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. The Ratiospectra derivative spectrophotometric method was developed for the simultaneous determination of Rosuvastatin (RSV) and Ezetimibe (EZE) in their fixed dosage forms. The method depends on the use of the first derivative of the ratio-spectra obtained by dividing the absorption spectrum of binary mixtures by a standard spectrum of one of the compounds. The first derivative amplitudes at 237.4 and 223.4 nm were selected for the determination of RSV and EZE respectively. The wavelength interval (Δλ) was selected as 4 nm. Methanol was used as the solvent. Both the drugs showed linearity in the range of 2.5-15µg mL⁻¹. The method was validated statistically and recovery studies were carried out. It was found to be accurate, precise and reproducible. The method was 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 method herein described can be successfully applied in quality control of combined pharmaceutical dosage forms.

Keywords: Rosuvastatin, Ezetimibe, Ratio Spectra Derivative Spectrophotometry.

INTRODUCTION

Rosuvastatin (RSV) is the calcium salt of (E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl (methylsulfonfonyl)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.¹

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.²

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.³ The chemical structures of RSV and EZE are shown in Fig.-1.

A detailed survey of analytical literature for RSV revealed several methods based on varied techniques, viz, HPLC⁴-⁶; Capillary Zone Electrophoresis⁷; Spectrophotometry⁸; High-Performance Thin- Layer Chromatography (HPTLC)⁹. Similarly, a survey of the analytical literature for EZE revealed methods based on HPLC¹⁰-¹³ for determination in pharmaceuticals, LC/tandem MS (LC/MS/MS)¹⁴,¹⁵ for determination in human plasma and serum, UV-spectrophotometric determination in
combination with other drug\textsuperscript{16} and stability-indicating LC\textsuperscript{17,18} for determination in combination drug products with other drugs.

A spectrophotometric method based on the use of the first derivative of the ratio spectra was first developed by Salinas et al.\textsuperscript{19,20}, for resolving binary mixtures. The objective of this work was to develop simple, precise and rapid ratio spectra derivative spectrophotometric method for combination drug products containing RSV and EZE.

2. EXPERIMENTAL
2.1 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 µm) were purchased from Millex-HN, Millipore (Mumbai, India).

2.2 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 UV Probe software, version 2.0 (Shimadzu). For scanning, the wavelength range selected was from 400 nm to 200 nm with medium scanning speed.

2.3 Standard and Test Solutions
2.3.1 Preparation of Standard Solution
The standard stock solutions containing 50µg mL\textsuperscript{-1} 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 2.5-15 µg mL\textsuperscript{-1} for each of the drugs.

2.3.2 Preparation of Test Solution
Twenty tablets were weighed and finely powdered in a mortar. A 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\textsuperscript{-1} each of RSV and EZE. The solution was further diluted with Methanol to get concentration of 10μg mL\textsuperscript{-1} of both drugs.

2.4 Ratio Spectra Derivative Method
This method works on two mechanisms viz. (1) Ratio and (2) Derivatization. In this method, the mixture spectra are divided with the divisor and first derivative spectra of these ratio spectra are generated. The main advantage of the ratio-spectra derivative spectrophotometry is the chance of doing easy measurements in correspondence of peaks so it permits the use of the wavelength of highest value of analytical signals (a maximum or a minimum). Moreover, the presence of a lot of maxima and minima is another advantage by the fact that these wavelengths give an opportunity for the determination of active compounds in the presence of other compounds and excipients which possibly interferes the assay.\textsuperscript{21}
For the determination of RSV, the spectra of RSV at increasing concentrations in methanol were divided by previously stored absorption spectrum standard solution of EZE (10 µg mL\(^{-1}\)) to obtain the corresponding ratio spectra. Then the first derivative of the obtained ratio spectra were traced with interval of \(\Delta \lambda = 4\) nm. In the binary mixtures, content of RSV was determined by measuring the first derivative amplitude at 237.4 nm, where there is no contribution or interference from EZE.

On the other hand, for the determination of EZE, an analogous procedure was followed. The spectra of EZE at increasing concentrations were divided by previously stored spectrum of 10 µg mL\(^{-1}\) solution of RSV and the first derivative of the developed ratio spectra were traced with \(\Delta \lambda = 4\) nm. In the binary mixtures, content EZE was determined by measuring the first derivative amplitude at 223.4 nm, where there is no contribution or interference from RSV.

First-derivative technique (D\(_1\)) traced with \(\Delta \lambda = 4\) 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 2.5-15 µg mL\(^{-1}\), for each of the drugs.

### 2.5 Method Validation

The method was validated as per ICH guidelines\(^{22}\) for parameters like Linearity, Accuracy and Precision. 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.

### 3. RESULTS AND DISCUSSION

Zero-order absorption spectra of 10 µg mL\(^{-1}\) of each of RSV and EZE showed overlapping peaks that interfere with the simultaneous determination of this formulation as shown in Figure-2. So it was thought of interest to develop the ratio spectra derivative spectrophotometry method for the simultaneous 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.

