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Development and Validation of a Stability-Indicating Reversed-Phase HPLC Method for Simultaneous Estimation of Rosuvastatin and Ezetimibe from Their Combination Dosage Forms

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Abstract

A simple, precise and rapid stability-indicating reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed and subsequently validated for the simultaneous estimation of Rosuvastatin (RSV) and Ezetimibe (EZE) from their combination drug product. The proposed method is based on the separation of the two drugs in reversed-phase mode using Hypersil C_{18} 150 x 4.6 mm, 5µ column maintained at a temperature of 40°C. The optimum mobile phase consisted of 0.05 M phosphate buffer (pH 2.5)-Methanol (45+55, v/v), mobile phase flow rate of 1.0 mL min^{-1} and UV detection was set at 242 nm. Rosuvastatin, Ezetimibe and their combination drug product were exposed to thermal, photolytic, hydrolytic and oxidative stress conditions and the stressed samples were analyzed by the proposed method. There were no interfering peaks from excipients, impurities or degradation products due to variable stress conditions and the proposed method is specific for the simultaneous estimation of RSV and EZE in the presence of their degradation products. The method was validated according to ICH guidelines. It was found to be accurate and reproducible. Linearity was obtained in the concentration range of 5-80 µg mL^{-1} for both RSV and EZE with correlation coefficients of 0.99999 and 0.99994 respectively. Mean percent recovery of triplicate samples at each level for both drugs were found in the range of 98% to 102% with RSD of less than 2.0%. The proposed method can be successfully applied in the quality control of bulk manufacturing and pharmaceutical dosage forms.

Keywords:

Stability-indicating; Rosuvastatin; Ezetimibe; Reversed-Phase HPLC

1. Introduction

Rosuvastatin (RSV) is the calcium salt of (E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl (methylsulfonyl)amino]pyrimidin-5-yl](3R,5S)-3,5-dihydroxyhept-6-enoic acid. RSV is a selective and competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme that converts 3-hydroxy-3-methylglutaryl-coenzyme A to Mevalonate, a precursor of cholesterol. RSV exhibits a high degree of specificity for uptake into the liver [1].

RSV is a member of the class of statins, used to treat hypercholesterolemia and related conditions and to prevent cardiovascular disease. It increases the number of hepatic LDL receptors on the cell-surface to enhance uptake and catabolism of LDL. Secondly, RSV

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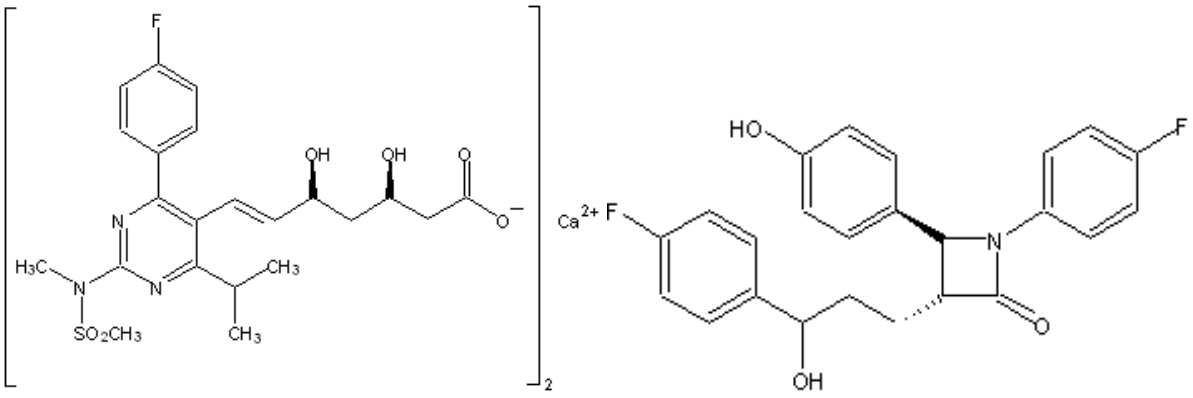
inhibits hepatic synthesis of VLDL, which reduces the total number of VLDL and LDL particles [2].

Ezetimibe (EZE), a selective inhibitor of intestinal cholesterol and related phytosterol absorption, is designated as 1-(4-fluorophenyl)-3(R)-[3-(4-fluorophenyl)-3(S) -hydroxy propyl] - 4(S) - (4 -hydroxy phenyl) -2-azetidinone. It selectively prevents the absorption of cholesterol from dietary and biliary sources by blocking the transport of cholesterol through the intestinal wall. This reduces the overall delivery of cholesterol to the liver, thereby promoting the synthesis of LDL receptors and the subsequent reduction in serum LDL-C [3,4].

EZE co-administered with HMG-CoA reductase inhibitors (statins) is licensed for the treatment of primary hypercholesterolaemia in patients, poorly controlled with a statin alone, and for homozygous familial hypercholesterolaemia. As monotherapy, it is licensed for primary hypercholesterolaemia where a statin is considered inappropriate or is not tolerated and for homozygous sitosterolaemia [5]. The chemical structures of RSV and EZE are shown in Fig.-1.



(A) (B)

Fig. 1. Chemical structures of (A) Rosuvastatin and (B) Ezetimibe

The importance and significance of a well-organized stability-testing program for the evaluation of the physical and chemical stability of a dosage form cannot be underestimated. Stability studies are indicative of the attributes of the pharmaceutical product that are susceptible to changes during storage or arising out of interactions between excipients and the active pharmaceutical ingredient(API), which are likely to influence the quality, safety and efficacy of the product. The stress testing aims at proving the quality of a drug substance or drug product with time under the influence of environmental factors, such as temperature, humidity and light and enables recommendation of appropriate storage conditions, retest periods and shelf life to be established, in addition to establishing the possible degradation pathways. The two main aspects of the drug product development that play an important role in shelf life determination are assay of the active drug and degradation products generated during the stability study. The drug product in a stability test sample needs to be determined using a stability-indicating method, as recommended by the International Conference on Harmonization (ICH) guidelines [6,7] and U.S. Pharmacopoeia 26 (USP) [8].

The literature survey for RSV revealed several methods based on different techniques, viz, HPLC for determination in plasma and serum [9]; Capillary Zone Electrophoresis for determination in pharmaceutical formulations [10]; Spectrophotometry for determination in tablets [11]; high-performance thin-layer chromatography (HPTLC) for determination in

pharmaceuticals [12] and HPLC for determination in human plasma in combination with Gemfibrozil [13]. Similarly, a survey of the analytical literature for EZE revealed methods based on HPLC for determination in pharmaceuticals [14-16], HPLC/tandem MS (LC/MS/MS) for determination in human plasma and serum [17-19] and stability-indicating HPLC method for determination in combination with Atorvastatin [20].

A stability-indicating HPLC method has been reported for the determination of RSV [21]. Similarly, a stability-indicating HPLC method with gradient elution has been reported for determination of EZE [22]. Only one method [23] has been reported for the simultaneous estimation of these two drugs in the binary mixture, but this method lacks stability indicating nature. In the present study, attempts were made to develop a simple, precise and rapid high performance liquid chromatographic method that would serve as a stability-indicating assay method for combination drug products of RSV and EZE.

2. Experimental

2.1 Materials and Reagents

Reference standards of Rosuvastatin Calcium and Ezetimibe were kindly supplied by Torrent Research Center, Gandhinagar, India with purity of 98.5% and 99.9% respectively. Tablet formulation containing 10 mg of RSV and 10 mg of EZE was procured from the local pharmacy. Methanol (HPLC grade) was purchased from Spectrochem (Mumbai, India). All other reagents and chemicals used in this study were of analytical grade. Potassium dihydrogen orthophosphate and hydrochloric acid (35%) were purchased from Ranbaxy Fine Chemicals (New Delhi, India). Orthophosphoric acid (88%), sodium hydroxide pellets and hydrogen peroxide solution (30% v/v) were purchased from Merck India Limited (Mumbai, India). Water was purified using Millipore system (Millipore Corp., Bangalore, India). Nylon syringe filters (0.45 µm) were purchased from Millex-HN, Millipore (Mumbai, India).

