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Development and Validation of Stability-Indicating Assay Method by RP-HPLC for Simultaneous Estimation of Rosuvastatin Calcium and Fenofibrate in Pharmaceutical Dosage Form

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ABSTRACT

A Simple, precise, and accurate stability-indicating reversed-phase high-performance liquid chromatography method has been established for the simultaneous estimation of rosuvastatin calcium and fenofibrate in combined bulk and tablet formulation. The chromatographic separation was performed on reverse phase Princeton (C18) (250 mm x 4.6 mm, 5µ) column with mobile phase as a mixture of water (pH adjusted to 3.0 with orthophosphoric acid) and acetonitrile in the ratio (40:60) v/v at the flow rate 1.0 ml/min. Detection was carried out at wavelength 240 nm. The retention time under the optimized condition of Rosuvastatin calcium and Fenofibrate was found to be 2.485 & 3.905 minutes respectively. The calibration curve was linear in the range of 6-16 µg/ml and 87-232 µg/ml for rosuvastatin calcium and fenofibrate with a correlation coefficient of 0.9999 and 0.9994 respectively. Relative standard deviation values for all key parameters were less than 2.0%. The percentage recovery was found to be 99.66-100.37% and 99.13-100.44% for rosuvastatin calcium and fenofibrate respectively. The developed reversed-phase high-performance liquid chromatography method was found to be simple, specific, sensitive, rapid, linear, accurate, precise, and economical, and could be used for regular quality control of rosuvastatin calcium and fenofibrate in bulk and tablet formulations.

Keywords: Rosuvastatin calcium, Fenofibrate, RP-HPLC, Method validation, ICH guidelines.

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INTRODUCTION

Rosuvastatin calcium (RSV), is chemically (3R,5S,6E)-7-[4-(4-fluorophenyl)-6-(1-methylene)-2[methyl (methyl sulphonyl amino)]-5pyrimidinyl]-3,5-dihydroxy-6-heptenoic acid calcium (Fig. 1). It is a synthetic lipid-lowering agent that blocks the production of cholesterol in the body, it is a competitive 3-hydroxy-3-methyl-glutaryl coenzyme-A reductase inhibitor effective in lowering LDL cholesterol and triglycerides, developed for the treatment of dyslipidemia [1,2]. Fenofibrate (FEN), is chemically propan-2-yl 2-{4-[(4-chlorophenyl) carbonyl] phenoxy} methyl propanoate (Fig. 2). It is mainly used to decreased cholesterol levels in patients at risk of cardiovascular disease. Like other fibrates, it reduces low-density. lipoprotein (LDL) and very-low-density lipoprotein (VLDL) levels, as well as increasing high-density lipoprotein (HDL) levels and reducing triglycerides levels. The drug is white to white-off powder, soluble in dimethylformamide, dimethyl sulfoxide, acetone, acetonitrile slightly soluble in water and methanol [3-5]. An extensive literature survey revealed that several HPLC should be methods were reported for the estimation of rosuvastatin calcium and fenofibrate in bulk and tablet formulation [6-17]. 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 [18]. 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 FEN, 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 FEN under acidic, alkaline, hydrolytic, oxidative, light, and thermal conditions. The reported method has the drawbacks of long runtime and less economical with a high proportion of organic phase. Hence, an attempt was made to develop reversed-phase high-performance liquid chromatography (RP-HPLC) which is a simple, rapid, accurate, precise, specific, economical, and sensitive method for the estimation of rosuvastatin calcium and fenofibrate in combined bulk and tablet dosage form.

