



**SIMULTANEOUS UV-SPECTROPHOTOMETRIC ESTIMATION OF ROSUVASTATIN AND EZETIMIBE IN THEIR COMBINED DOSAGE FORMS**

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**ABSTRACT**

Rosuvastatin is an HMG Co-A inhibitor and Ezetimibe is an intestinal cholesterol absorption inhibitor. The combination formulation is used for the treatment of hypercholesterolemia. Three new, simple, accurate and precise UV spectrophotometric methods have been developed and validated for the simultaneous determination of Rosuvastatin (RSV) and Ezetimibe (EZE) in their combined dosage forms. First method is based on Q-absorption Ratio method using two wavelengths, 232.4 nm ( $\lambda_{\max}$  of EZE) and 237 nm (Isoabsorptive point). The second method is the dual wavelength method, where 226.4 nm and 239.2 nm were selected as  $\lambda_1$  and  $\lambda_2$  for the determination of Rosuvastatin and 232.6 nm and 252.4 nm were selected similarly for the determination of Ezetimibe. The last method involves the use of First order derivative technique. Here 232.4 nm, the zero crossing point of Ezetimibe, was selected for the determination of Rosuvastatin and 223.4 nm, the zero crossing point of Rosuvastatin, was selected for the determination of Ezetimibe. Methanol was the solvent used in all three methods. Both drugs showed linearity in the range of 1-25 $\mu$ g/mL in all the methods. All methods were validated statistically and recovery studies were carried out. All methods were found to be accurate, precise and reproducible. These methods were applied to the assay of the drugs in marketed formulation, which were found in the range of 98.0% to 102.0% of the labeled value for both Rosuvastatin and Ezetimibe. Hence, the methods herein described can be successfully applied in quality control of combined pharmaceutical dosage forms.

**Keywords:** Rosuvastatin, Ezetimibe, UV-Spectrophotometric.

**INTRODUCTION**

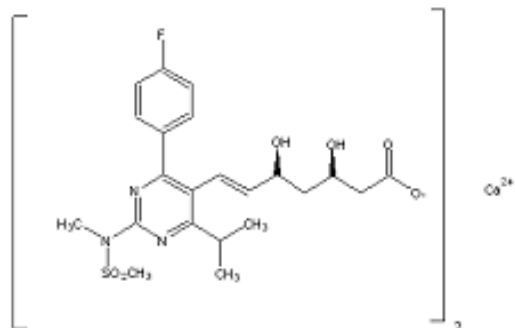
Rosuvastatin (RSV) is the calcium salt of (E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulfonyl)amino]pyrimidin-5-yl](3R,5S)-3,5-dihydroxyhept-6-enoic acid. RSV is a selective and competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme that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

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 (Low Density Lipoprotein) receptors on the cell-surface to enhance uptake and catabolism of LDL. Secondly, RSV inhibits hepatic synthesis of VLDL (Very Low Density Lipoprotein), which reduces the total number of VLDL and LDL particles<sup>2</sup>.

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-Cholesterol<sup>3,4</sup>.

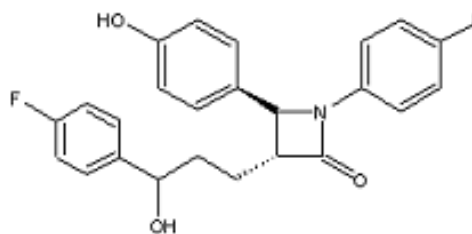
EZE co-administered with HMG-CoA reductase inhibitors (statins) is licensed for the treatment of primary hypercholesterolemia in patients, poorly controlled with a statin alone, and for homozygous familial hypercholesterolemia. As monotherapy, it is licensed for primary hypercholesterolemia where a statin is considered inappropriate or is not

tolerated and for homozygous sitosterolemia<sup>5</sup>. The chemical



(A)

structures of RSV and EZE are shown in Fig.1.



