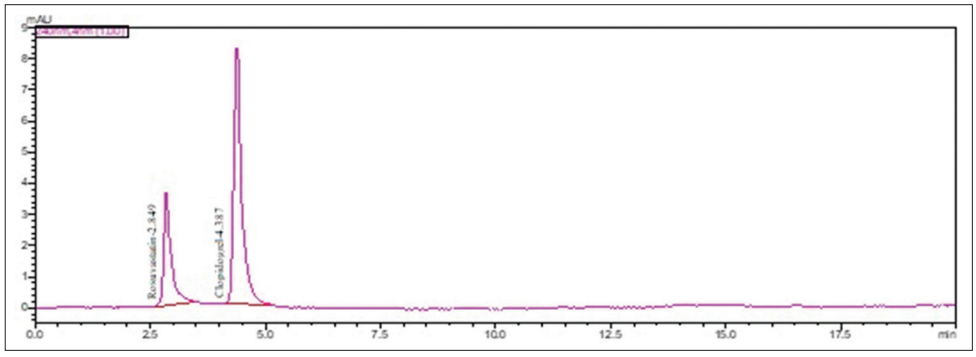

Fig. 6: Chromatogram of mixture of sample RSV and CLO. Rosuvastatin calcium (RSV) and clopidogrel bisulfate (CLO) with Rt of 2.849 min and 4.387 min, respectively

0.8 for RSV and CLO, respectively (Table 2). All the system suitability parameters were well within limits, indicating that the system was well suitable for performing the analysis.

Linearity

Linearity was established by the least-squares linear regression analysis of the calibration data. Calibration plots were linear over the concentration range of 6–16 µg/ml for RSV and 45–120 µg/ml for CLO. Peak areas were plotted against the respective concentrations and linear regression analysis performed on the resulting curves. The linear curve of RSV calcium and CLO was shown in Figs. 3 and 4, respectively. The linear regression equation obtained was Y=47831x+3050.8 for RSV and Y=7103.4x+6366 for CLO with correlation coefficient 0.9999 and 0.9996, respectively. The results of linearity are shown in Table 3.

Accuracy

Accuracy was computed by recoveries studies. The mean percentage recoveries values for three levels were found to be between 100.12–101.37% and 99.72–101.09% for RSV and CLO, respectively. The percentage recoveries values within limits, indicating the method developed was accurate. The results of recovery are shown in Table 4.

Precision

The results of intraday precision and interday precision were 0.9 and 0.6 for RSV. The results of intraday precision and interday precision were 0.8 and 0.4 for CLO. The percentage RSD of system, method, and intermediate precision study was well within limits (<2%), indicate that the method was precise.

LOD and LOQ

The LOD was found to be 0.65 µg/ml for RSV and 6.90 µg/ml for CLO. The LOQ was found to be 1.98 µg/ml for RSV and 20.91 µg/ml for CLO. The values of LOD and LOQ indicate that the method was greatly sensitive (Table 5).

Robustness

Robustness of the method was designed by changing the optimized condition adequately. To evaluate the robustness of the developed method, the chromatographic conditions were deliberately altered and the resolution between RSV and CLO was evaluated. On the assessment

Table 2: System suitability results

Parameter	RSV	CLO
Theoretical plate	2816	3811
Retention time (Rt)	2.844	4.388
Tailing factor	1.60	1.54
% RSD	0.9	0.8

Rt: Retention time, %RSD: Percentage relative standard deviation. RSV: Rosuvastatin, CLO: Clopidogrel bisulfate

Table 3: Linearity results

Parameter	RSV	CLO
Concentration range (µg/ml)	6–16	45–120
Slope (m)	47831	7103.4
Tailing factor	3050.8	6366
Coefficient correlation (r^2)	0.9999	0.9996

RSV: Rosuvastatin, CLO: Clopidogrel bisulfate

Table 4: Recovery results

Drug	Level (%)	Amount taken (µg/ml)	Amount found* (µg/ml)	% Recovery*
RSV	80	8	8.11	101.37
	100	10	10.01	100.12
	120	12	12.05	100.41
CLO	80	60	59.83	99.72
	100	75	75.81	101.09
	120	90	90.75	100.83