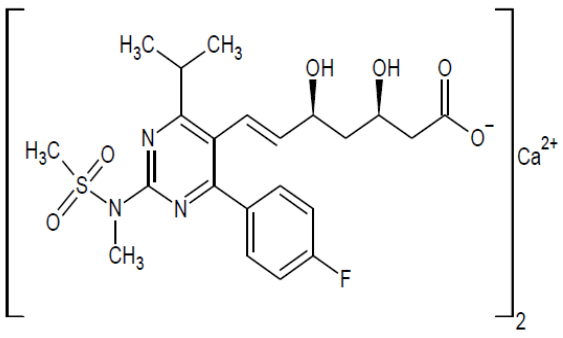

Figure 1: Chemical structure of rosuvastatin calcium (RSV)

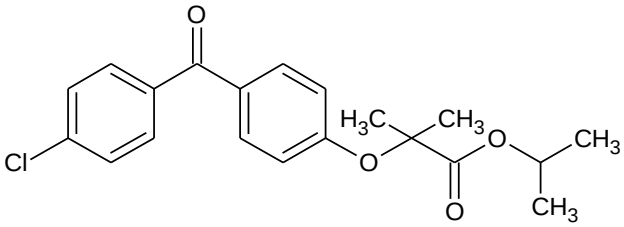

Figure 2: Chemical structure of fenofibrate (FEN)

MATERIALS AND METHODS

Chemicals and Reagents

Pharmaceutical grade Rosuvastatin Calcium and Fenofibrate were procured as a gift sample from Cadila Pharmaceuticals Ltd., Ahmedabad (India), Zyrova F-10 a tablet formulation, obtained commercially.

Acetonitrile, 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 reverse-phase column Princeton C18 (250 mm × 4.6 mm, 5µ) at ambient temperature using a mobile phase consisting of a mixture of buffer (pH 3.0, adjusted with orthophosphoric acid) and acetonitrile in the ratio of (40:60) 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, Injection volume was 10 µl. The results of the optimized chromatographic condition are shown in Table 1.

Table 1: Optimized chromatographic condition

Chromatographic condition	
Mobile phase	Water (pH adjusted to 3.0 with ortho phosphoric acid):Acetonitrile (40:60) v/v
Flow rate	1.0 ml/min.
Column	Princeton C18 (250 mm × 4.6 mm, 5µ)
Detector wavelength	240 nm
Column temperature	30 °C
Injection volume	10 µl
Runtime	20 minutes
Diluent	Acetonitrile:Water (50:50)
Retention time	About 2.485 minutes for Rosuvastatin calcium peak & 3.905 minutes for Fenofibrate peak

Preparation of standard solution of RSV and FEN

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 diluent. The volume was completed to 10.0 ml with diluent. 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 diluent to furnish a solution of concentration 10 µg/ml of RSV.

For FEN, an accurately weighed 14.5 mg of FEN was transferred to a 10.0 ml volumetric flask and dissolved in 5.0 ml of diluent. The volume was completed to 10.0 ml with diluent. 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 diluent to furnish a solution of concentration 145 µg/ml of FEN.

For the working mixed standard solution, an accurately weighed 1.0 mg of RSV and 14.5 mg of FEN were transferred to a 10.0 ml volumetric flask and dissolved in 5.0 ml of diluent. The volume was completed to 10.0 ml with diluent. 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 diluent to furnish a solution of concentration 10 µg/ml and 145 µg/ml of RSV and FEN respectively.

Preparation of sample solution of RSV and FEN

Twenty tablets were weighed and finely powdered. An accurately weighed amount of powder equivalent to 1.0 mg of RSV and 14.5 mg of FEN was transferred into a 10.0 ml volumetric flask. Then 5.0 ml of diluent 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 diluent. 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 diluent to furnish a sample solution containing 10 µg/ml of RSV and 145 µg/ml of FEN. Six replicate of tablet powder equivalent to 1.0 mg of RSV and 14.5 mg of FEN were 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), robustness [19].

