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VALIDATED ABSORPTION CORRECTION METHOD FOR SIMULTANEOUS ESTIMATION OF ROSUVASTATIN CALCIUM AND EZETIMIBE IN TABLET DOSAGE FORM

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ABSTRACT

A new, simple, accurate and sensitive UV-Spectrophotometric absorption correction method has been developed and validated for simultaneous determination of Rosuvastatin calcium (RSV) and Ezetimibe (EZE) in a tablet dosage form. Methanol and distilled water (70:30) was used as a solvent. The wavelengths selected for analysis were 300 nm and 242.80 for Rosuvastatin calcium and Ezetimibe respectively. Beer's law obeyed the concentration range of 10-35 mcg/ml for Rosuvastatin calcium and 5-30 mcg/ml for Ezetimibe. The percentage recovery was found in the range of 99.34% - 100.2% for Rosuvastatin calcium and 100.02% - 102.49% for Ezetimibe. The mean percentage drug content for Rosuvastatin calcium and Ezetimibe was found to be 103% and 108% respectively and % RSD value was found to be less than 2 which shows the precision of method. The developed method was validated statistically and by recovery studies. Thus the proposed method is simple, precise, economic, accurate and can be successfully applied for simultaneous determination of Rosuvastatin calcium and Ezetimibe in tablet dosage form.

KEYWORDS: Rosuvastatin calcium, Ezetimibe, Absorption correction method, Spectrophotometer, Validated.

INTRODUCTION

Rosuvastatin (RSV) calcium is chemically (3R,5S,6E)-7-[4-(4-flurophenyl)-2-(N-methylmethane sulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid] calcium salt. It is a lipid lowering drug. It inhibits the enzyme 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) to mevalonate, a precursor of cholesterol and thereby

checks the synthesis of cholesterol. Ezetimibe (EZE) is chemically 1-(4-fluro phenyl)-3(R)-[3-(4-fluro phenyl)-3-(S)-hydroxy propyl]-4-(S)-(4-hydroxy phenyl)-2-azetidinone.It is a selective absorption inhibitor that effectively blocks intestinal absorption of dietary and biliary cholesterol. Both drugs are used in combination to treat dyslipidemia, hyperlipidemia, hypercholesterolemia and to prevent cardiovascular disease including artherosclerosis. The chemical structures of RSV calcium and EZE are shown in **Fig 1 and 2** respectively.