2.2 The Chromatographic System

A Shimadzu (Kyoto, Japan) model LC-2010C chromatograph with autoinjector, sample cooler, UV-Visible and Photodiode array (PDA) detector (SPD 10mA vp) was used. The equipment was connected to data processing system software (Class-VP 6.13 SP_2). In the developed method, a Hypersil (Thermo electron corporation) C_{18} column (150 mm x 4.6 mm i.d., 5µm particle size) was kept at temperature of 40°C. The column oven temperature was controlled with column thermostat compartment. The chromatographic separations were achieved under isocratic conditions using a mobile phase consisting a mixture of KH_2PO_4 buffer (pH 2.5; 50 mM) – Methanol (45:55, v/v). Mobile phase was filtered through 0.45µ Millipore filter and degassed by sonication. pH of buffer solution was measured using a PHAN, LABINDIA pH meter (Mumbai, India). The UV detector was set at 242 nm, as both components showed reasonably good response at this wavelength. During the course of method development, the PDA detector was set in the 190 to 400 nm range. The flow rate was adjusted to 1.0 mL min^{-1}. 10 µL of standard and test solutions were injected.

2.3 Standard and Test Solutions

2.3.1 Preparation of Stock and Standard Solutions

The standard stock solutions containing 0.5 mg mL^{-1} each of RSV and EZE were prepared separately by dissolving reference standards in water-methanol (50:50, v/v) and diluting with the same diluent. Five mL aliquots from the standard stock solutions of RSV and EZE were transferred to 50 mL calibrated volumetric flask and the volume was made up to

the mark with the same solvent mixture to prepare a mixed standard preparation having a concentration of 50 µg mL^{-1} for both drugs. Calibration curve solutions containing 5-80 µg mL^{-1} each of RSV and EZE were prepared by diluting the standard stock solution to the appropriate volume with the same diluent.

2.3.2 Preparation of Test Solution

Twenty tablets were weighed and finely powdered in a mortar. Tablet powder equivalent to 10 mg each of RSV and EZE was accurately weighed and transferred to a 200 mL calibrated volumetric flask. Around 150 mL of water-methanol (50:50, v/v) mixture was added, and the solution sonicated for 30 min. Volume was made up to the mark with the same solvent mixture. The solution was filtered through 0.45 µm nylon syringe filter. This solution contains 50 µg mL^{-1}, each of RSV and EZE.

2.4 Forced Degradation Study of drug substance and drug product.

In order to establish whether the analytical method and the assay were stability-indicating, tablets and pure active pharmaceutical ingredient (API) of both RSV and EZE were stressed under thermolytic, photolytic, hydrolytic and oxidative stress conditions as shown by Snyder et al. [24]. The degradation conditions were optimized to obtain target degradation between 10-30% as per ICH guidelines.

The hydrolytic degradation of RSV, EZE and their combination dosage form was done using 5 mL each of 0.01 N aqueous hydrochloric acid solution and 0.01 N aqueous sodium hydroxide solution to study the acidic and alkaline degradation pattern respectively. Degradation was done on 10 mg each of RSV & EZE and tablet powder equivalent to 10 mg of the two drugs. Acidic degradation was done by heating with 5 mL of 0.01 N aqueous hydrochloric acid solution at 80°C for 1h on a constant temperature water bath (Matalab, Mumbai). Alkali degradation was done by heating with 5 mL of 0.01 N aqueous sodium hydroxide solution at 60°C for 10 min on constant temperature water bath. After the degradation, solutions were neutralized to pH 7.0 after cooling to room temperature. Solutions were then diluted using water-methanol (50:50, v/v) mixture to obtain solutions in a concentration of 50 µg mL^{-1}.

The oxidative degradation of RSV, EZE and their combination dosage form was done using 5 mL of 10% v/v hydroxide peroxide solution by heating at 80°C for 1h on a constant water temperature bath. Degradation was done on 10mg each of RSV and EZE and tablet powder equivalent to 10 mg of each of the drugs. After the degradation, solutions were allowed to attain room temperature and diluted with water-methanol (50:50, v/v) mixture to obtain solutions in concentration of 50 µg mL^{-1}.

For thermal stress, 10 mg each of RSV and EZE and tablet powder equivalent to 10 mg of both drugs were placed in hot air oven (Labline, Mumbai, India) at 105°C for 24 h under dry heat conditions in open petridishes. Solutions of the solid samples were subsequently prepared after quantitative transfer and dilution using water-methanol (50:50, v/v) to give solutions having a concentration of 50 µg mL^{-1}.

For photodegradation, 10 mg each of RSV and EZE and tablet powder equivalent to 10 mg of two drugs were irradiated with a UV lamp (254nm) (Camag, Muttenz, Switzerland) in a cabinet for 12 h. Solutions of the solid samples were then prepared after quantitative transfer and diluting using water-methanol (50:50, v/v) to give solutions having a concentration of 50 µg mL^{-1}.

2.5 Validation of the method

The developed method was validated according to ICH [25] guidelines. To check the system performance, the system suitability parameters were measured. System precision was determined on five replicate injections of standard preparations. Number of theoretical plates and asymmetry were measured. Linearity was performed with eight concentrations, ranging from 5 μg mL^{-1} to 80 μg mL^{-1} for both RSV and EZE. The peak areas versus concentration of drug were plotted and a linear least-square regression analysis was conducted to determine the slope, intercept and correlation coefficient (r) to demonstrate the linearity of the method.

The limit of detection (LOD) and limit of quantitation (LOQ) of RSV and EZE were determined by calculating the signal-to-noise (S/N) ratio of 3:1 and 10:1, respectively. Precision (repeatability) of the developed method was evaluated by assaying six sets of test samples prepared for assay determination, all on the same day (intraday precision). System precision and method precision were also determined by performing the same procedures on a different day on another instrument under the same experimental conditions (intermediate precision).

Recovery experiments were carried out by addition of standards of RSV and EZE. Three different levels of 50%, 100% and 150% of standards were added to pre-analyzed tablet samples in triplicate. The percentage recoveries of RSV and EZE at each level were determined. The mean recoveries and the relative standard deviation were then calculated. The robustness of the method was evaluated by assaying the test solutions after slight but deliberate changes in the analytical conditions- flow rate ($\pm$ 0.1 mL min^{-1}), proportion of buffer and methanol (47:53 and 43:57, v/v), and pH of the buffer ($\pm$ 0.2). Stability of standard and test solution (prepared from the dosage form) was established by storage at 25°C and 15°C for 48 h. During the storage period, the test solutions were re-analyzed at intervals of 6, 12, 24, 36 and 48 h and assay was determined against appropriate fresh standard preparations.

3. Results and Discussion

3.1 HPLC method development and Optimization

A stability-indicating HPLC method has been developed for the determination of both RSV and EZE in presence of their degradation products. Various development trials were taken for the development of a chromatographic system for the estimation of RSV and EZE in their fixed dosage form. On the basis of their structural formula, the reversed-phase liquid chromatography was selected. Application of the forced degradation study was considered an integral aspect for the development of a stability-indicating assay method for the simultaneous determination of RSV and EZE. In the developed method, all of the generated impurities were separated from the main peaks with good resolution along with the closely eluting impurities.

Various columns are available for RP-HPLC method. A C_{18} Hypersil BDS (150 x 4.6 mm i.d.) having 5 μm particle size column was preferred over other columns, as the main aim of the method was to resolve both the compounds in the presence of their degradation products and impurities. The column provided excellent peak symmetry, better reproducibility, lower back pressure, high resolution power and high degree of retention. The column temperature was kept at 40°C to separate the major impurity found during acid degradation near to RSV peak from the main peak of RSV.