### 3.1 Ratio spectra Derivative Spectrophotometry Method

The absorption spectra of RSV prepared at increasing concentrations in Methanol were recorded in the spectral region of 200.0-300.0 nm and divided by the previously stored spectrum of 10.0µg mL\(^{-1}\) EZE in the same solvent and their ratio spectra were obtained as seen in the Figure-3a. Then, the first derivatives of ratio-spectra were recorded as shown in Fig. 3b which were plotted with the interval of \(\Delta \lambda = 4\) nm and the values of the derivatives were measured at suitably selected wavelength for the determination of RSV. The influence of the \(\Delta \lambda\) for obtaining the first derivative was tested and \(\Delta \lambda = 4\) nm was considered as suitable. The concentration of divisor can be modified, and different calibration graphs are then obtained. A concentration of 10.0 µg mL\(^{-1}\) of EZE was considered as suitable. The calibration graph was established by measuring at the amplitude at 237.4 nm corresponding to a maximum wavelength.

For determining EZE, an analogous procedure was followed. The ratio spectra were obtained by dividing the spectra of EZE with previously stored spectrum of a 10 µg mL\(^{-1}\) RSV solution as shown in figure-4a and their first derivatives were calculated with the interval of \(\Delta \lambda = 4\) nm as shown in figure-4b. The values of the derivatives were measured at suitably selected wavelength for the determination of RSV. A concentration of 10.0 µg mL\(^{-1}\) of RSV was considered as suitable. The calibration graph was established by measuring at the amplitude at 223.4 nm corresponding to a maximum wavelength.

### 3.2 Method validation

The developed method was validated for parameters like linearity, precision and accuracy. The method was found to be linear in the range of 2.5-15 µg mL\(^{-1}\) for both the drugs with correlation coefficient of 0.99995 and 0.99977 for RSV and EZE respectively. The data for linearity and precision are presented in the Table-1. The data for recovery study are shown in the Table-2. The low value of %R.S.D. indicates that the method is precise and accurate.

### 3.3 Application of method to Tablet dosage form

The proposed method after validation was applied to the simultaneous estimation of RSV and EZE in tablet dosage forms available commercially. The results obtained show the high reliability and reproducibility of the method. The results of the study are presented in Table-3.
Table-1: Data showing linearity and precision of the developed method

<table>
<thead>
<tr>
<th>Method</th>
<th>Ratiospectra Derivative Method</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>RSV</td>
<td>EZE</td>
</tr>
<tr>
<td>Linearity Range</td>
<td>2.5-15 µg mL⁻¹</td>
<td>2.5-15 µg mL⁻¹</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0349</td>
<td>0.0485</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.0073</td>
<td>0.0495</td>
</tr>
<tr>
<td>Correlation Co-efficient</td>
<td>0.99995</td>
<td>0.99977</td>
</tr>
<tr>
<td>Intraday Precision (% assay)</td>
<td>100.3</td>
<td>100.0</td>
</tr>
<tr>
<td>Intraday Precision (% R.S.D.)</td>
<td>0.17</td>
<td>0.91</td>
</tr>
<tr>
<td>Interday Precision (% assay)</td>
<td>100.4</td>
<td>99.8</td>
</tr>
<tr>
<td>Interday Precision (% R.S.D.)</td>
<td>0.85</td>
<td>0.37</td>
</tr>
</tbody>
</table>

* Average of six determinations  

b Estimated on six determinations

Table-2: Data showing recovery of the developed method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>RSV µg mL⁻¹</th>
<th>EZE µg mL⁻¹</th>
<th>STD. RSV added µg mL⁻¹</th>
<th>STD. EZE added µg mL⁻¹</th>
<th>RSV found µg mL⁻¹</th>
<th>EZE found µg mL⁻¹</th>
<th>% RSV recovered</th>
<th>% EZE recovered</th>
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<tr>
<td>Ratiospectra Derivative Spectrophotometry</td>
<td>10</td>
<td>10</td>
<td>2.52</td>
<td>2.53</td>
<td>12.63</td>
<td>12.51</td>
<td>100.9</td>
<td>99.8</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
<td>5.04</td>
<td>5.06</td>
<td>14.93</td>
<td>14.89</td>
<td>99.3</td>
<td>98.9</td>
</tr>
</tbody>
</table>

* Average of three determinations

Table-3: Results of analysis of tablet dosage forms containing RSV and EZE

<table>
<thead>
<tr>
<th>Method</th>
<th>Ratiospectra Derivative Method</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>RSV</td>
<td>EZE</td>
</tr>
<tr>
<td>%Assay*</td>
<td>99.7</td>
<td>99.2</td>
</tr>
<tr>
<td>SD*</td>
<td>0.35</td>
<td>0.49</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.35</td>
<td>0.49</td>
</tr>
</tbody>
</table>

* Average of six determinations  

SD: Standard Deviation  
RSD: Relative Standard Deviation
Figure-2: Overlain spectrum of 10 µg mL$^{-1}$ of RSV and EZE

Figure-3a: Ratio spectra of RSV when 10 µg mL$^{-1}$ solution of EZE is used as a divisor

Figure-3b: First Derivative Ratio spectra for RSV
Figure-4a: Ratio spectra of EZE when 10 µg mL⁻¹ solution of RSV is used as a divisor

Figure-4b: First Derivative Ratio spectra for EZE

4. ACKNOWLEDGEMENT
The authors are grateful to Torrent Research Center, Gandhinagar, India, for providing free drug standards of RSV and EZE.

5. REFERENCES
7. Suslu I., Celebier M. and Altinoz S., Determination of Rosuvastatin in Pharmaceutical

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