IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
April 2018 Vol.:12, Issue:1

Development and Validation of Spectrophotometric Method for Simultaneous Determination of Rosuvastatin and Ezetimibe in Capsule Dosage Form

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Submission: 20 March 2018
Accepted: 27 March 2018
Published: 30 April 2018

www.ijppr.humanjournals.com

Keywords: Rosuvastatin, Ezetimibe, UV Spectrophotometry, Simultaneous Equation Method, First Order Derivative method.

ABSTRACT

Two precise, accurate, simple and cost-effective UV spectrophotometric methods have been developed and validated for simultaneous estimation of Rosuvastatin and Ezetimibe in Capsule dosage form. Method I is estimation using simultaneous equation method at 242 nm (λ max of Rosuvastatin) and 232 nm (λ max of Ezetimibe). Method II is First Order Derivative method. Here 231.5 nm, the zero crossing point of Ezetimibe, was selected for the determination of Rosuvastatin and 224.2 nm, the zero crossing point of Rosuvastatin, was selected for the Determination of Ezetimibe. Both methods obeys Beer's law in the concentration range of 2-20 µg /ml for Rosuvastatin and Ezetimibe. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of the proposed method. The method was successfully applied to the determination of these drugs in capsule dosage form.

INTRODUCTION:

Rosuvastatin (RST) is chemically designated as (3R, 5S, 6E) - 7 - [4 - (4 - fluorophenyl) - 2 - (N – methyl methane sulfonamido) - 6 - (propane - 2 - yl) pyrimidin - 5 - yl] - 3, 5 - dihydroxyhept - 6 - enoic acid. It is a member of the drug class of statins. It is used in the treatment of Hyperlipidemia (Figure-1).

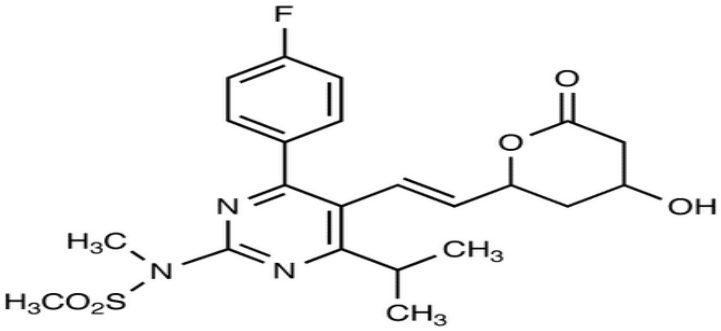

Figure 1: Chemical Structure of Rosuvastatin

Rosuvastatin Calcium is a selective and competitive inhibitor of hydroxyl methyl glutaryl coenzyme A (HMG Co-A) reductase (a precursor of cholesterol), the rate-limiting enzyme that converts 3-hydroxyl-3-methylglutaryl coenzyme A to mevalonate. It reduces levels of low-density lipoprotein, apolipoprotein B and triglycerides in the blood while increasing levels of high-density lipoprotein in the management of hyperlipidaemias. Ezetimibe (EZT) chemically designated as (3R, 4S) - 1 - (4 - fluorophenyl) - 3 - [(3S) - 3 - (4 - fluorophenyl) - 3 - hydroxypropyl] - 4 - (4 - hydroxyphenyl) azetidin - 2 – one (Figure-2).

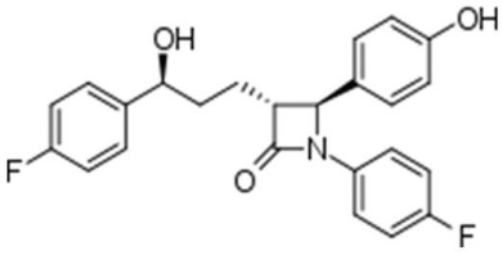

Figure 2: Chemical Structure of Ezetimibe

It is a selective cholesterol absorption inhibitor, used for the treatment of hyperlipidemia, which potentially inhibits the absorption of biliary and dietary cholesterol. Ezetimibe prevents intestinal absorption of cholesterol without affecting absorption of triglycerides, fatty acids, and fat-soluble vitamins.