A variety of mobile phases were tried to develop a suitable stability-indicating method for the simultaneous estimation of RSV and EZE. 0.05 M phosphate buffer, pH 2.5 (pH adjusted to 2.5 with 10% v/v orthophosphoric acid solution) showed the optimum separation of the drugs from their degradation products under all types of stress conditions studied.

Different solvents were tried with the buffer to get better separation but methanol showed a better resolution as compared to acetonitrile. The compositions of the mobile phase were varied to optimize the separation conditions. The optimum mobile phase consisting of buffer–methanol (45+55, v/v) was selected after analyzing all the degraded samples with PDA detector and evaluating the stability-indicating ability of the method.

The flow rate was set at 1.0 mL min^{-1}, as it was found to ideally resolve the peaks of RSV and EZE and give the complete separation of their degradation products. Wavelength at 242nm gave good response at the specified concentration. Under the described experimental conditions, all the peaks were well defined and free from tailing. The effects of small deliberate changes in the mobile phase composition, pH and flow rate were evaluated as a part of testing for method robustness.

3.2 Forced degradation study

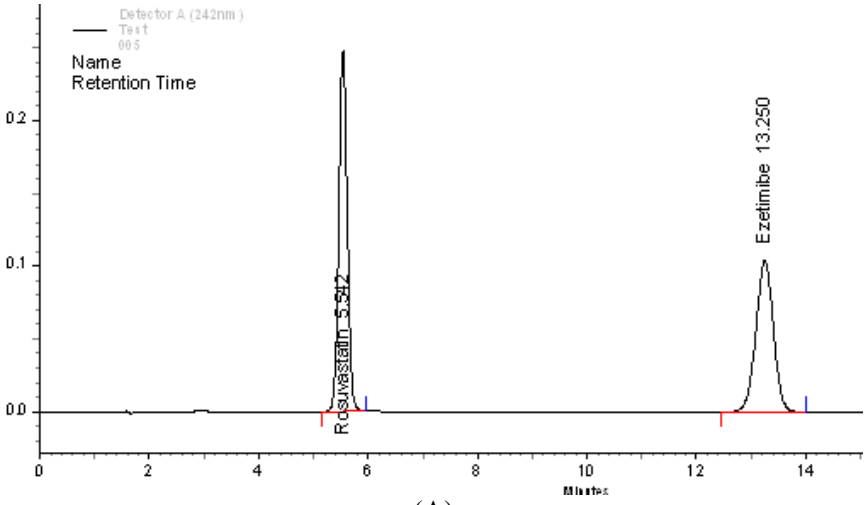
As per ICH guidelines, the target degradation between 10-30% should be there for the stability-indicating ability of the assay method and the same was tried in the present study. Fig. 2 shows the chromatogram of untreated drugs in solution, of the tablet powder.

3.2.1 Acid degradation study

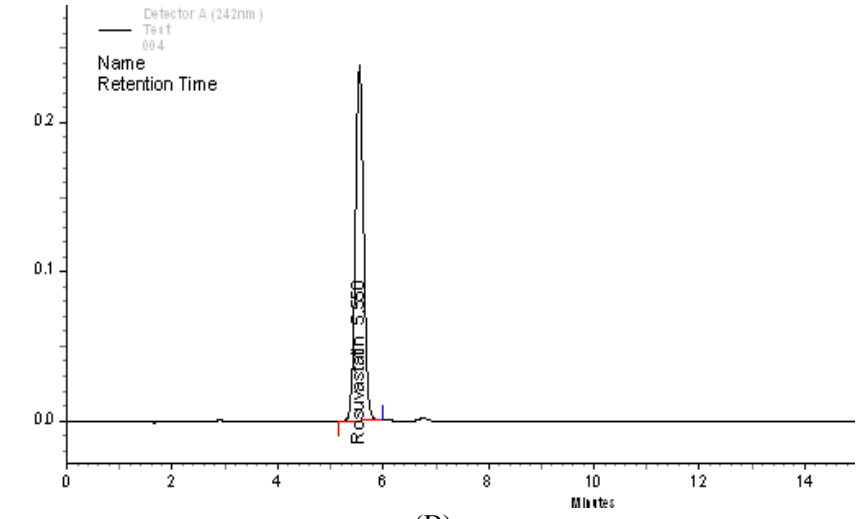
RSV showed extensive degradation in acidic hydrolytic conditions. RSV was found to be unstable in acidic conditions, while EZE was found to be stable. During the initial forced degradation experiments, it was observed that high degree of degradation of RSV occurred on heating with 0.1 N aqueous hydrochloric acid solution at 80°C for 1 h. Therefore, in later experiments, the acid hydrolysis of drug product in solution state was conducted by heating with 0.01 N aqueous hydrochloric acid solution at 80°C for 1 h. Major degradation product of acid hydrolysis of RSV was found at Relative Retention Time (RRT) of 1.11 as shown in Fig. 3.

3.2.2 Alkali degradation study

Extensive degradation of EZE was occurred in basic hydrolytic conditions, while RSV was found to be stable in these conditions. EZE is highly unstable in basic conditions. Complete degradation of EZE occurred on heating with 0.1 N aqueous sodium hydroxide solution at 80°C for 30 min. Subsequently base hydrolysis of drug product in solution state was performed by heating with 0.01 N aqueous sodium hydroxide solution at 60°C for 10 min. Major degradation product of alkali hydrolysis of EZE was found at RRT of 0.80 as shown in Fig. 4.



(A)



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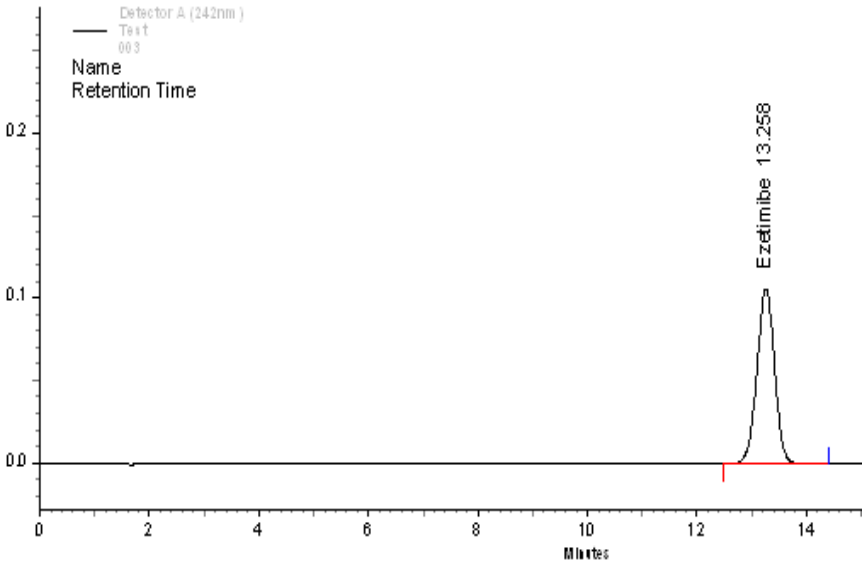
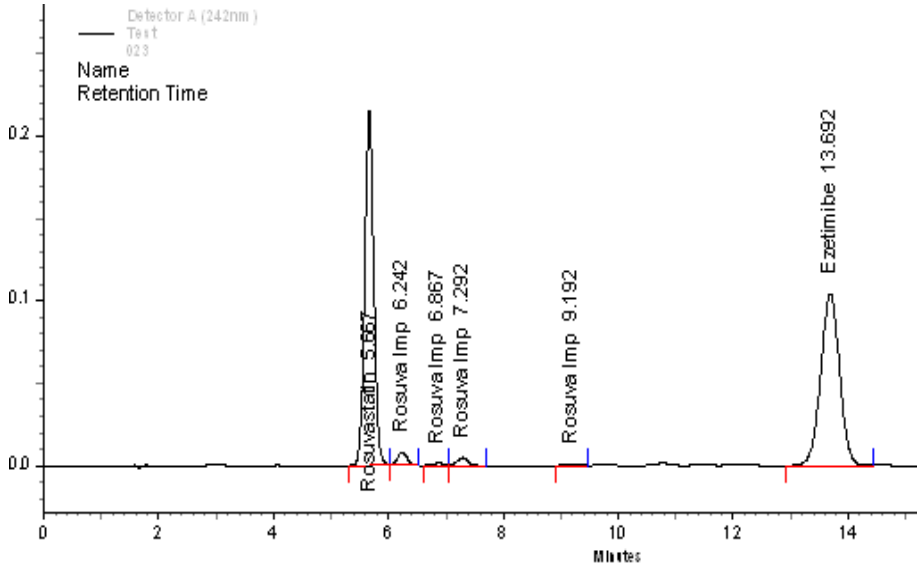
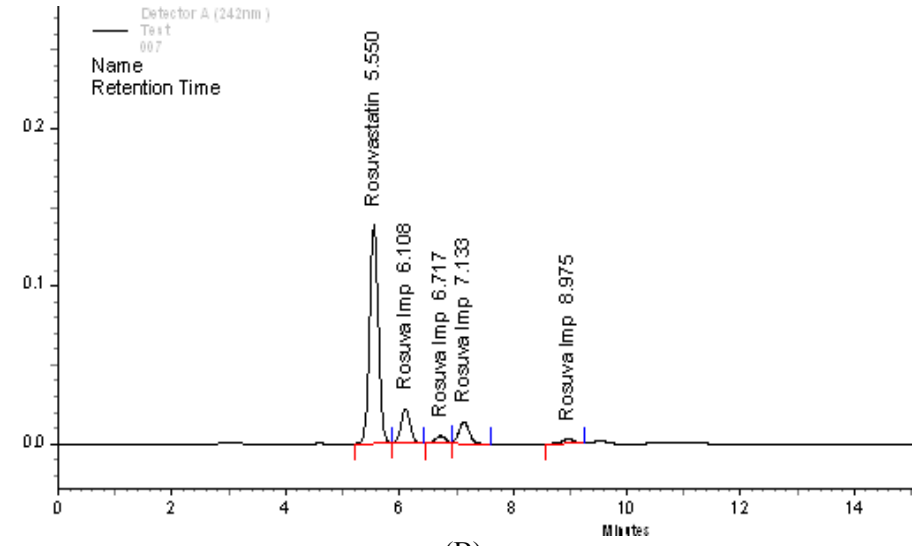