Stress studies and specificity:

Stress testing of drug substances can help to identify the likely degradation products, which can, in turn, help to work out the degradation pathways and thus the intrinsic stability of the drug substances. Specificity is the ability of the method to measure the responses of the analyte in the presence of its related substances. All stress degradation studies were performed at initial drug concentrations of 0.1 mg/ml for RSV and 1.45 mg/ml for FEN. Acid hydrolysis was performed in 0.1N HCl at 80˚C for 24 hours. Alkali hydrolysis was performed in 0.1N NaOH at 80˚C for 24 hours. Neutral hydrolysis was performed at 80˚C for 24 hours. Oxidation studies were conducted at room temperature in 30% hydrogen peroxide for 24 hours. For photodegradation studies, the drug sample was exposed to sunlight for 30 days. Thermal hydrolysis was performed on the sand bath at 50°C for 24 hrs. Samples were withdrawn at appropriate times and subjected to HPLC analysis after suitable dilution to evaluate the ability of the proposed method to separate RSV and FEN from their degradation products.

System Suitability:

Specificity is the ability of the method to measure the responses of the analyte in the presence of its related substances. 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 3636 and 4997, tailing factor of 1.30 and 1.45, and %RSD of peak area was 0.9 for both RSV and FEN respectively.

Linearity

Linearity test solutions of RSV and FEN were prepared at concentration levels of 6-16 µg/ml and 87-232 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 linearity curve for RSV and FEN was summarized by Fig. 3 and Fig. 4 respectively.

Precision

The system precision was evaluated by measuring the area of six qualified working standards for RSV and FEN 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 FEN against qualified working standards and calculating the percentage of relative standard deviation (RSD).

Accuracy

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

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 limits of detection (LOD) and quantification (LOQ) were calculated from the standard deviation of the response and the slope of the calibration plot. LOD and LOQ were established, following ICH definitions, by use of the equations LOD = 3.3σ/S and LOQ = 10σ/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 FEN was evaluated. To study the effect of wavelength on the estimation, the wavelength was altered by ± 2 nm, i.e., 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 i.e., 0.9 and 1.1 ml/min from the actual flow rate, 1.0 ml/min.

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 acetonitrile in the ratio of (40:60) v/v at a flow rate of 1.0 ml/min. gives well-resolved peaks of drugs. Detection was carried out at 240 nm. The retention time under the optimized condition of rosuvastatin calcium and fenofibrate was found to be 2.485 min. & 3.905 min. respectively. The total run time of the chromatogram was about 20 minutes. A typical chromatograph of a mixture of standard and sample rosuvastatin calcium and fenofibrate is summarized by Fig. 5. and Fig. 6 respectively.