MATERIALS AND METHODS:

Materials:

A double beam UV- visible spectrophotometer (Shimadzu, UV – 1800, Japan), attached to a computer software UV prob 2.0, with a spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 1 cm matched quartz cells, Analytical balance (CP224S, Sartorius, Germany), Ultrasonic cleaner (Frontline FS 4, Mumbai, India), Corning volumetric flasks and pipettes of borosilicate glass were used in the study.Methanol AR Grade was procured from S. D. Fine Chemicals Ltd., Mumbai, India. Whatman filter paper no 41 (Millipore, USA) was also used in the study. Rosuvastatin and Ezetimibe standard powder were gifted by Zydus Cadila Healthcare Ltd.The capsule formulations containing 10 mg rosuvastatin and 10 mg ezetimibe were procured From Zydus Cadila Healthcare Ltd.

Preparation of standard stock solution

An accurately weighed standard powder of 10 mg of RSV and EZE were transferred in 100 ml volumetric flask separately, dissolved and diluted up to the mark with methanol, to get final concentration 100µg/ml of RSV and EZE. From this standard stock solution, different aliquots were transferred into 10 ml volumetric flask and volume were made up to the mark with methanol. This solution was used as a working standard solution (WSS).

Selection of analytical wavelength

10 µg /ml solution of RSV and EZE were prepared in methanol and spectrum was recorded between 200-400 nm.The overlain spectrum of both drugs was recorded.

Preparation for a calibration curve

For the construction of calibration curve, two series of concentration in the range of 2-20 µg/ml for RSV and EZE were prepared in Methanol from a stock solution. These solutions were scanned in the range of 200-400 nm and absorbances were measured at the selective wavelength and calibration curve was plotted for absorbance vs. concentration.

Method I (Simultaneous Equation Method)

λ maximum of individual drugs was at 242 nm for Rosuvastatin and 232 nm for Ezetimibe. Different aliquots of the standard solution of Rosuvastatin and Ezetimibe were transferred

into the volumetric flask. The solutions were then made up to the volume with diluents, so the final concentration for Rosuvastatin was in the range of 2-20 µg/mL and for Ezetimibe was in the range of 2-20 µg/mL At the absorbance of these standard solutions, calibration curves were plotted at these wavelengths. Two simultaneous equations were formed using these absorptivity coefficient values.(Figure.3)

$Cx = (A_1 a_{y2} - A_2 a_{y1}) / (a_{x1} a_{y2} - a_{x2} a_{y1})$ and

$Cy = (a_{x1} A_2 - a_{x2} A_1) / (a_{x1} a_{y2} - a_{x2} a_{y1})$

Cx and Cy = Concentration of RSV and EZE respectively (gm/100 ml)

ax1 and ax2= Absorptivity of RSV at λ1 and λ2 respectively

ay1 and ay2= Absorptivity of EZE at λ1 and λ2 respectively

A1 and A2= Absorbance of a test at λ1 and λ2 respectively

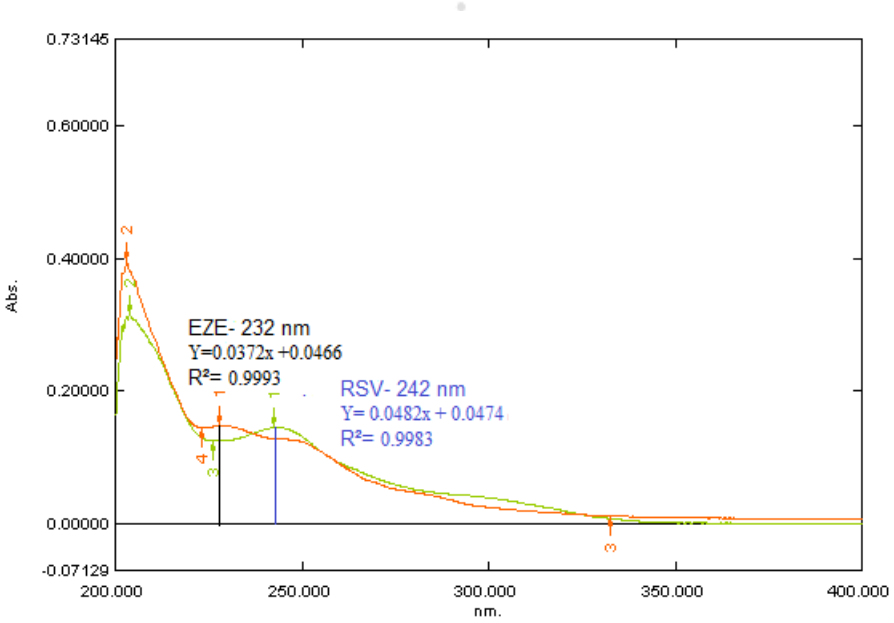

Figure 3: Simultaneous Equation Method

Method II (First Order Derivative Method)