(C)

Fig. 2. Chromatograms of untreated (A) Tablet (B) RSV and (C) EZE solution



(A)



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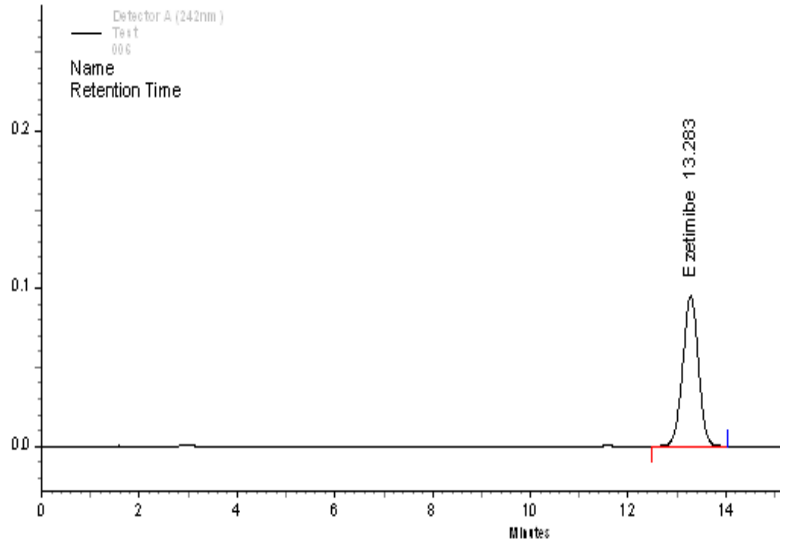
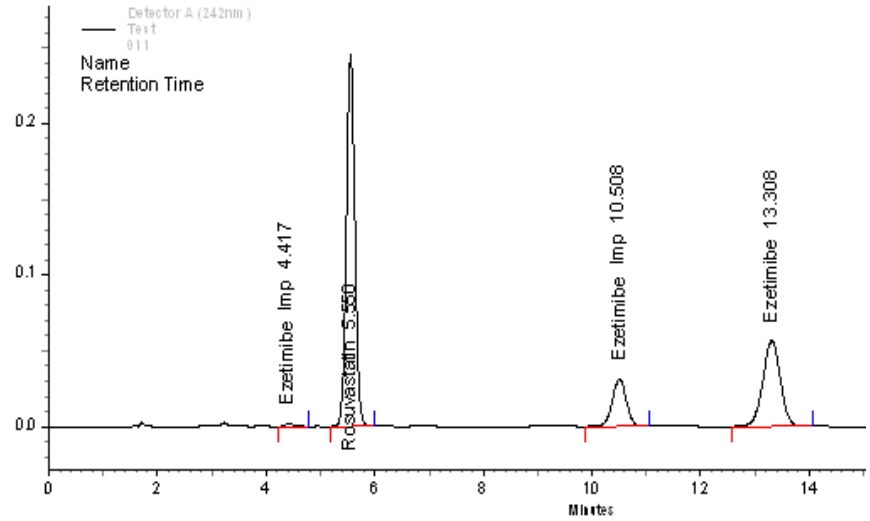
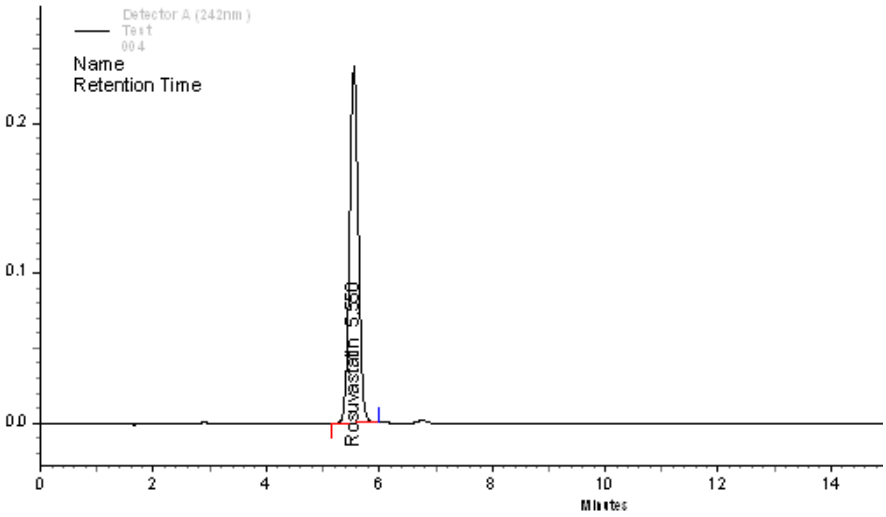


(C)

Fig. 3. Chromatograms of acid degraded (A) Tablet (B) RSV and (C) EZE solution



(A)