(B)

**Fig. 1: Chemical structure of (A) Rosuvastatin and (B) Ezetimibe**

A detailed survey of analytical literature for RSV revealed several methods based on varied techniques, viz, HPLC<sup>6-8</sup>, Capillary Zone Electrophoresis<sup>9</sup>, Spectrophotometry<sup>10</sup> and High-Performance Thin-Layer Chromatography (HPTLC)<sup>11</sup>. Similarly, a survey of the analytical literature for EZE revealed methods based on HPLC for determination in pharmaceuticals<sup>12-15</sup>, LC/tandem MS (LC/MS/MS) for determination in human plasma and serum<sup>16,17</sup>, UV-spectrophotometric determination in combination with other drugs<sup>18</sup> and stability-indicating HPLC method for determination in combination drug products with other drugs<sup>19,20</sup>.

Till date, none of the reported analytical procedures describes a simple and satisfactory UV spectrophotometric method for simultaneous determination of RSV and EZE in their combined dosage forms. So the objective of this work was to develop simple, precise and rapid spectrophotometric methods for combination drug products containing RSV and EZE.

## EXPERIMENTAL

### Materials and reagents

Reference standards of Rosuvastatin Calcium and Ezetimibe were 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). Nylon syringe filters (0.45  $\mu\text{m}$ ) were purchased from Millex-HN, Millipore (Mumbai, India).

### Instrumentation

A double-beam Shimadzu (Kyoto, Japan) UV-Visible spectrophotometer, Model UV-2450 PC, equipped with 1 cm quartz cells, with a fixed slit width (1 nm), wavelength accuracy of  $\pm 0.5$  nm (with automatic wavelength correction) was connected to IBM-PC compatible computer loaded with UVProbe software, version 2.0 (Shimadzu). For scanning, the wavelength range selected was from 400 nm to 200 nm with medium scanning speed.

### Standard and Test Solutions

#### Preparation of standard solution

The standard stock solutions containing 50 $\mu\text{g}/\text{mL}$  each of RSV and EZE were prepared separately by dissolving reference standards in Methanol and diluting with the same diluent. Standard solutions of both the drugs were

prepared individually by dilution of the standard stock solutions with Methanol to obtain the concentration range of 1-25 µg/mL for each of the drugs.

### 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 Methanol was added, and the solution was sonicated for 30 min. Volume was made up to the mark with the same solvent. The solution was filtered through 0.45 µm nylon syringe filter. The resultant solution contained 50µg/mL each of RSV and EZE. The solution was further diluted with Methanol to get concentration of 10µg/mL of both drugs.

### Methods

#### Q-Absorption Ratio Method

This method is applicable to the drugs that obey Beer's law at all wavelengths and the ratio of absorbances at any two wavelengths are a constant value, independent of concentration or pathlength<sup>21,22</sup>. The solutions of 10µg/mL each of RSV and EZE were scanned in the wavelength range of 400 to 200 nm to obtain overlain spectra (Figure-2). Two wavelengths, 237 nm (Isoabsorptive point) and 232.4 nm ( $\lambda_{max}$  of EZE) were selected for the formation of Q-absorbance equation. The calibration curves were determined in the concentration range of 1-25 µg/mL, for each of the drugs. The absorptivity co-efficient of each drug at both the wavelengths were determined. The concentration of individual components,  $C_{EZE}$  and  $C_{RSV}$  may be calculated using the following equations

$$C_{EZE} = (Q_m - Q_{RSV} / Q_{EZE} - Q_{RSV}) * A_1 / ax_1 \dots\dots\dots (1)$$

$$C_{RSV} = (Q_m - Q_{EZE} / Q_{EZE} - Q_{RSV}) * A_1 / ay_1 \dots\dots\dots (2)$$

$$Q_m = A_2 / A_1 \dots\dots\dots (3)$$

$$Q_{EZE} = ax_2 / ax_1 \text{ \& } Q_{RSV} = ay_2 / ay_1 \dots\dots\dots (4)$$

where,  $A_1$  and  $A_2$  are absorbance of sample solution at Isoabsorptive point (237 nm) and  $\lambda_{max}$  of EZE (232.4 nm) respectively;  $ax_1$  and  $ax_2$  are the absorptivities of EZE at 237.0 and 232.4 nm respectively and  $ay_1$  and  $ay_2$  are the absorptivities of RSV at the two wavelengths respectively.