In this method, Rosuvastatin and Ezetimibe standard stock solutions were prepared and scanned in the spectrum mode from 200 nm to 400 nm. The absorption spectra obtained were derivatized from first to fourth order. First order derivative spectra were selected for analysis

of drug.From spectra of a drug, the absorbance was measured at 231.5 nm for Rosuvastatin and 224.2 nm for Ezetimibe, amplitude difference (dA) with respect to wavelength difference (dλ) was measured for the respective concentration of standard and was plotted against concentrations and the regression equation was calculated. All the validation tests were conducted in above-prepared range. (Figure. 4)

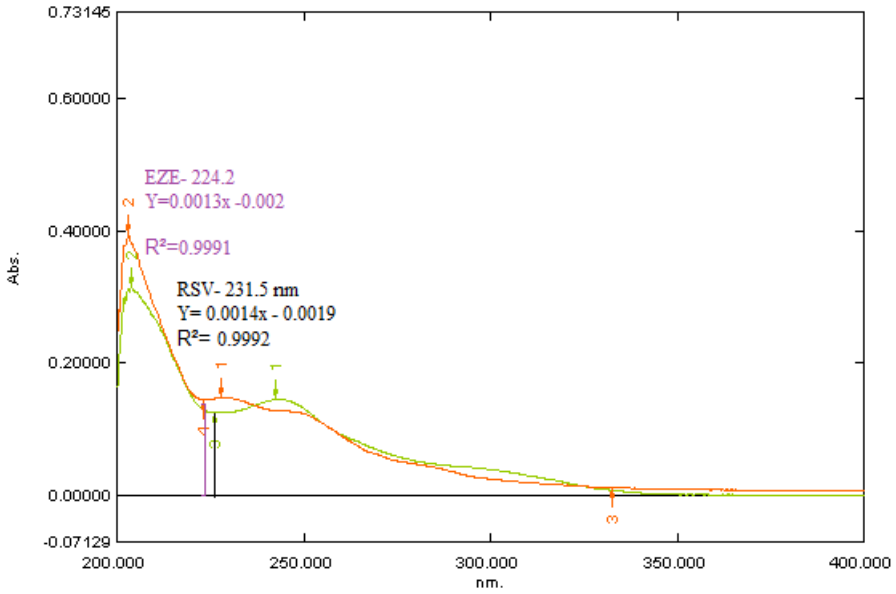

Figure 4: First Order Derivative Method

Procedure for Analysis of Capsule Formulation

To determine the content of Rosuvastatin and Ezetimibe simultaneously in Capsule (label claim: 10 mg Rosuvastatin and 10 mg Ezetimibe); twenty tablets were weighed and average weight was calculated. The capsules were crushed to obtain a fine powder. Capsule powder equivalent to 10 mg of Rosuvastatin and 10 mg of Ezetimibe was transferred to 100 ml volumetric flask and diluted to 10 ml with methanol. Sonication for 15 min and was diluted up to the mark.It was mixed and filtered the resulting solution with what Mann filter paper. 1.0 ml of resulting solution was taken and diluted to 10 ml with methanol mixed properly. The resulting solution appropriate diluted with methanol to obtain 10µg/ml of Rosuvastatin and 10µg/ml of Ezetimibe. The concentration of both Rosuvastatin and Ezetimibe were determined by measuring the absorbance of the sample at 232 nm, 242 nm (for Simultaneous equation) and 224.2 nm& 231.5nm (for First order derivative).The results of the capsule analysis were calculated against the calibration curve in quantitation mode.The result is shown in table.1.

Table 1: Analysis of marketed formulation by the proposed method

Drug	label claim mg/capsule	Method I (% Assay)	Method II (% Assay)
RSV	10mg	101.67%	100.38%
EZE	10 mg	98.00%	99.5%

METHOD VALIDATION

The proposed methods were validated according to ICH Q2 (R1) guidelines for linearity, precision, accuracy, limit of detection, limit of quantification. The results are shown in table 2.