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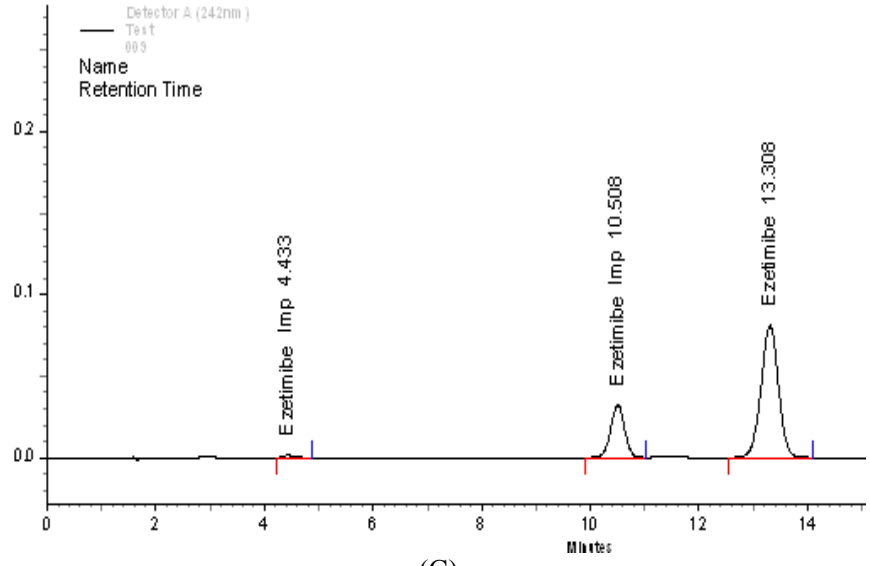
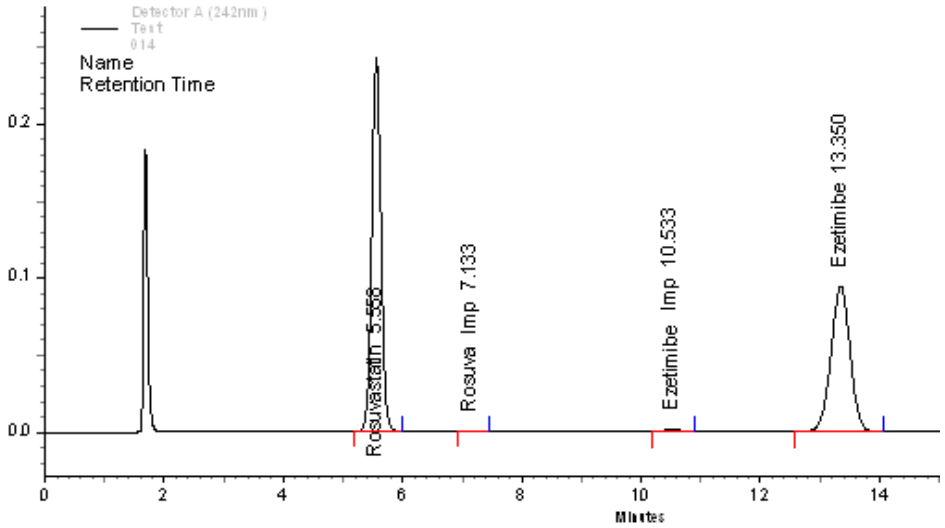


(C)

Fig. 4. Chromatograms of alkali degraded (A) Tablet (B) RSV and (C) EZE solution



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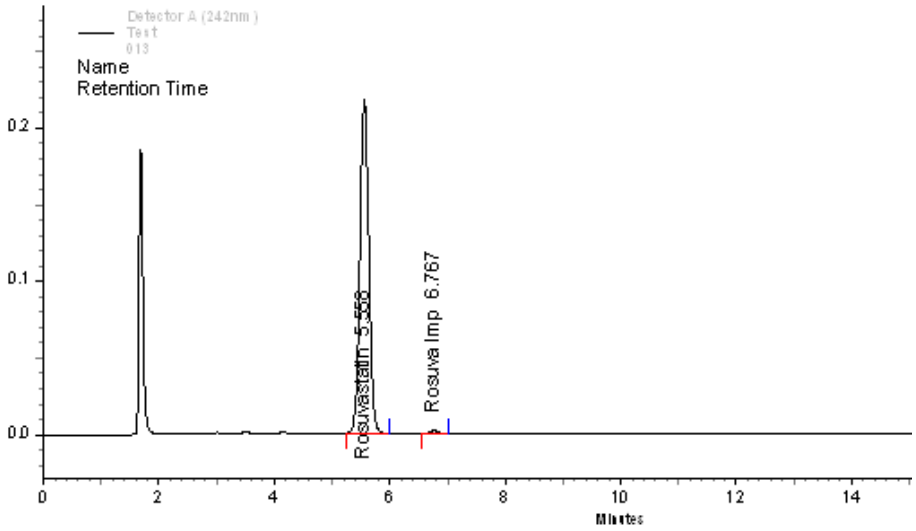

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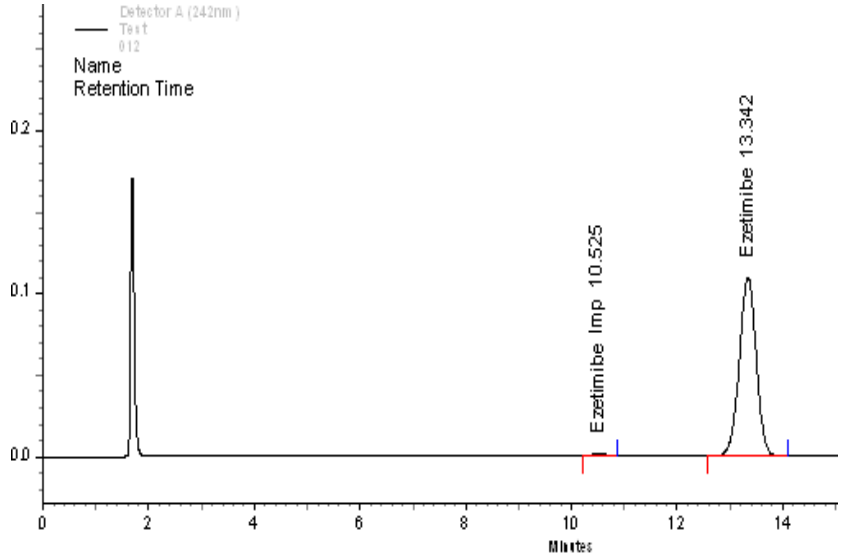

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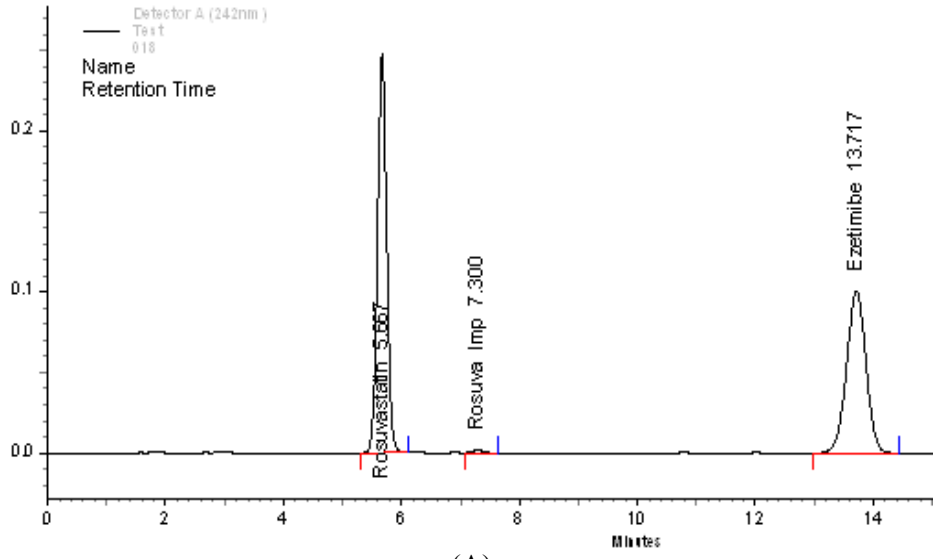
Fig. 5. Chromatograms of oxidation degraded (A) Tablet (B) RSV and (C) EZE solution