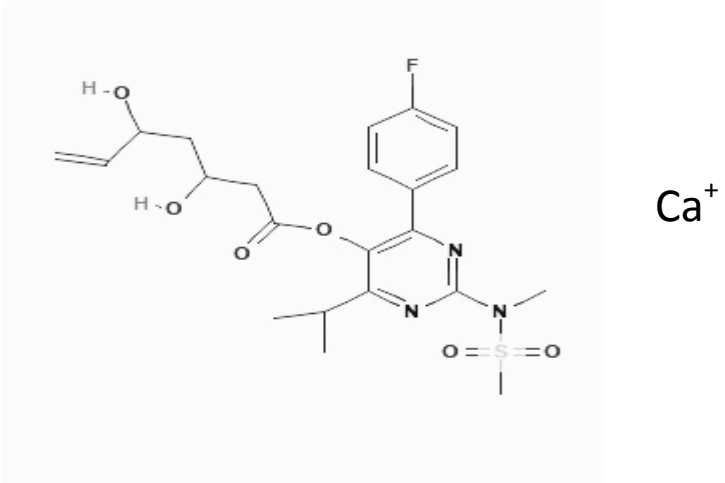

Fig 1: Structure of rosuvastatin calcium

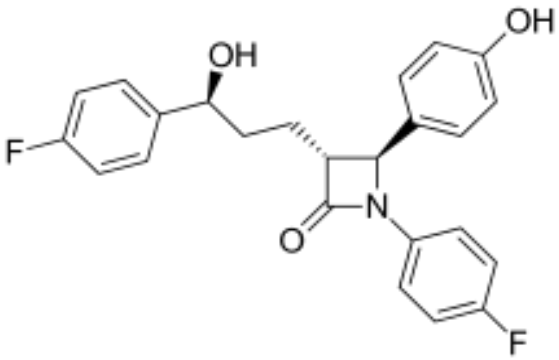

Fig 2: Structure of ezetimibe

Literature survey revealed number of reported methods for individual determination of both the drugs and in combination with other drugs. RSV calcium alone has been determined by spectrophotometric methods[1,2,3], stability indicating method[4], HPTLC and RP-HPLC.[5] EZE was also estimated using UV-method[6], Derivative spectroscopy[7], HPLC, HPTLC and LC-MS/MS[8] for determination in human plasma and serum. Methods for simultaneous estimation of both the drugs in tablets which has been developed include simultaneous

equation method[9], Q absorption ratio, dual wavelength method and first derivative method.[10]

Till date, there is no reported method for simultaneous estimation of both the drugs in a dosage form by absorption correction method. So the objective of this work was to develop simple, precise and rapid absorption correction method for combination drug products containing RSV calcium and EZE.

MATERIALS AND METHOD

Instrumentation

The present work was carried out on Shimadzu UV-2700 UV/VIS Spectrophotometer, with a pair of 1.0 cm matched quartz cells.

Reagents and Chemicals

Pharmaceutically pure samples of Rosuvastatin calcium and Ezetimibe were obtained from Zydus cadila, Goa and Glenmark Pharmaceuticals, Goa respectively. Razel EZ 10 tablets were obtained from Salcette Farmacia, Goa. All chemicals were of analytical grade.

METHODOLOGY (PRELIMINARY STUDY)

Determination of Solubility

Solubility of Rosuvastatin calcium and Ezetimibe was checked in methanol, distilled water, 0.1N HCL, 0.1 and 0.2 N NaOH, Ethanol. According to the solubility characteristics, the common solvent for drug was found to be methanol and distilled water in the ratio 70:30.

Principle[11]

In this method, two wavelengths are selected, one wavelength at which both the drugs shows absorbance, whereas the other wavelength at which only one drug shows absorbance and the other drug shows zero absorbance. If the identity, concentration and absorptivity of the absorbing interferents are known, it is possible to calculate their contribution to the total absorbance of the mixture. The concentration of absorbing component of interest is than calculated from the corrected absorbance i.e. difference between total absorbance and absorbance of the interfering substances.

Preparation of standard stock solutions

10 mg of Rosuvastatin calcium and Ezetimibe were weighed accurately and each transferred in 10 ml amber colored volumetric flask. The stock solution was prepared by dissolving both

the drugs in 70:30 solvent mixture of methanol and water. The final concentration obtained were 1000 mcg/ml for both the drugs.

Study of Spectral characteristics

The aliquots portions of standard stock solutions of Rosuvastatin calcium and Ezetimibe were further diluted with methanol-water (70:30) solvent mixture to get the concentration of 10 mcg/ml of each drug and the solutions were scanned between the range 400-200 nm in 1 cm cell against blank and the overlain spectra was recorded. From the overlain spectra it was observed that Ezetimibe has zero absorbance at 300 nm whereas Rosuvastatin calcium has substantial absorbance. Thus Rosuvastatin calcium was directly estimated at 300 nm without interference of Ezetimibe. At 242.80 nm both the drugs showed substantial absorbance. Absorbance of Ezetimibe was measured at 242.80 nm. The contribution of Rosuvastatin calcium was deducted from total absorbance of the sample mixture at 242.80 nm. The calculated absorbance was called as corrected absorbance for Ezetimibe. Overlay spectra of RSV calcium and EZE is given in **Fig 3**.

