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Simultaneous Estimation Method for the Determination of Rosuvastatin and Ezetimibe from their Combination Tablet Dosage form Using RP-HPLC

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A B S T R A C T

Rosuvastatin is an HMG Co-A inhibitor and Ezetimibe is an intestinal cholesterol absorption inhibitor. The combination formulation is used for the treatment of hypercholestrolemia. A simple, accurate and precise assay 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 Water's C18 250 x 4.6 mm, 5µ column maintained at an ambient temperature. The optimum mobile phase consisted of Acetonitrile: water: 0.02 M phosphate buffer pH 8 (40:10:50 v/v), flow rate of mobile phase was set 1.0 mL min^{-1} and PDA detection was performed at 230 nm. The method was validated according to ICH guidelines. It was found to be accurate and reproducible. Linearity was obtained in the concentration range of 30-90 µg mL^{-1} for both RSV and EZE with correlation coefficients of 0.999 and 0.998 respectively. Mean percent recovery of triplicate samples at each level for both drugs were found in the range of 98% to 100% with RSD of less than 2.0%. 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 proposed method can be successfully applied in the quality control and stability samples of bulk manufacturing and pharmaceutical dosage forms.

Keywords: Rosuvastatin, Ezetimibe, hypercholesterolemia, Acetonitrile, PDA detection.

A R T I C L E   I N F O

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1. Introduction

Rosuvastatin calcium is chemically (3*R*, 5S, 6E)-7-[4- (4-fluorophenyl)-2-(*N*-methyl methane sulfonamido)-6-(propan-2-yl) pyrimidin-5-yl]-3, 5-dihydroxyhept-6-enoic acid. It is a competitive inhibitor of the enzyme HMG-CoA reductase, the rate–limiting enzyme that converts 3-hydroxy -3-methylglutaryl coenzyme A to mevalonate, precursor for cholesterol. It is a cholesterol lower agent. Ezetimibe (EZE), (3R, 4S)-1-(4- fluorophenyl)-3-[(3S)-(4-fluorophenyl)-3 hydroxypropyl]- 4-(4-hydroxyphenyl)-2-azetidinone, is a class of lipidlowering compound that selectively inhibits the intestinal absorption of cholesterol and related phytosterols [1].
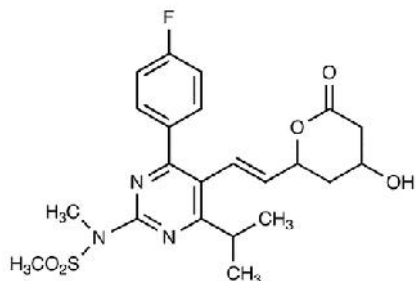
Figure 1: Chemical structure of Rosuvastatin
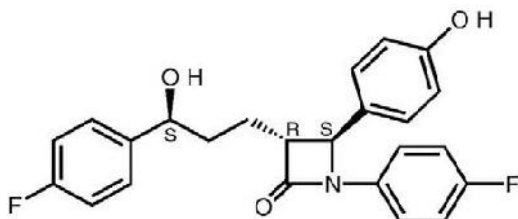
Figure 2: Chemical structure of Ezetimibe

Rosuvastatin calcium alone has been determined by Spectrophotometric methods [2,3,4] Stability indicating HPTLC method5 and RP-.HPLC [6,7,8] Ezetimibe was also estimated using UV- method [9,10,11], Derivative Spectroscopy [12,13], HPLC [14,15], HPTLC [16] and LC-MS/MS [17]. To the best of knowledge, only one method has been developed for the simultaneous determination of both the drugs in tablets include Q ratio and first derivative methods [18]. The present research work describes the rapid, accurate, sensitive and reproducible spectroscopic method for simultaneous estimation of Rosuvastatin Calcium and Ezetimibe from the tablet formulation.

2. Materials and Methods
Experimental
Instrumentation

The HPLC system consisted of a LC Waters (Waters, Milford, MA, USA) using a Water's C18 250 x 4.6 mm, 5μ column, a quaternary gradient system (600 Controller), in line degasser (Waters, model AF). The system was equipped with a photodiode array detector (Water, 2998 model) and auto sampler (Waters, model 717 plus). Data was processed using Empower Pro software (Waters, Milford, MA, USA). The mobile phase was pumped at a flow rate of 1.2 mL min−1. The detection wavelength for Rosuvastatin and Ezetimibe was 253 nm and 230 nm respectively.

Chemicals and Reagents

Reference standards of Rosuvastatin Calcium and Ezetimibe were kindly supplied by TSK laboratories, Hyderabad, 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 Anukar pharmacy, Hyderabad. Acetonitrile (HPLC grade) was purchased from Spectrochem (Mumbai, India). All other reagents and chemicals used in this study were of analytical grade. Potassium dihydrogen phosphate and sodium hydroxide pellets were purchased from Ranbaxy Fine Chemicals (New Delhi, India). Water was purified using Millipore system (Millipore Corp., Bangalore, India).

Preparation of Stock and Standard Solutions

The standard stock solutions containing 1 mg mL-1 each of RSV and EZE were prepared separately by dissolving reference standards in mobile phase (Acetonitrile: water: 0.02 M phosphate buffer pH 8 (40:10:50 v/v)) and diluting with the same diluent. 3 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 60 μg mL-1 for both drugs. Calibration curve solutions containing 30-90 μg mL-1 each of RSV and EZE were prepared by diluting the standard stock solution to the appropriate volume with the same diluent.