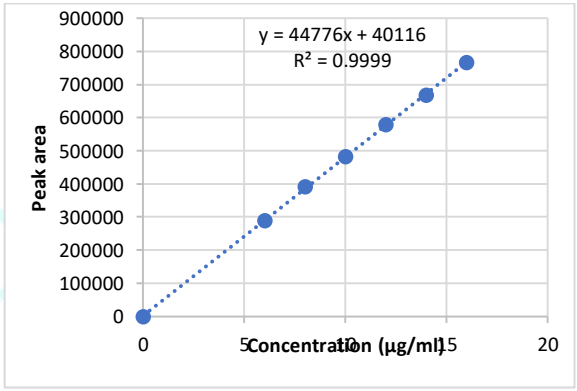

Figure 3: Linearity curve of rosuvastatin calcium

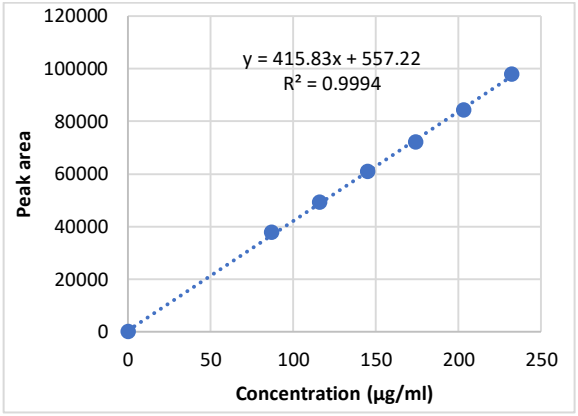

Figure 4: Linearity curve of fenofibrate

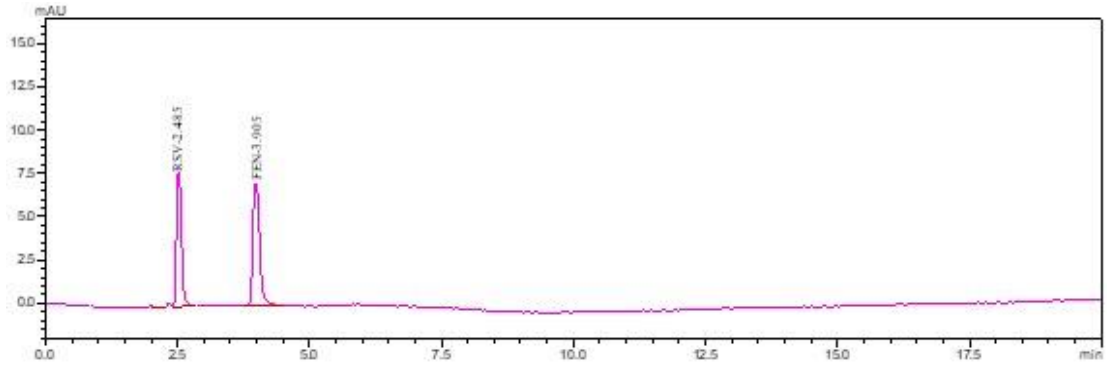

Figure 5: Chromatograph of mixture of standard Rosuvastatin calcium and Fenofibrate

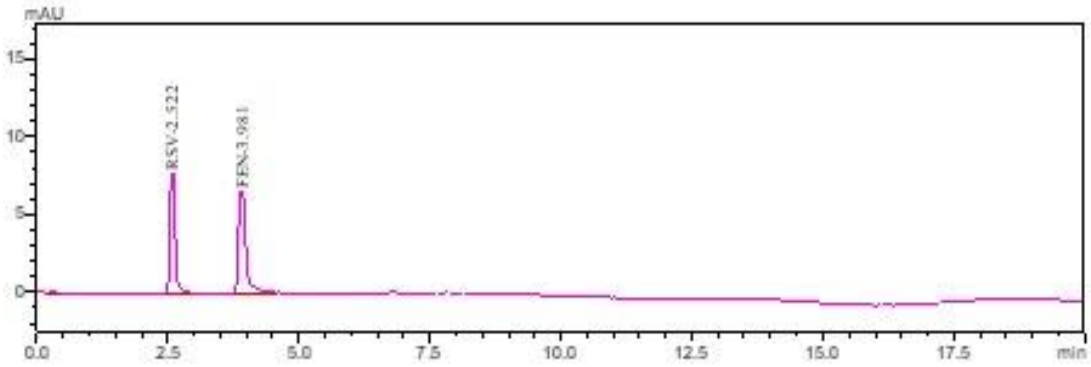

Figure 6: Chromatograph of mixture of sample Rosuvastatin calcium and Fenofibrate

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 3636 and 4997, tailing factor of 1.30 and 1.45, and %RSD of peak area was 0.9 for both RSV and FEN respectively (Table 2). All the system suitability parameters were well within the limits, indicating that the system was well suitable for performing the analysis.

Table 2: **System suitability results**

Parameter	RSV	FEN
Theoretical Plate	3636	4997
Retention Time (Rt)	2.485	3.905
Tailing factor	1.30	1.45
Resolution	2.115	6.874
% RSD	0.9	0.9

Rt: Retention time, %RSD: Percentage relative standard deviation

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 87-232 µg/ml for FEN. Peak areas were plotted against the respective concentrations and linear regression analysis performed on the resulting curves. The linear curve of rosuvastatin calcium and fenofibrate was shown in Fig. 3 and Fig. 4 respectively. The linear regression equation obtained was Y=44776x+40116 for RSV and Y=415.83x+557.22 for FEN with correlation coefficient 0.9999 and 0.9994 respectively. The results of linearity are shown in Table 3.