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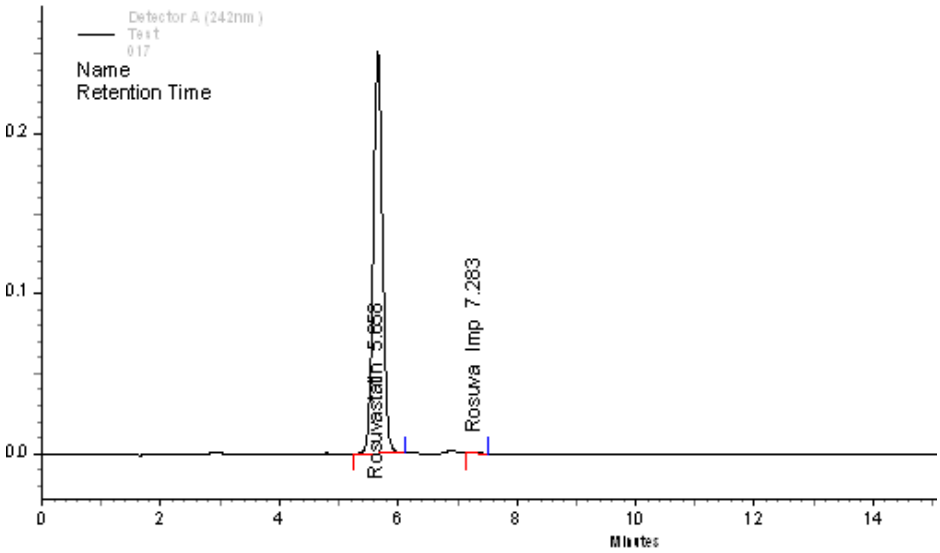

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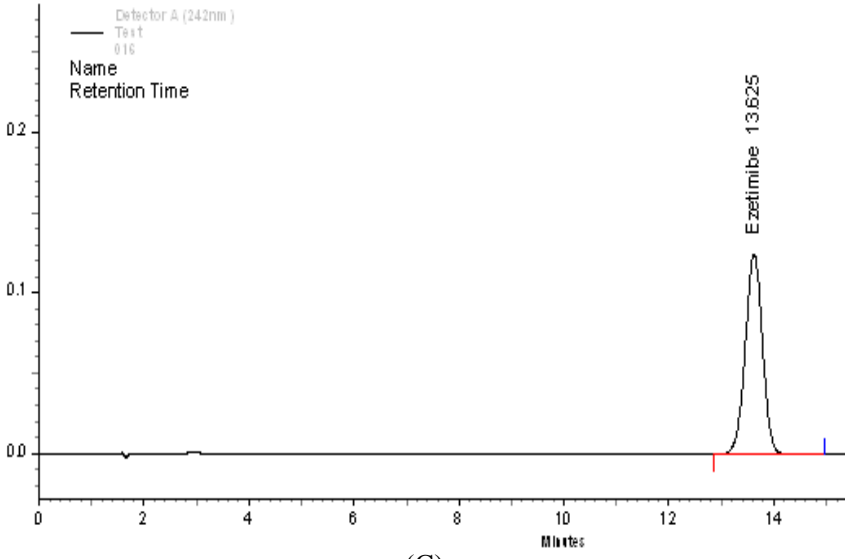
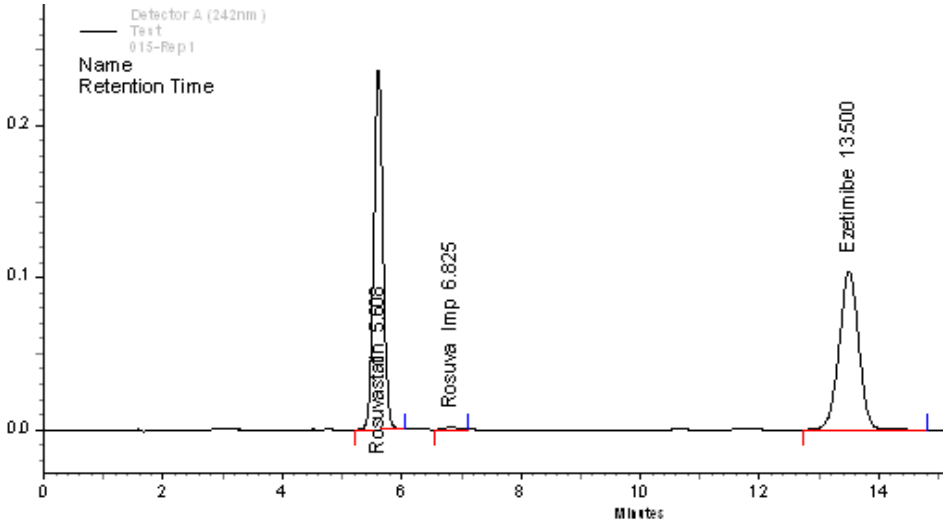
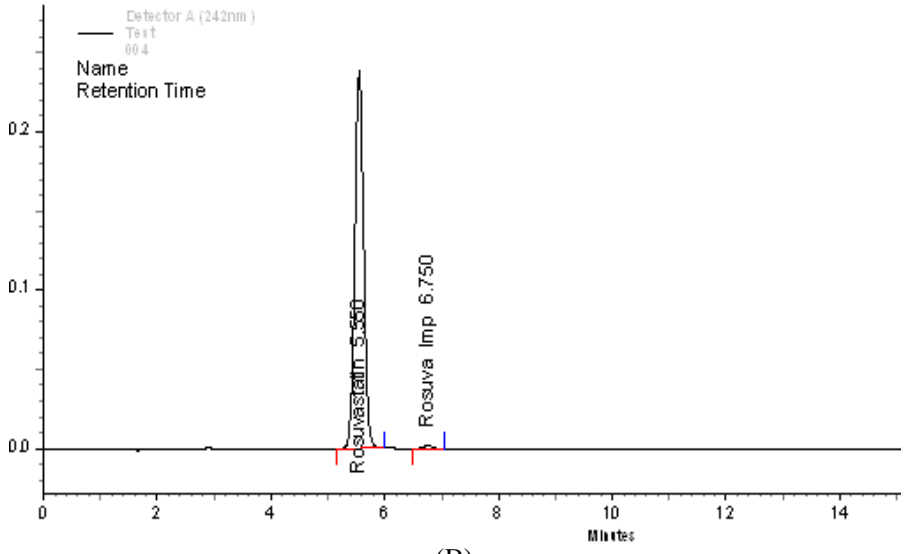


(C)

Fig. 6. Chromatograms of thermal degraded (A) Tablet (B) RSV and (C) EZE solution



(A)



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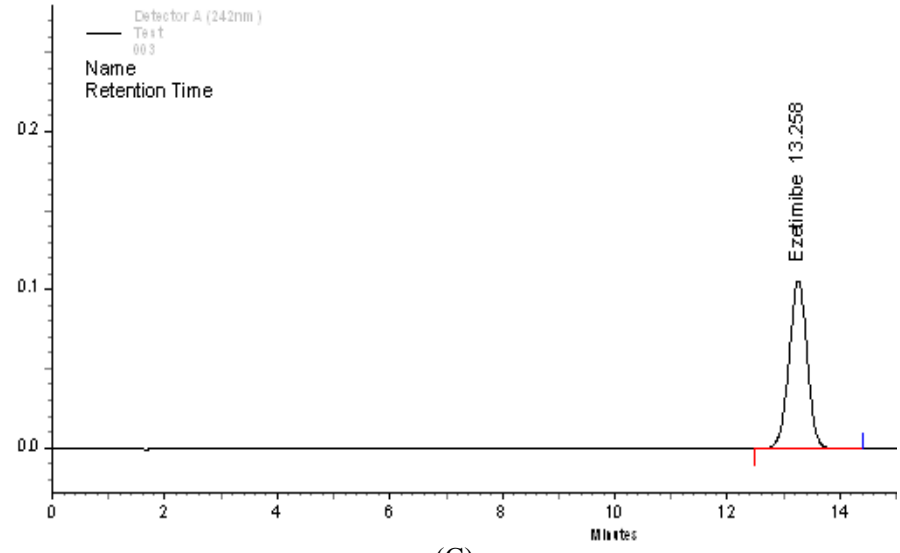

(C)

Fig. 7. Chromatograms of photo degraded (A) Tablet (B) RSV and (C) EZE solution

3.2.3 Oxidation degradation study

Both drugs exhibited very low degree of degradation in oxidative conditions on heating with 10% v/v hydrogen peroxide solution in water at 80°C for 1 h as shown in Fig. 5.