Table 2: Summary of Linear regression analysis and optical characteristics of RSV and EZE

Parameters	Method I		Method II	
	RSV	EZE	RSV	EZE
Wavelength	242 nm	232 nm	231.5 nm	224.2 nm
Linearity range (mcg/ml)	2-20 µg /ml	2-20 µg /ml	2-20 µg /ml	2-20 µg /ml
Regression equation	Y=0.0482x + 0.0474	Y=0.0372x +0.0466	Y= 0.0014x - 0.0019	Y=0.0013x -0.002
Correlation co-efficient (R^2)	0.9983	0.9993	0.9992	0.9991
Intraday Precision (% RSD)	0.4- 0.7%	0.2- 0.75%	0.28- 0.59%	0.48- 0.91%
Interday Precision (% RSD)	0.2- 0.57%	0.46- 0.8%	0.49- 0.72%	0.50- 0.74%
Repeatability (% RSD)	0.39%	0.7%	0.48%	0.99%
Detection Limit	0.0948	0.138	0.088	0.1299
Quantification Limit	0.316	0.460	0.296	0.433
(By calculation)				

Linearity & Range

The standard stock solution containing 100µg/ml each of RSVand EZE were further diluted to get linearity conc. of 2-20 µg/ml of RSV and EZE respectively. Calibration curve was plotted by taking absorbance on Y –Axis and concentration on X –axis the relation between drug and its absorbance is expressed by the equation Y = MX + C, where 'M' is slope and 'C' is intercept, linear regression equation.

Accuracy

Recovery studies were performed to validate the Accuracy of a developed method by adding an 50 %, 100 %, 150 %. of a standard drug in a Pre-analyzed sample solution. These results summarized in table 3.

Table 3: Results of Recovery Study

Name of drug	% level	Amount of drug Taken (µg /ml)	Amount of drug Added (µg /ml)	Method I (% Recovery)	Method II (% Recovery)
RSV	50	5	2.5	99.98%	101.66%
	100	5	5	101.12%	99.74%
	150	5	10	100.69%	99.00%
EZE	50	5	2.5	98.23%	98.56%
	100	5	5	99.59%	99.41%
	150	5	10	98.03%	99.72%

Precision

Repeatability

Six dilutions in three replicate of concentration were analyzed in the same day for repeatability and result were found within the acceptable limit (RSD < 2) as shown in table4.

Table 4: Result of Precision Study

	RSV and EZE (µg /ml)	Method I		Method II	
		RSV	EZE	RSV	EZE
Intraday	2	0.49%	0.28%	0.59%	0.48%
(% RSD)	4	0.7%	0.59%	0.28%	0.57%
	6	0.24%	0.75%	0.45%	0.91%
Interday	2	0.57%	0.7%	0.56%	0.50 %
(%RSD)	4	0.29%	0.80%	0.72%	0.52%
	6	0.41%	0.46%	0.49%	0.74%

Intermediate Precision (Reproducibility)

Three dilutions in the three Replicate were analyses on two different days, two analysts for day to day & analyst to analyst variation. All result fell within acceptable limits (RSD < 2) as shown in table 4.

Limit of Detection and Quantification

The limit of detection and limit of quantification were estimated from the std. calibration curve. The residual standard deviation of regression line or std. deviation of Y –intercepts of regression lines were used to calculate LOD and LOQ.

Here LOD = 3.3*D/S and LOQ = 10*D/S. Where D is the Standard deviation of Y-intercept of regression line S is the slope of calibration curve table 2.

RESULT AND DISCUSSION:

For both the methods linearity was observed in the concentration range of 2-20 µg/ml for both Rosuvastatin and Ezetimibe. Marketed brand of the capsule was analyzed and amount of drug determined by proposed methods ranges from 99.5 to 100.38 % as shown in Table- 1. The proposed methods were validated as per ICH guideline. The accuracy of the method was determined by calculating mean percentage recovery. It was determined at 50,100 and 150 % level. The % recovery ranges from 98.03 to 101.66% for both the methods and are presented in Table 3.Precision was calculated as repeatability (% RSD is less than 1) and inter and intraday variations (%RSD is less than 1) for both drugs. The repeatability data are presented in Table-4. The proposed methods were found to be simple, accurate and rapid for the routine determination of Rosuvastatin and Ezetimibe in the capsule formulation. To study the validity and reproducibility of proposed methods, recovery studies were carried out. The methods were validated in terms of linearity, accuracy, precision, and reproducibility. Both methods can be successfully used for simultaneous estimation of Rosuvastatin and Ezetimibe in capsule dosage form.

CONCLUSION

All the validation parameters were studied as per the ICH guidelines. All the methods were found to be accurate, simple, Precise, Selective, Specific and reproducible. Hence, the

methods can be used for routine analysis of both the drugs in their combined capsule dosage form.

ACKNOWLEDGMENT

We are very thankful to Zydus Cadila Healthcare Ltd, Ahmedabad for providing standard API of Rosuvastatin and Ezetimibe respectively and to S. K. Patel College of Pharmacy, Ganpat University, Kherva for providing the necessary facilities and constant encouragement.

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