#### Dual wavelength method

The utility of dual wavelength data processing programme is to calculate the unknown concentration of a component of interest present in a mixture containing both the components of interest and an unwanted interfering component by the mechanism of the absorbance difference between two points on the mixture spectra. This is directly proportional to the concentration of the components of interest, independent of the interfering components. The pre-requisite for dual wavelength method is the selection of two such wavelengths where the interfering component shows same absorbance whereas the component of interest shows significant difference in absorbance with concentration<sup>23</sup>. Based on this criterion, two wavelengths 239.2 nm and 226.4 nm were selected as  $\lambda_1$  and  $\lambda_2$  for the estimation of RSV. EZE shows the same absorbance at these wavelengths. Similarly, wavelengths 232.6 nm and 252.4 nm were selected as  $\lambda_1$  and  $\lambda_2$  for estimation of EZE. For calibration curve, the standard stock solutions of these drugs were diluted in

the concentration range of 1-25  $\mu\text{g}/\text{mL}$  and absorbances were recorded at selected wavelengths.

### First derivative spectroscopy method

First derivative spectroscopy on the basis of zero-crossing measurements involves measurement of the absolute value of the total derivative spectrum at an abscissa value corresponding to the zero-crossing wavelength of the derivative spectrum of another component<sup>24,25</sup>. In this method, 232.4 nm was selected for the determination of RSV, which is the zero crossing point of EZE and 223.4 nm, the zero crossing point of RSV, was selected for the determination of EZE. First-derivative technique ( $D_1$ ) traced with  $\Delta\lambda = 2$  nm was used to resolve the spectral overlapping. The calibration curves were checked for linearity and linear behavior was observed in the concentration range of 1-25  $\mu\text{g}/\text{mL}$ , for each of the drugs.

### Method validation

All the methods were validated as per ICH guidelines for parameters like Linearity, Accuracy and Precision<sup>26</sup>. The accuracy studies were carried out at different concentrations by spiking a known concentration of standard drug to the pre-analyzed sample and contents were reanalyzed by the developed method. Precision was studied by analyzing six replicates of sample solutions. Intermediate precision was determined in a similar manner on the next day using a different instrument.

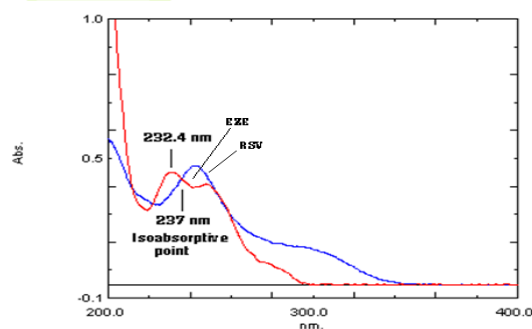
## RESULTS AND DISCUSSION

In the present work, three methods, namely, Q-absorption ratio method, dual wavelength method and first derivative

spectroscopy method were developed for the simultaneous spectroscopic estimation of RSV and EZE in commercially available tablet dosage forms. Methanol was used as the solvent since both the drugs exhibit good solubility in it and no interference due to excipients of the tablet formulation were observed.