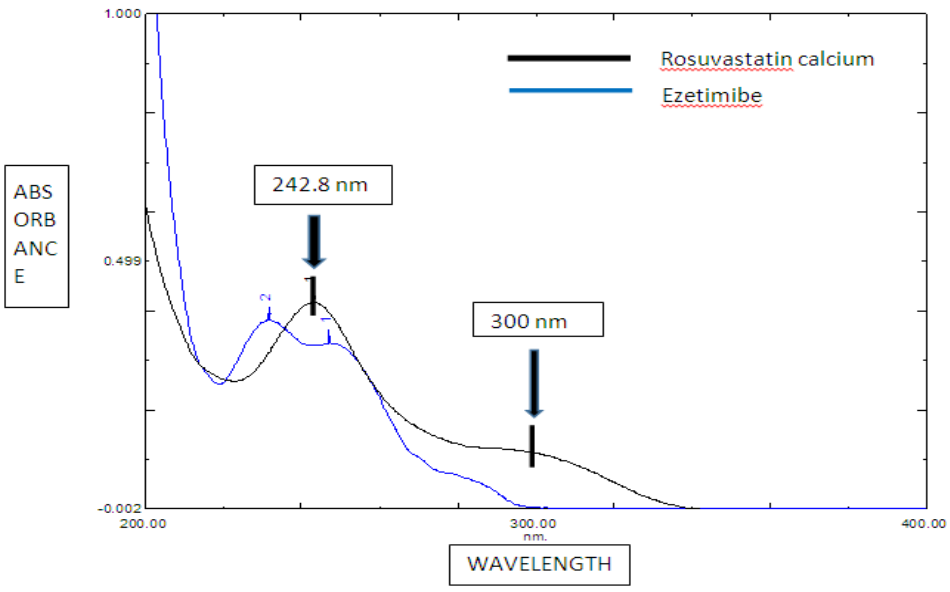

Fig 3: Overlay spectra of rosuvastatin calcium and ezetimibe

The concentration of the solutions were calculated using the Beer-Lamberts Law: A= a x b x c

Where,

'A ' is the absorbance of the solution, 'a' is the absorptivity, 'b' is the pathlength and 'c' is the concentration.

Thus, concentration of Rosuvastatin calcium was calculated at 300 nm using the equation,

$$C_x = A_x / a_x$$

Where,

C_x = concentration of Rosuvastatin calcium

A_x = Absorbance of Rosuvastatin calcium at 300 nm

a_x = absorptivity of Rosuvastatin calcium at 300 nm

Absorbance of Rosuvastatin calcium at 242.80 nm was calculated using the equation,

$$A_{x1} = C_x \times a_{x1}$$

Where,

A_{x1} = Absorbance of Rosuvastatin calcium at 242.80 nm

a_{x1} = absorptivity of Rosuvastatin calcium at 242.80 nm

Corrected absorbance of Ezetimibe at 242.80 nm was calculated using the equation,

$$A_y = A_{total} - A_{x1}$$

Where,

A_y = Absorbance of Ezetimibe at 242.80 nm

A_{total} = Total absorbance of sample at 242.80 nm

Concentration of Ezetimibe at 242.80 nm was calculated using the equation,

$$C_y = A_y / a_y$$

Where,

C_y = Concentration of Ezetimibe

a_y = Absorptivity of Ezetimibe at 242.80 nm

Analysis of Tablet Formulation

5 tablets were weighed and their average weight was determined. The tablets were triturated to fine powder. An accurately weighed quantity of powder equivalent to 10 mg of both the drugs was transferred to 100 ml calibrated amber colored volumetric flask and 75 ml of solvent mixture of methanol:water (70:30) was added to dissolve the substance. The solution was shaked for 30 minutes and sonicated for 3 minutes and made up to the volume with the solvent mixture. The final concentration obtained was 100 mcg/ ml. The solution was filtered through Whatman filter No 42. An aliquot portion of obtained filtrate was diluted to 10 ml with solvent mixture to obtain a concentration of 10 mcg/ml. At all the selected wavelengths

the absorbance of the sample solution was accurately measured. The concentrations of Rosuvastatin calcium and Ezetimibe in the tablet formulation were thus calculated. Six solutions using the same procedures were analysed. The results of analysis are shown in **Table 1**.