3.2.4 Thermal degradation study

Both drugs were found to be relatively stable when the drug substances and drug product were kept for thermal degradation in solid state at 105°C for 24 h under dry heat, as shown in Fig. 6.

3.2.5 Photo degradation study

Both drugs were found to be stable to photolytic stress when the drug product, in solid state, was irradiated for 12 h at 254 nm in a cabinet with a UV lamp as shown in Fig. 7.

Table 1 indicates the extent of degradation of RSV and EZE in the dosage form under various stress conditions. The specificity of the method was also evaluated by checking its peak purity of the analyte peaks during the forced degradation study. The peak purity indexes of RSV and EZE responses in standard solution and all the degraded sample solutions are shown in Table-2. No other co-eluting peaks were found with the main peaks during forced degradation study suggesting the specificity of the method for the simultaneous estimation of RSV and EZE in the presence of their degradation products, excipients from the formulation and impurities.

Table 1. Data indicating % Assay of tablet formulation and % degradation under stressed conditions using the proposed method.

Stress Condition / Duration	Assay, %		Degradation, %	
	RSV	EZE	RSV	EZE
As such (unstressed)	100.4	100.8	-NA-	-NA-
Acidic/0.01 M HCl/80°C/1 h	87.9	98.0	12.5	2.8
Basic/0.01 M NaOH/80°C/10 min	99.3	77.7	1.1	23.0
Oxidative/10%H_2O_2/80°C /1 h	90.4	96.8	9.9	4.0
Thermal (dry heat)/105°C /24 h	97.9	100.7	2.5	0.1
Photolytic/UV 254nm/12 h	96.0	100.8	4.4	0.1

NA = Not Applicable

Table 2. Data indicating specificity of the proposed method

Sample Solutions	Peak Purity Index	
	RSV	EZE
Standard Solution	1.0000	0.9999
Untreated Sample	0.9999	0.9999
Acid- degraded Sample	0.9993	0.9999
Base- degraded Sample	1.0000	1.0000
Oxidative- degraded Sample	0.9995	0.9999
Thermal- degraded Sample	1.0000	0.9999
Photo-degraded Sample	1.0000	0.9999

3.3 Method Validation

The method was evaluated to demonstrate its suitability for its intended purpose with adequate validation characteristics.

3.3.1 System Suitability

RSD of area of five replicate injections of standard preparation was under 2% indicating the suitability of the system. The efficiency of the column was expressed by the number of theoretical plates and the tailing factor. The system suitability data for RSV and EZE are shown in Table 3 and 4 respectively.

Table 3. Data indicating system suitability parameters and robustness of RSV using proposed method

Conditions for RSV	% Assay of RSV in tablet	Theoretical Plates[a]	Asymmetry[a]	RSD of areas of five replicate of standard preparations
No Change (repeatability)	101.4	5879	1.07	0.10
Flow rate : -0.1 mL min^{-1}	101.6	6226	1.07	0.66
Flow rate : +0.1 mL min^{-1}	100.4	5599	1.08	0.10
Buffer-Methanol (47-53)	101.3	6101	1.08	0.10
Buffer-Methanol (43-57)	101.5	5693	1.07	0.11
pH of buffer : -0.2 unit	100.1	6294	1.08	0.21
pH of buffer : +0.2 unit	100.6	6070	1.10	0.38

[a] = average of five standard replicates

Table 4. Data indicating system suitability parameters and robustness of EZE using proposed method

Conditions for EZE	% Assay of EZE in tablet	Theoretical Plates[a]	Asymmetry[a]	RSD of areas of five replicate of standard preparations
No Change (repeatability)	99.2	8410	1.00	0.07
Flow rate: -0.1 mL min^{-1}	99.9	8349	1.01	0.25
Flow rate: +0.1 mL min^{-1}	100.3	7643	1.02	0.21
Buffer-Methanol (47-53)	99.9	8103	1.00	0.12
Buffer-Methanol (43-57)	100.2	8015	1.02	0.18
pH of buffer : -0.2 unit	99.6	7941	1.03	0.29
pH of buffer : +0.2 unit	99.4	8178	1.03	0.17

[a] = average of five standard replicates

3.3.2 Linearity and Range

The calibration curve constructed was evaluated by its correlation coefficient. The peak area versus concentration of drug were plotted and a linear least square regression analysis was conducted to determine the slope, intercept and correlation coefficient (r) to demonstrate the linearity of the method. The method was found linear over the concentration range of 5-80 µg mL^{-1} for both RSV and EZE. The parameters for the regression analysis are given in Table 5.

Table 5. Data indicating linearity of the proposed method

Parameters	RSV	EZE
Linearity range	5-80 µg mL^{-1}	5-80 µg mL^{-1}
Regression equation	y=50037x-10786	y=42753x+6098
Slope	50037	42753
Intercept	-10786	6098
Correlation coefficient	0.99999	0.99994

3.3.3 Limit of Detection (LOD) & Limit of Quantitation (LOQ)

The LOD and LOQ of the method were determined by calculating the signal to noise (S/N) ratio of 3:1 and 10:1 respectively. LOD and LOQ values for RSV were found to be 0.04 µg mL^{-1} and 0.12 µg mL^{-1}, respectively and similarly LOD and LOQ values for EZE were found to be 0.06 µg mL^{-1} and 0.19 µg mL^{-1}, respectively.

3.3.4 Precision

The average % assay (n=6) of RSV was 101.0% and of EZE was 99.9% in the tablet formulation with RSD of 1.38% and 1.02% respectively. The data for precision study are shown in Table 6 along with intermediate precision data. Low values of RSD indicate that the method is precise.

Table 6. Data indicating precision of the proposed method

Parameters	RSV	EZE
Method Precision (repeatability)[a] (RSD)	0.10	0.07
Intermediate Precision (reproducibility)[a] (RSD)	0.19	0.34
Method Precision (% assay)[b]	101.0	99.9
Method Precision (RSD)[c]	1.38	1.02
Intermediate Precision (% assay)[b]	100.7	100.4
Intermediate Precision (RSD)[c]	0.95	0.91

RSD= Relative Standard Deviation
[a] Determined on five replicate of standard injections
[b] Average of six determinations
[c] Determined on six determinations

3.3.5 Recovery

Mean percent recovery of triplicate samples at each level for both the drugs were found to range from 98% to 102% with RSD of less than 2.0%, indicating the accuracy of the method. The data for the recovery study are presented in Table 7.

3.3.6 Robustness

There was no significant effect on system suitability parameters such as asymmetry and number of theoretical plates of both the peaks and no significant difference was observed in % assay of both RSV and EZE, when small but deliberate changes were made to the chromatographic conditions. The data for robustness for RSV and EZE are presented in Table 3 and 4 respectively along with system suitability parameters of normal methodology.

3.3.7 Solution Stability

During stability study of stored solutions of test preparation for assay determination, the solutions were found to be stable for up to 24 h at 25°C and up to 48 h at 15°C. % Assay for the drugs in the tablet formulation obtained after above mentioned period were statistically identical with the initial % assay value as mentioned in Table 8.