*Average of three determinations. RSV: Rosuvastatin, CLO: Clopidogrel bisulfate

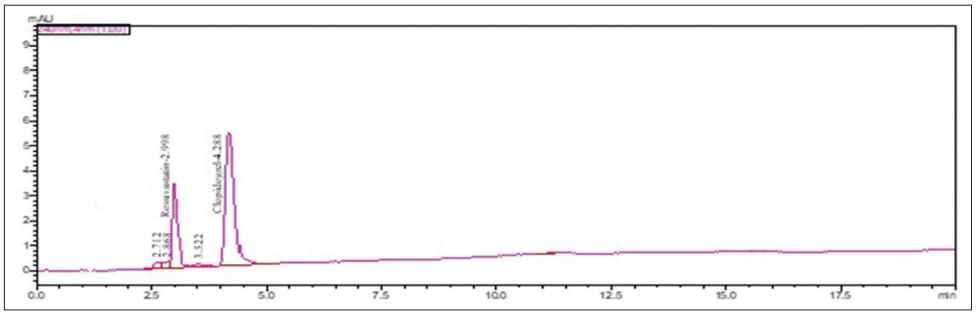

Fig. 7: Chromatogram of RSV and CLO degraded with acid hydrolysis. Rosuvastatin calcium (RSV) and clopidogrel bisulfate (CLO) with Rt of 2.998 min and 4.288 min, respectively, and degradation products of RSV with Rt2.712 and 2.868 min and degradation products of CLO with Rt3.522 min

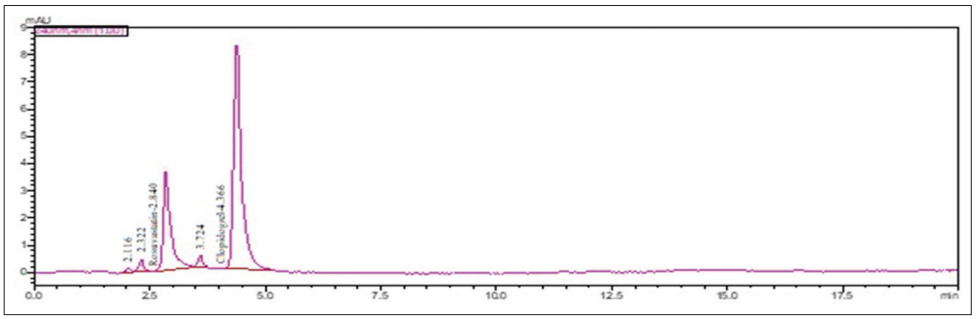

Fig. 8: Chromatogram of RSV and CLO degraded with alkali hydrolysis. Rosuvastatin calcium (RSV) and clopidogrel bisulfate (CLO) with Rt of 2.840 min and 4.366 min, respectively, and degradation products of RSV with Rt 2.322 min and degradation products of CLO with Rt 2.116 and 3.724 min

Table 5: Summary of validation parameter

Parameter	RSV	CLO
Calibration range (µg/ml)	6–16	45–120
Optimized wavelength (nm)	240	240
Retention time	2.844	4.388
Regression equation (Y)	Y = 47831x+3050.8	Y = 7103.4x+6366
Slope	47831	7103.4
Intercept	3050.8	6366
Coefficient correlation (r^2)	0.9999	0.9996
Precision (%RSD)		
Intraday	0.6	0.8
Interday	0.9	0.4
% Assay*	98.99	99.92
LOD (µg/ml)	0.65	6.90
LOQ (µg/ml)	1.98	20.91

*Average of five determinations, LOD: Limit of detection, LOQ: Limit of quantification. RSV: Rosuvastatin, CLO: Clopidogrel bisulfate, RSD: Relative standard deviation

of the result, it can be deduced that the variation in the changing wavelength, flow rate does not affect the method significantly. %RSD <2% specifies that the developed method was robust. The results of robustness are shown in Table 6.

Analysis of RSV calcium and CLO from marketed tablets

The percentage assays of tablet formulation were found to be 98.99 and 99.92% for RSV and CLO, respectively. The stability of the drug solution was observed for 24 h. In degradation studies, the drug was exposed to various stress conditions. From the chromatograms of stressed samples, it was found that no interference from degradants was observed at the retention time of RSV calcium and CLO. Optimum degradation was observed in the presence of acid and alkali. Substantial degradation was observed in the presence of water, light, and peroxide. No degradation was observed in the presence of heat for RSV and CLO. The results of the percentage of degradation are presented in Table 7 and Figs. 7-12. Hence, the method was found to be specific.