METHOD VALIDATION[12]

Following were the parameters which were performed in order to validate the method – Linearity, Accuracy, Precision, LOD (Limit of detection), LOQ (Limit of quantitation).

Linearity

Six different concentrations were checked for linearity by diluting the stock solutions. The final concentrations Rosuvastatin calcium were in the range of 10 -35 mcg/ ml and for Ezetimibe in the range 5-30 mcg/ml and absorbance was measured at wavelengths 300 nm and 242.80 nm respectively. Concentrations of Rosuvastatin calcium were also measured at 242.80 nm. Calibration curves (n = 6) were plotted between the concentration of solution and absorbance of drug. The calibration plot for RSV calcium and EZE are shown in **figure 4, 5** and **6** respectively.

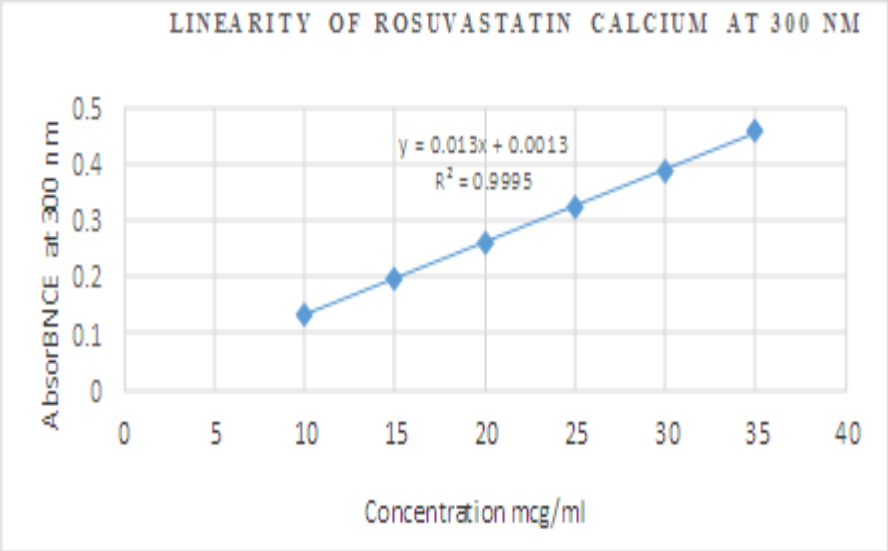

Fig 4: Linearity of rosuvastatin calcium at 300 nm

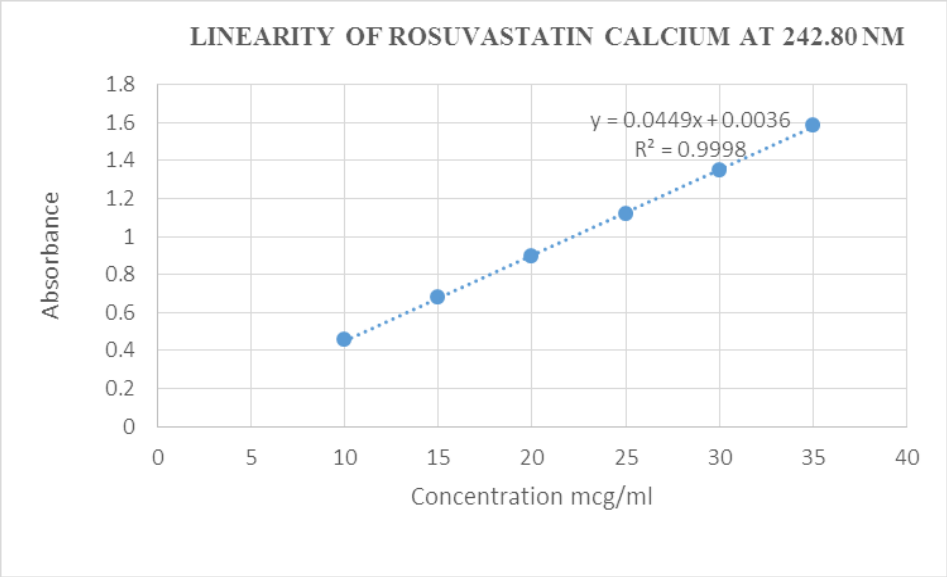

Fig 5: Linearity of rosuvastatin calcium at 242.8 nm

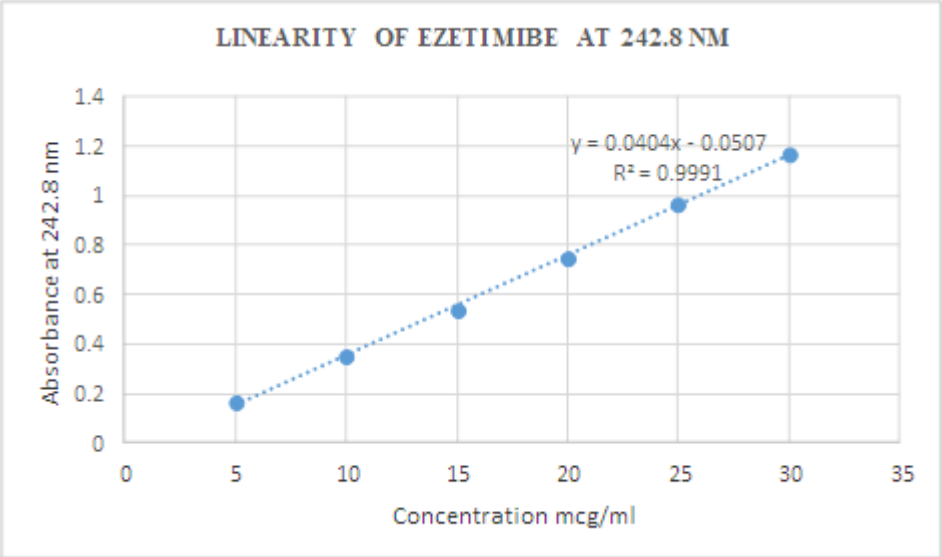

Fig 6: Linearity of ezetimibe at 242.80 nm

Precision

The precision of the method was confirmed by repeatability and intermediate precision. The repeatability was done by analysis of formulation for six times. The amount of each drug present in tablet formulation was calculated. The % RSD was calculated. Intermediate precision of the method was confirmed by interday analysis i.e the analysis of formulation was repeated on three successive days. The amount of drug and % RSD was calculated. The repeatability data is tabulated in **Table 3.** The intermediate precision data is tabulated in **Table 4.**