### Q-Absorption ratio Method

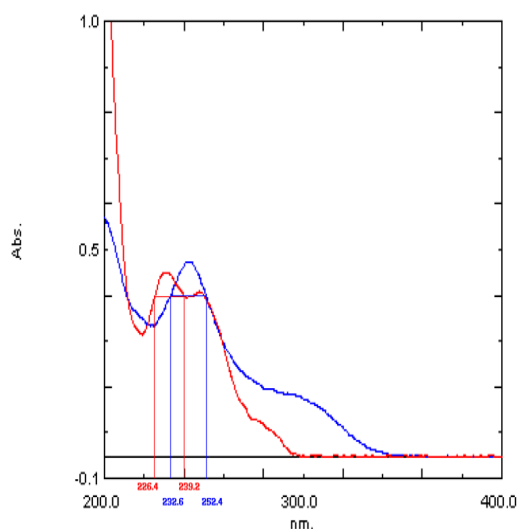
As shown in Figure-2, the overlain spectra of both drugs in 1:1 ratio (10 $\mu\text{g}/\text{mL}$  of each drug) show a reproducible Iso-absorptive point at 237.0 nm. Thus estimation of drugs by Q-absorbance ratio equation method was carried out at 237.0 nm (Iso-absorptive point) and 232.4 nm ( $\lambda_{\text{max}}$  of EZE). The standard solutions of RSV and EZE were prepared to determine the absorptivity values of the subject analyte at the two selected wavelengths. The method showed good linearity in the range of 1-25 $\mu\text{g}/\text{mL}$ . The absorptivity values of RSV were found to be 442.5 and 379.8 at the wavelengths of 237 nm and 232.4 nm respectively and similarly the absorptivity values of EZE were found to be 443.0 and 455.3 at the wavelengths of 237 nm and 232.4 nm respectively.



**Fig. 2: Overlain spectra of 10  $\mu\text{g}/\text{mL}$  of RSV and EZE**

### Dual Wavelength Method

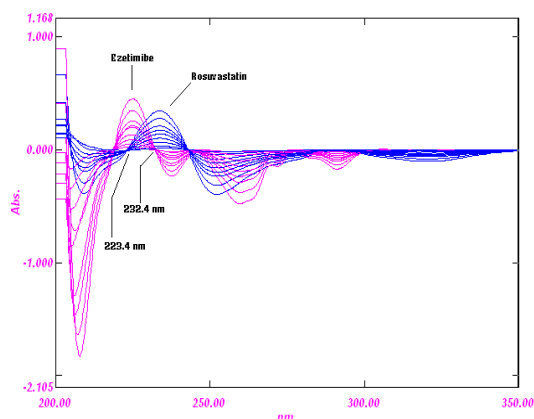
In this method, standard solutions of RSV and EZE were scanned in the entire range from 200 nm to 400 nm. As shown in Figure-3, at wavelengths of 239.2 nm and 226.4 nm, EZE shows same absorbance, and hence were selected for the estimation of RSV. Similarly, at wavelengths of 232.6 nm and 252.4 nm, RSV shows same absorbance, which were selected for the estimation of EZE. For estimation of RSV, difference in absorbances at 239.2 nm and 226.4 nm were plotted against the concentrations, while for the estimation of EZE, difference in absorbances at 232.6 nm and 252.4 nm were plotted against the concentrations to construct two separate calibration curves for both RSV and EZE. The method showed good linearity in the concentration range of 1-25 $\mu$ g/mL for both RSV and EZE.



**Fig. 3: Selection of wavelengths for dual wavelength method**

### First derivative spectroscopy method

In this method, the absorption spectra of standard solutions of RSV and EZE were recorded in the range of 200 nm to 400 nm as shown in Figure-2. The 1<sup>st</sup> derivative spectra, obtained were traced with smoothing at  $\Delta\lambda=2$  nm for determining zero cross points for both the drugs as shown in Figure-4. It was found that the 1<sup>st</sup> derivative spectrum of RSV crosses zero point at 223.4 nm and that of EZE crosses zero point at 232.4 nm. The amplitudes at 232.4 nm were plotted against the respective concentrations of RSV and the amplitudes at 223.4 nm were plotted against the respective concentrations of EZE. The method showed good linearity in the range of 1-25 $\mu$ g/mL for both the drugs.