Table 6: Robustness results

Condition	RSV		CLO	
	Amount estimated* (%)	RSD (%)	Amount estimated* (%)	RSD (%)
Change in wavelength 238 nm	97.28	0.5698	100.16	0.0900
(240±2 nm) 242 nm	98.21	0.5733	100.08	0.1417
Change in flow rate 0.9 ml/min	99.55	0.6381	99.37	0.2068
(1.0±0.1 ml/min) 1.1 ml/min	99.24	0.5078	99.56	0.2009

*Average of three determinations, %RSD: Percentage relative standard deviation. RSV: Rosuvastatin, CLO: Clopidogrel bisulfate, RSD: Relative standard deviation

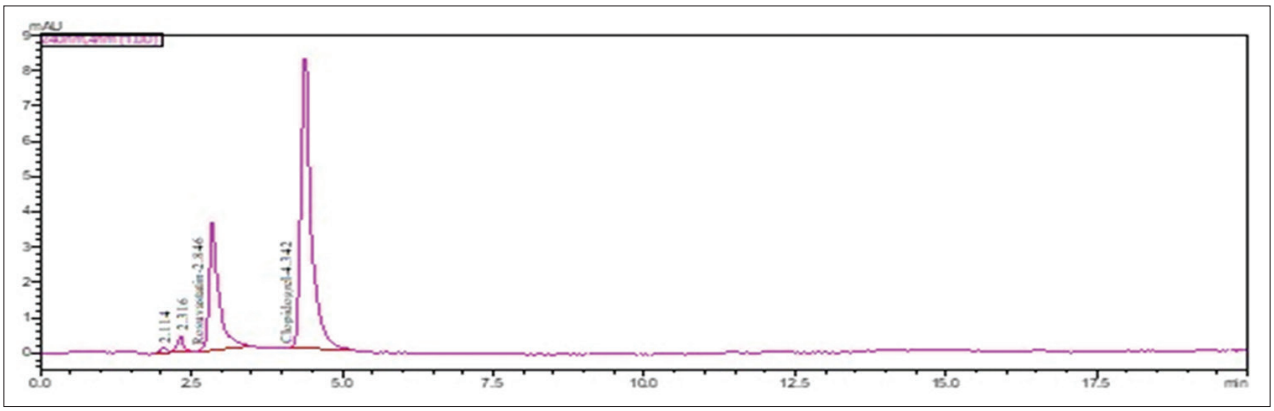

Fig. 9: Chromatogram of RSV and CLO degraded with neutral hydrolysis. Rosuvastatin calcium (RSV) and clopidogrel bisulfate (CLO) with Rt of 2.846 min and 4.342 min, respectively, and degradation products of RSV with Rt 2.114 and 2.316 min and no degradation products of CLO

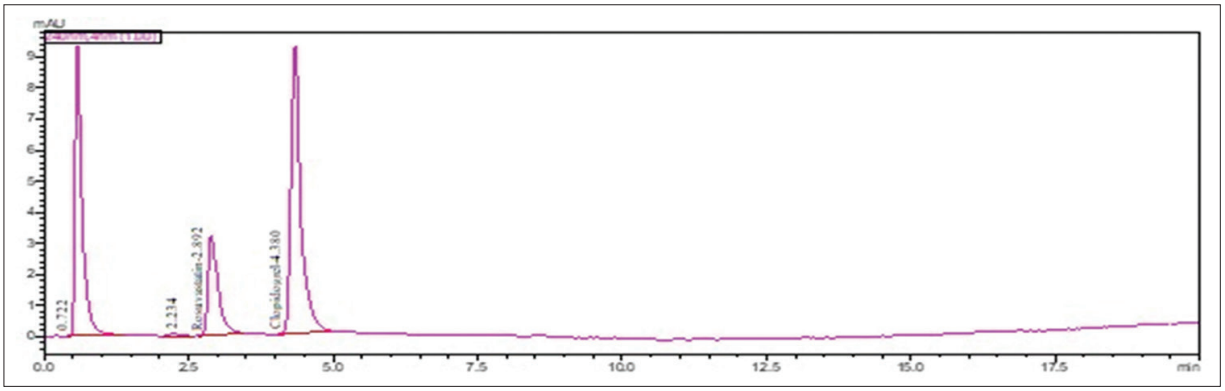

Fig. 10: Chromatogram of RSV and CLO degraded with oxidative hydrolysis. Rosuvastatin calcium (RSV) and clopidogrel bisulfate (CLO) with Rt of 2.892 min and 4.380 min, respectively, and degradation products of RSV with Rt0.722 and 2.234 min and no degradation products of CLO