Accuracy

The recovery studies were carried to check interference of the excipients and other interferences at three different concentrations for which standard addition method was used. Reference standard solution of each drug was added to tablet samples at three different concentration level. At each level, samples were prepared in triplicate and the mean percentage recoveries and % RSD values were calculated. The results are tabulated in **Table 5.**

Sensitivity

The limit of detection (LOD) and limit of quantitation (LOQ) parameters were calculated using the following equations:

$$LOD = 3.3\sigma/s$$

$$LOQ = 10\sigma/s$$

Where, σ is standard deviation and s is slope.

RESULTS AND DISCUSSION

An attempt has been made to develop a rapid, sensitive, economic, precise and accurate analytical method for simultaneous estimation of Rosuvastatin calcium and Ezetimibe in tablet dosage forms by UV spectrophotometric absorption correction method. Simultaneous estimation for both the drugs was carried out using methanol and water in the ratio 70:30 at wavelengths 300 nm and 242.80 nm. At 300 nm only Rosuvastatin calcium showed absorbance and thus its concentration was determined directly from the absorbance, whereas at 242.80 nm both Rosuvastatin calcium and Ezetimibe showed absorbance. Thus correction of absorbance helps estimate the concentration of Ezetimibe, the other drug in the combination by subtracting the known absorbance of Rosuvastatin calcium at 242.80 nm.