**Fig. 4: First derivative overlain spectra of RSV and EZE at concentrations of 1-25 $\mu$ g/mL**

### 3.4 Method validation

The developed methods were validated for parameters like linearity, precision and accuracy; the data for which are presented in the Tables-1 to 3. The low value of R.S.D. value indicates that all the methods are precise and accurate.

**Table 1: Data showing linearity of the developed methods**

Methods	Q-Absorption ratio method		Dual wavelength method		First derivative spectroscopy method	
	RSV	EZE	RSV	EZE	RSV	EZE
Linearity range	1-25 µg/mL	1-25 µg/mL	1-25 µg/mL	1-25 µg/mL	1-25 µg/mL	1-25 µg/mL
Slope	0.0441(at 237 nm) 0.0377(at 232.4 nm)	0.0441 (at 237 nm) 0.0474 (at 232.4 nm)	0.0152	0.0104	0.0137	0.0163
Intercept	0.0017 (at 237 nm) 0.00188 (at 232.4 nm)	-0.0001 (at 237 nm) -0.00570(at232.4 nm)	0.00114	0.00028	0.00194	0.00710
Correlation co-efficient	0.99996 (at 237 nm) 0.99986(at232.4 nm)	0.99990 (at 237 nm) 0.99950(at 232.4 nm)	0.99975	0.99798	0.99984	0.99729

**Table 2: Data showing precision of the developed methods**

Methods	Q-Absorption ratio method		Dual wavelength method		First derivative spectroscopy method	
	RSV	EZE	RSV	EZE	RSV	EZE
Intraday Precision (% assay) <sup>a</sup>	99.8	99.0	100.5	100.2	100.7	99.8
Intraday Precision (% R.S.D.) <sup>b</sup>	0.67	0.59	0.12	0.25	0.94	0.81
Interday Precision (% assay) <sup>a</sup>	99.2	99.5	101.5	99.5	101.0	99.1
Interday Precision (% R.S.D.) <sup>b</sup>	0.28	0.79	0.71	0.82	0.17	0.58

<sup>a</sup> Average of six determinations, <sup>b</sup> Estimated on six determinations

**Table 3: Data showing recovery of the developed methods**

Parameters	RSV	EZE	STD. RSV added	STD. EZE added	RSV found	EZE found	% RSV recovered	% EZE recovered
	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL		
<b>Methods</b>								
Q-Absorption Ratio Method	10	10	2.52	2.53	12.41	12.41	99.1	99.0
	10	10	5.04	5.06	15.06	14.91	100.1	99.0
Dual Wavelength Method	10	10	2.52	2.53	12.35	12.39	98.6	98.9
	10	10	5.04	5.06	14.83	14.93	98.6	99.1
First Derivative Spectroscopy Method	10	10	2.52	2.53	12.59	12.48	100.6	99.6
	10	10	5.04	5.06	15.12	14.96	100.5	99.3

**Table 4: Results of analysis of tablet dosage forms containing RSV and EZE**

Methods	Q-Absorption ratio method		Dual wavelength method		First derivative spectroscopy method	
	RSV	EZE	RSV	EZE	RSV	EZE
%Assay*	100.5	99.1	100.7	99.7	99.3	99.8
SD*	0.29	0.38	0.81	0.19	0.35	0.68
%RSD	0.29	0.38	0.80	0.19	0.35	0.68
%Assay*	100.5	99.1	100.7	99.7	99.3	99.8

\* Average of six determinations, SD: Standard Deviation, RSD: Relative Standard Deviation

## Application of methods to Tablet dosage form

The developed methods after validation were applied to the estimation of RSV and EZE in tablet dosage forms available commercially. The results of the study are presented in Table-4.

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