Table 7. Data indicating recovery of the proposed method

Drug	Level	Amount of Drug Added, µg mL^{-1}	Amount of Drug Found, µg mL^{-1}	Recovery, %	Mean Recovery,%	RSD
RSV	50%	25.43	25.16	98.9	100.0	1.20
		25.15	25.48	101.3		
		25.02	24.97	99.8		
	100%	37.57	37.65	100.2	99.6	0.53
		37.87	37.58	99.2		
		37.47	37.24	99.4		
	150%	50.23	50.03	99.6	99.9	0.60
		50.19	50.48	100.6		
		50.47	50.21	99.5		
EZE	50%	24.50	24.31	99.2	99.6	0.73
		25.61	25.41	99.2		
		25.11	25.23	100.5		
	100%	37.57	37.58	100.0	99.5	0.49
		37.79	37.52	99.3		
		37.56	37.22	99.1		
	150%	50.31	49.81	99.0	99.4	1.07
		50.69	49.97	98.6		
		50.67	50.97	100.6		

Table 8. Data indicating solution stability using the proposed method

Time	Difference between % assay value from initial for RSV		Difference between % assay value from initial for EZE	
	At 15°C	At 25°C	At 15°C	At 25°C
After 6 h	0.15	0.67	0.12	0.58
After 12 h	0.52	1.05	0.45	0.91
After 24 h	0.89	1.81	1.05	1.75
After 36 h	1.49	2.37	1.55	2.16
After 48 h	1.71	3.05	1.81	2.81

4. Conclusions

A simple and rapid isocratic stability-indicating RP-HPLC method was developed for simultaneous determination of Rosuvastatin and Ezetimibe in tablet dosage form. This study is a typical example of development of a stability-indicating assay, established following the recommendations of ICH guidelines. In this study, the drug product containing RSV and EZE was subjected to hydrolytic (both acidic and basic), oxidative, thermal and photo degradation and the degraded samples were analyzed by the described method. Based on the peak purity results obtained from the analysis of forced degradation samples using the described method, it can be concluded that there is no co-eluting peak with the main peaks, and so the method can be termed as specific for the simultaneous estimation of RSV and EZE in the presence of degradation products. The method was validated for various statistical parameters (linearity, accuracy, precision and robustness) and was found to be accurate and precise as indicated from the recovery study and the low RSD. It can be concluded that the proposed method has great promise as a rapid analytical tool for the simultaneous estimation of RSV and EZE in their combined pharmaceutical formulations, especially for quality control laboratories.

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References

1. Lennernas H and Fager G (1997) Pharmacodynamic and pharmacokinetics of the HMG-CoA reductase inhibitors. Clin Pharmacokinet 32: 403.
2. Nissen S E, Nicholls S J, Sipahi I, Libby P, Raichlen J S, Ballantyne C M, Davignon J, Erbel R, Fruchart J C, Tardif J C, Schoenhagen P, Crowe T, Cain V, Wolski K, Goormastic M and Tuzcu E M (2006) Effect of very high-intensity statin therapy on regression of coronary atherosclerosis: the ASTEROID trial. JAMA 295(13): 1556.
3. Leitersdorf E (2002) Selective cholesterol absorption inhibition: a novel strategy in lipid-lowering management. Int J Clin Pract 56: 116.
4. Heek M V, Farley C, Compton D, Hoos L and Davis H R (2001) Ezetimibe selectively inhibits intestinal cholesterol absorption in rodents in the presence and absence of exocrine pancreatic function. Br J Phamacol 134(2): 409.
5. Dujovne A, Ettinger M P, McNeer F, Lipka L, Lebeaut A P, Suresh R, Yang B and Veltri E P (2002) Efficacy and Safety of a Potent New Selective Cholesterol Absorption Inhibitor, Ezetimibe, in Patients With Primary Hypercholesterolemia. Am J Cardiol 90: 1092.
6. ICH (1993) Proceedings of the International Conference on Harmonisation of Technical Requirement for Registration of Pharmaceuticals for Human Use. Stability Testing of New Drug Substances and Products, Q1A. Geneva, Switzerland.
7. ICH (1996) Proceedings of the International Conference on Harmonisation of Technical Requirement for Registration of Pharmaceuticals for Human Use. Photostability Testing of New Drug Substances and Products, Q1B. Geneva, Switzerland.
8. United States Pharmacopoeia 26 (2003) U.S. Pharmacopoeial Convention Inc, Rockville: p 1151.

9. Kumar T R, Shitut N R, Kumar P K, Vinu M C, Kumar V V, Mullangi R and Srinivas N R (2006) Determination of Rosuvastatin in rat plasma by HPLC: Validation and its application to pharmacokinetic studies. Biomed Chromatogr 20: 881.

10. Suslu I, Celebier M and Altinoz S (2007) Determination of Rosuvastatin in Pharmaceutical Formulations by Capillary Zone Electrophoresis. Chromatographia 66: 65.

11. Uyar B, Celebier M and Altinoz S (2007) Spectrophotometric determination of Rosuvastatin calcium in tablets. Pharmazie 62: 411.

12. Sane R T, Kamat S S, Menon S N, Inamdar S R and Mote M R (2007) Determination of Rosuvastatin calcium in its bulk drug and pharmaceutical preparations by high-performance thin-layer chromatography. J Planar Chromatogr 18: 194.

13. Vittal S, Kumar T R, Shitut N R, Vinu M C, Kumar V V and Mullangi R (2006) Simultaneous quantitation of Rosuvastatin and Gemfibrozil in human plasma by high-performance liquid chromatography and its application to a pharmacokinetic study. Biomed Chromatogr 20: 1252.

14. Sistla R, Tata V S, Kashyap Y V, Chandrasekar D and Diwan P V (2005) Development and validation of a reversed-phase HPLC method for the determination of Ezetimibe in pharmaceutical dosage forms. J Pharm Biomed Anal 39: 517.

15. Doshi A S, Kachhadia P K and Joshi H (2008) Validation of a stability-indicating LC method for assay of Ezetimibe in tablets and for determination of content uniformity. Chromatographia 67: 137.

16. Akmar S K, Kothapalli L, Thomas A, Jangam S and Deshpande A D (2007) Reverse Phase HPLC method for the estimation of Ezetimibe in bulk and pharmaceutical formulations. Indian J Pharm Sci 69(5): 695.

17. Oswald S, Scheuch E, Cascorbi I and Siegmund W (2006) A LC–MS/MS method to quantify the novel cholesterol lowering drug Ezetimibe in human serum, urine and feces in healthy subjects genotyped for SLCO1B1. J Chromatogr B Anal Technol Biomed Life Sci 830: 143.

18. Li S, Liu G, Jia J, Li X and Yu C J (2006) Liquid chromatography–negative ion electrospray tandem mass spectrometry method for the quantification of Ezetimibe in human plasma. J Pharm Biomed Anal 40(4): 987.

19. Oliveira P R, Brum L L, Fronza M, Bernardi L S, Masiero S M and Dalmora S L (2006) Development and validation of a Liquid Chromatography-Tandem Mass Spectrometry method for the determination of Ezetimibe in human plasma and pharmaceutical formulations. Chromatographia 63(7-8): 315.

20. Chaudhari B G, Patel M N, Shah P B, Patel L J and Patel V P (2007) Stability-indicating reversed-phase liquid chromatography method for simultaneous determination of Atrovastatin and Ezetimibe from their combination drug products. J AOAC Int 90(6): 1539.

21. Mehta T N, Patel A K, Kulkarni G M and Suubbaiah G (2005) Determination of Rosuvastatin in the presence of its degradation products by a stability-indicating LC method. J AOAC Int 88(4): 1142.

22. Singh S, Singh B, Bahuguna R, Wadhwa L and Saxena R (2006) Stress degradation studies on Ezetimibe and development of a validated stability-indicating HPLC assay. J Pharm Biomed Anal 41: 1037.

23. Rajkondawar V V (2006) Simultaneous Estimation of Rosuvastatin and Ezetimibe by Reverse Phase High Performance Liquid Chromatography. Asian J Chem 18(4): 3230.

24. Snyder L R, Kirkland J J, Glajch L J (1997) Practical HPLC Method Development 2nd edition, John Wiley and Sons Inc: New York, p 709.

25. ICH (1996) Proceedings of the International Conference on Harmonisation of Technical Requirement of Registration of Pharmaceuticals for Human Use (ICH Harmonised Tripartite Guidelines). Validation of Analytical Procedures: Methodology, Q2B, Geneva, Switzerland.