In the range of 10-35 mcg/ml for Rosuvastatin calcium at 300 nm and 242.80 nm and 5-30 mcg/ml for Ezetimibe at 242.80 nm the Beer's law was obeyed. The absorbances of the drugs were found to be linear with concentration as the values for correlation coefficient were above 0.999 at all the wavelengths. The results are tabulated in **Table 1.** Absorptivity values for Rosuvastatin calcium and Ezetimibe is tabulated in **Table 2** and **3** respectively.

Table 1: Data for spectral and linearity characteristics

Parameters	Rosuvastatin calcium	Ezetimibe
λmax (nm)	300	242.80
Linearity range (mcg/ ml)	10-35	5-30
Correlation coefficient (r2)	0.9995	0.9991
Slope (m)	0.013	0.0404
Intercept	0.0013	0.0507

Table 2: Absorptivity values for rosuvastatin calcium

Concentration mcg/ml	Absorptivity at 242.8 nm	Absorptivity at 300 nm
10	0.0457	0.0134
15	0.0453	0.01306
20	0.0449	0.0131
25	0.04484	0.01304
30	0.0449	0.0129
35	0.045285	0.0132
Mean	0.04515	0.0131

Table 3: Absorptivity values for ezetimibe

Concentration mcg/ml	Absorptivity at 242.8 nm	Absorptivity at 300 nm
5	0.033	0
10	0.0351	0
15	0.0358	0
20	0.03745	0
25	0.03856	0
30	0.0389	0
Mean	0.03646	0

The percentage label claim present in tablet formulation was found to be 103% and 108% for Rosuvastatin calcium and Ezetimibe respectively **Table 4.**

Table 4: Results of assay of tablet formulation

Parameters	Rosuvastatin calcium	Ezetimibe
Label claim	10 mg	10 mg
% Assay	103%	108%
Standard deviation	0.002041242	0.0080663
% RSD	1.51%	1.02%

Precision was confirmed by repeated analysis of formulation for six times. The % RSD values were found to be 0.94% and 1.49% for Ezetimibe and Rosuvastatin Calcium

respectively. Data of repeatability studies is given in **Table 5.** Data of intermediate precision is given in **Table 6.**

Table 5: Data of repeatability study

Sr NO	Absorbance	
	Rosuvastatin calcium at 300 nm	Ezetimibe at 242.80 nm
1	0.137	0.849
2	0.136	0.847
3	0.135	0.850
4	0.141	0.868
5	0.137	0.860
6	0.137	0.852
Mean	0.13716	0.85433
%Assay	104.7%	106.3%
% RSD	1.49%	0.94%

Table 6: Data of intermediate precision

Sr No	Absorbance			
	Day I		Day II	
	242.8 nm	300 nm	242.8 nm	300 nm
1	0.853	0.136	0.846	0.136
2	0.853	0.138	0.861	0.139
3	0.877	0.141	0.869	0.138
4	0.874	0.139	0.860	0.136
5	0.866	0.137	0.867	0.136
6	0.867	0.141	0.854	0.137
Mean	0.865	0.13866	0.859	0.1365
% Assay (%)	108	105	108	104
% RSD (%)	1.18	1.49	0.99	0.92

The recovery studies were carried out using standard addition method .The results are tabulated in **Table 7.**

Table 7: Data of accuracy studies

% Recovery	% Mean recovery (%)		% RSD (%)	
	RSV calcium	EZE	RSV calcium	EZE
80	99.76	102.49	1.86	0.55
100	100.2	101.7	1.61	0.37
120	99.34	99.99	1.09	0.33

LOD and LOQ results are tabulated in **Table 8**.

Table 8: Lod and Loq

DRUGS	LOD mcg/ml	LOQ mcg/ml
Rosuvastatin calcium	0.786709	2.383965
Ezetimibe	1.0483	3.176667

CONCLUSION

The proposed analytical UV Spectrophotometric method was developed and validated for quantitative determination of Rosuvastatin calcium and Ezetimibe in tablets. The developed method was found to be simple, rapid, accurate, precise and economical, which can be directly and easily applied to the analysis of RSV calcium and EZE in pharmaceutical tablet dosage form.

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