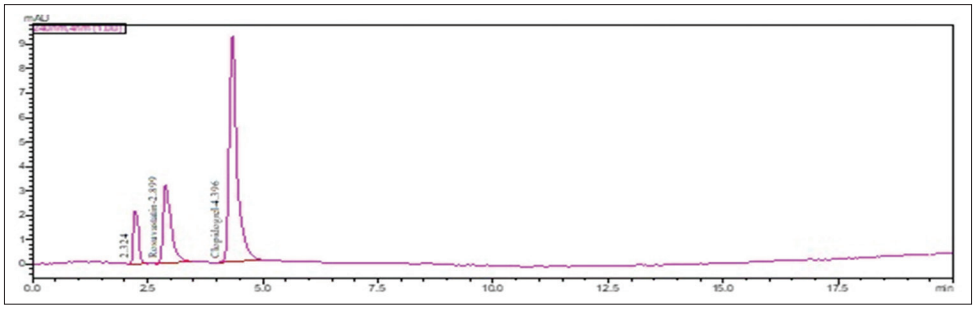

Fig. 11: Chromatogram of RSV and CLO degraded with exposed to direct sunlight. Rosuvastatin calcium (RSV) and clopidogrel bisulfate (CLO) with Rt of 2.899 min and 4.396 min, respectively, and degradation products of RSV with Rt 2.324 min and no degradation products of CLO

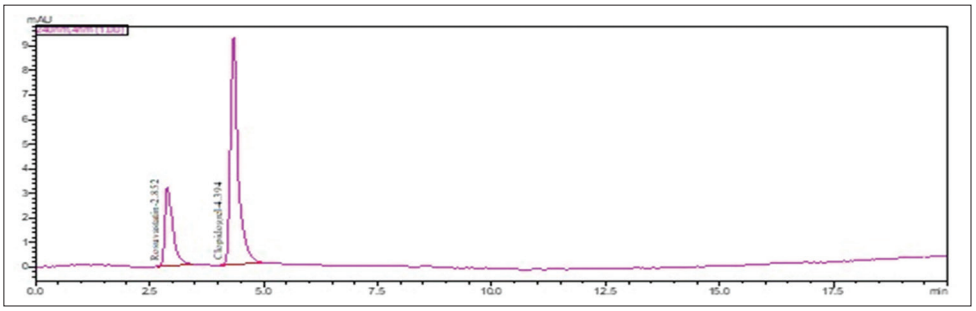

Fig. 12: Chromatogram of RSV and CLO degraded with thermal hydrolysis. Rosuvastatin calcium (RSV) and clopidogrel bisulfate (CLO) with Rt of 2.852 min and 4.394 min, respectively, and no degradation products of RSV and CLO

Table 7: Stability-indicating method data for RSV and CLO

Stress condition	RSV (%Degradation)	CLO (%Degradation)
Acidic (0.1N HCl for 24 h)	9.29	8.26
Alkaline (0.1N NaOH for 24 h)	8.72	6.24
Hydrolytic (HPLC waters for 24 h)	6.16	No degradation
Oxidative (0.3% H_2O_2 for 24 h)	7.82	No degradation
Photo (Sunlight for 30 days)	1.45	No degradation
Thermal (Sand bath at 50°C for 24 h)	No degradation	No degradation

HPLC: High-performance liquid chromatography, RSV: Rosuvastatin, CLO: Clopidogrel bisulfate

CONCLUSION

The method enables simple, rapid, accurate, precise, specific, economical, and sensitive analysis of RSV calcium and CLO in bulk and tablet dosage form. This method was validated as per ICH guidelines. The method can, therefore, be used for routine quality control analysis RSV calcium and CLO in bulk and tablet dosage form.

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AUTHORS' CONTRIBUTIONS

AP and RK designed the study. AP performed the experiment, and analyzed the data, and reviewed it. RK supervised the experiment, reviewed the data, and supported for writing the manuscript.

CONFLICTS OF INTEREST STATEMENT

Authors declare that they have no conflicts of interest that exist in this investigation.

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