Table 3: **Linearity results**

Parameter	RSV	FEN
Concentration Range (µg/ml)	6-16	87-232
Slope (m)	44776	415.83
Intercept	40116	557.22
Coefficient correlation (r^2)	0.9999	0.9994

Accuracy

Accuracy was computed by recoveries studies. The mean percentage recoveries values for three levels were found to be between 99.66-100.37%cand 99.13-100.44% for RSV and FEN respectively. The percentage of recoveries values within the limits, indicating the method developed was accurate. The results of recovery are shown in Table 4.

Table 4: Recovery results

Drug	Level (%)	Amount taken (µg/ml)	Amount found* (µg/ml)	% Recovery*
RSV	80	8	7.99	99.98
	100	10	9.96	99.66
	120	12	12.04	100.37
FEN	80	116	116.51	100.44
	100	145	143.74	99.13
	120	174	172.55	99.16

*Average of three determinations

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 0.9 and 0.8 for FEN. The percentage RSD of system, method, and intermediate precision study was well within the limits (<2%), indicate that the method was precise.

LOD and LOQ

The LOD was found to be 0.33 µg/ml. for RSV and 16.51 µg/ml. for FEN. The LOQ was found to be 1.00 µg/ml. for RSV and 50.05 µg/ml. for FEN. The values of LOD and LOQ indicate that the method was greatly sensitive (Table 7).

Robustness

The 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 FEN was evaluated. On the assessment of the result it can be deduced that the variation in the changing wavelength, the 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 5.

Table 5: Robustness results

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

*Average of three determinations, %RSD: Percentage relative standard deviation

Analysis of rosuvastatin calcium and fenofibrate from marketed tablets

The percentage assay of tablet formulation was found to be 98.69 and 99.96% for RSV and FEN respectively. The stability of the drug solutions 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 rosuvastatin calcium and fenofibrate. 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 peroxide and thermal for RSV and thermal for FEN. The results of the percentage of degradation are presented in Table 6 and Fig. 7-12. Hence, the method was found to be specific.