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 100 mL calibrated volumetric flask. Around 50 mL of mobile phase mixture was added, and the solution sonicated for 10 min. Volume was made up to the mark with the same solvent mixture. The solution was filtered through 0.45 μm membrane filter. This solution contains 60 μg mL-1, each of RSV and EZE.

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. [19].

The degradation conditions were optimized to obtain target degradation between 10-30% as per ICH guidelines.

Validation of the method
The developed method was validated according to ICH guidelines [20]. To check the system performance, the system suitability parameters were measured. System precision was determined on six replicate injections of standard preparations. Number of theoretical plates and asymmetry were measured.

Linearity:
Calibration graphs were constructed by plotting peak area Vs concentration of RSV and EZE and the regression equations were calculated. The calibration graphs were plotted over 5 different linear concentrations in the range of 30-90 µg/ml for both drugs. Aliquots (20µl) of each solution were injected under the operating chromatographic condition described above [Number of replicates (n=6)].

Accuracy
The accuracy of the method was established by recovery studies i.e external standard addition method. The known amount of standard was added at three different levels to pre-analyzed sample. Each determination was performed in triplicate.

Precision
The intraday and interday precision of the proposed method was determined by analyzing mixed standard solution of RSV and EZE at concentration 60 µg/ml 3 times on the same day and on 3 different days. The results are reported in terms of relative standard deviation.

Limit of detection (LOD) & limit of quantitation (LOQ)
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 according to International Conference on Harmonization guidelines [20].

Robustness:
The robustness of the method was evaluated by assaying the test solutions after slight but deliberate changes in the analytical conditions like flow rate (+ 0.1 mL min-1), and pH of the buffer (+ 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.

Analysis of RSV and EZE in tablet dosage form
The response of sample solutions were measured at 230 nm for quantitation of RSV and EZE by the method described above. The amount of RSV and EZE present in the sample solution were determined by applying values of peak area to regression equation of the calibration graph. The results of assay was given in table 3.

3. Results and Discussion
An assay and 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 performed 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.
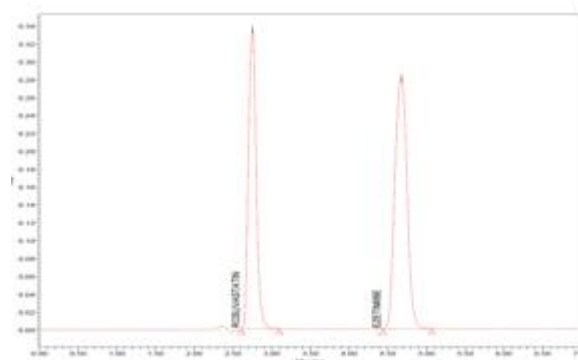

Figure 3: Typical Chromatogram of RSV and EZE with detection at 230 nm

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. No interfering peaks were found due to degradation products at the drugs Rt's.

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

Linearity and Range
The calibration curve constructed was evaluated by its correlation coefficient. The method was found linear over the concentration range of 30-90 µg mL-1 for both RSV and EZE. The parameters for the regression analysis are given in Table 1.

Table 1: Data indicating linearity of the proposed method

S. No	Parameter	RSV	EZE
1	Linearity Range	30-90 µg mL-1	30-90 µg mL-1
2	Regression equation	y = 38907x + 14460	y = 53942x + 23972
3	Slope	38907	53942
4	Intercept	14460	23972
5	Correlation (R²)	$R^2 = 0.9999$	$R^2 = 0.9998$

Accuracy: The recovery experiments were performed by standard addition method. The mean recoveries obtained were 98.00% and 100.00 % for RSV & EZE respectively (Table 2).

Method precision
The RSD values for RSV and EZE were found to be 1.07 % and 1.11 % respectively (Table 2).

LOD and LOQ: LOD values for RSV and EZE were found to be 0.08 and 0.006µg/ml respectively. LOQ values for

RSV and EZE were found to be 0.08 and 0.05μg/ml respectively. (Table 2).

Table 2: Summary of validation parameter

S. No	Parameter	Result	
		RSV	EZE
1	Accuracy (%Recovery)	98 %	100 %
2	Precession (% RSD)	1.07	1.11
3	LOD	0.05μg/ml	0.006μg/ml
4	LOQ	0.08μg/ml	0.05μg/ml

Assay of the tablet dosage form
The proposed validated method was successfully applied to determine ROS and EZE in tablet dosage form. The result obtained for ROS and EZE were comparable with corresponding labeled amounts (Table 3)

Table 3: Assay of tablet dosage form

RSV		EZE	
Labeled amount (mg/tablet)	Amount found (mg/tablet)	Labeled amount (mg/tablet)	Amount found (mg/tablet)
10	99.53	10	101
	99.38		99
	101.83		99
	99.65		101
	100.78		101
	98.98		99
Mean	100	Mean	100
SD	1.07	SD	1.11
% RSD	1.07	% RSD	1.11

4. Conclusion

The proposed method has advantage of simplicity and convenience for the separation and quantitation of RSV and EZE in the combination and cab be used for the assay of their dosage form. Also, the low solvent consumption and short analytical run time lead to environmentally friendly chromatographic procedure. The method is accurate, precise, rapid and selective for simultaneous estimation of Rosuvastatin Calcium and Ezetimibe in tablet dosage form. Hence it can be conveniently adopted for routine analysis.

5. Acknowledgements

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