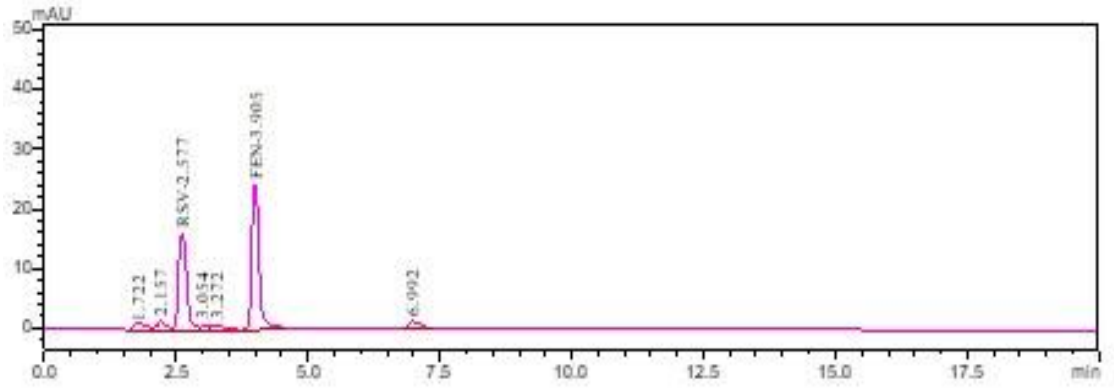

Fig. 7: Chromatogram of RSV and FEN degraded with acid hydrolysis

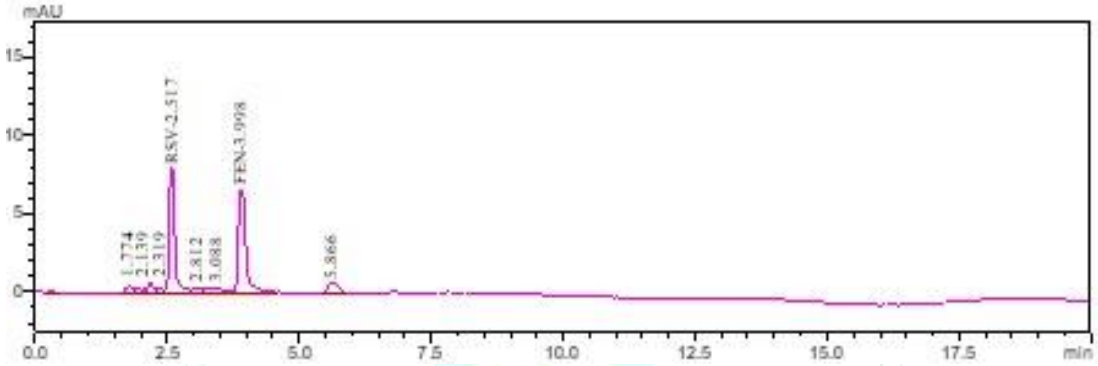

Fig. 8: Chromatogram of RSV and FEN degraded with alkali hydrolysis

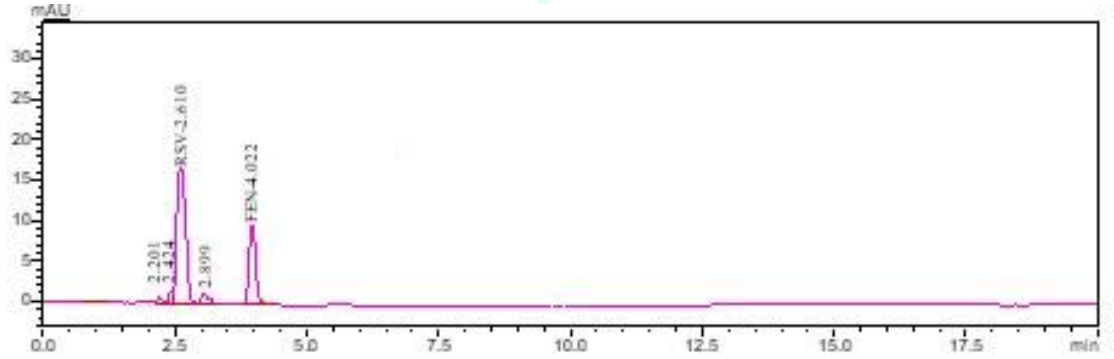

Fig. 9: Chromatogram of mixture of RSV and FEN degraded with neutral hydrolysis

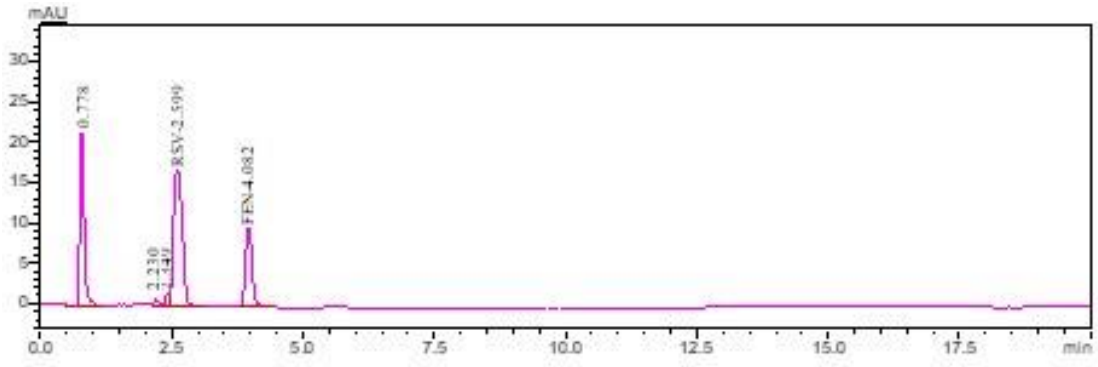

Fig. 10: Chromatogram of RSV and FEN degraded with oxidative hydrolysis

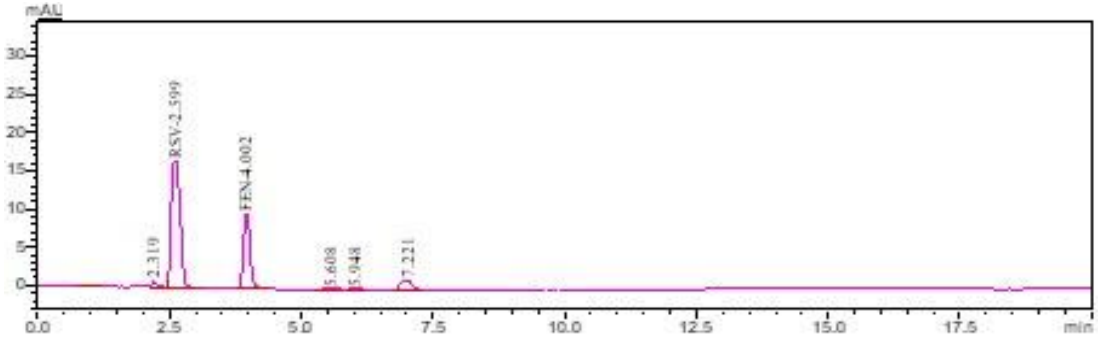

Fig. 11: Chromatogram of RSV and FEN degraded with exposed to direct sunlight

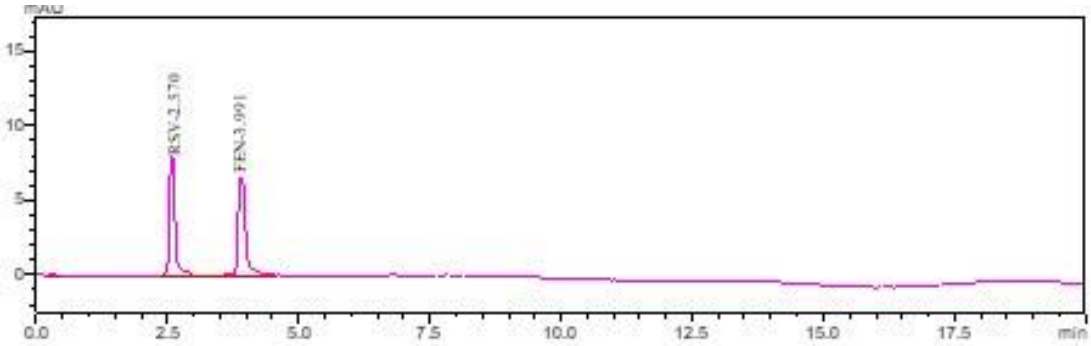

Fig. 12: Chromatogram of RSV and FEN degraded with thermal hydrolysis

Table 6: Stability-indicating method data for RSV and FEN

Stress condition	RSV (% Degradation)	FEN (% Degradation)
Acidic (0.1 N HCl for 24 hrs.)	10.06	9.29
Alkaline (0.1 N NaOH for 24 hrs.)	12.96	8.72
Hydrolytic (HPLC waters for 24 hrs.)	8.45	6.16
Oxidative (30% H_2O_2 for 24 hrs.)	No degradation	7.82
Photo (Sun light for 30 days)	6.21	1.45
Thermal (Sand bath at 50°C for 24 hrs.)	No degradation	No degradation

HPLC: High Performance Liquid Chromatography

Table 7: Summary of validation parameter

Parameter	RSV	FEN
Calibration range (µg/ml)	6-16	87-232
Optimized wavelength (nm)	240	240
Retention Time	2.485	3.905
Regression equation (Y)	Y = 44776x+40116	Y = 415.83x+557.22
Slope	44776	415.83
Intercept	40116	557.22
Coefficient correlation (r^2)	0.9999	0.9994
Precision (% RSD)		
Intraday	0.9	0.9
Interday	0.6	0.8
% Assay*	98.69	99.96
LOD (µg/ml)	0.33	16.51
LOQ (µg/ml)	1.00	50.05

*Average of five determinations, LOD: Limit of detection, LOQ: Limit of quantification

CONCLUSION

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

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CONFLICT OF INTEREST STATEMENT

Authors declare that they have no conflict of interest exists in this investigation.

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