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Simultaneous estimation of Rosuvastatin calcium and Ezetimibe in tablet dosage form by reverse phase high performance liquid chromatography

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This paper described validated high performance liquid chromatographic (HPLC) method for estimation of Rosuvastatin Calcium (ROS) and Ezetimibe (EZE) in tablet dosage form. HPLC separation was achieved on Licrosphere C_{18} column (250 x 4.6mm) using Methanol: Acetonitrile: Phosphate buffer, pH 3.5(60:20:20 v/v) at flow rate of 1.0ml/min at 25^{o}C temperature. Quantitation was achieved by UV detection at 279 nm over the concentration range 5-10 mg/ml for both the drugs with mean recoveries of 99.01% $\pm$ 0.12 and 100.64% $\pm$ 0.20 for ROS and EZE respectively. This method is simple, precise and sensitive and it is applicable for the simultaneous estimation of ROS and EZE in tablet dosage form.

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Keywords: Reverse phase -HPLC, Rosuvastatin calcium, Ezetimibe

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[1], the rate –limiting enzyme that converts 3-hydroxy -3-methylglutaryl coenzyme A to mevalonate, precursor for cholesterol. It is a cholesterol lower agents. In recent years some HPLC method were reported for the quantification of rosuvastin calcium in human plasma by automated solid phase extraction using tandem mass spectrometric detection. [2, 3, 4] Its approximate elimination half life is 19 hours and its time to peak plasma concentration are reached in 3–5 hours following oral administration.Ezetimibe[5] (EZTB), (3R, 4S)-1-(4-fluorophenyl)-3-[(3S)-(4-fluorophenyl)-3 hydroxypropyl]-4-(4-hydroxyphenyl)-2-azetidinone, is a class of lipid-lowering compound that selectively inhibits the intestinal absorption of cholesterol and related phytosterols.Several analytical methods have been developed for the quantification of Ezetimibe .the methods include HPLC[6] and spectrophotometry[7] .Literature survey revealed that no HPLC method has been reported for the estimation of in combined dosage form.Because of the absence of an official pharmacopoeial method for the simultaneous estimation of ROS and EZE in tablet dosage form, efforts were made to develop an analytical method for the estimation of ROS and EZE in tablet dosage form using HPLC method.

2. Experimental

2.1 Apparatus

High performance liquid chromatograph, Shimadzu pump LC-10AT VP equipped with Rheodyne inject ROS with 20µl fixed loop, Photo Diode Array detec ROS. SPD-MXA software was used.

2.2 Reagent and material

ROS and EZE pure powder were procured as gifts sample from Sun pharma Dadra. Rozavel EZ tablets (Sun Pharmaceuticals Ltd) were procured from local market. Label claim of Rozavel EZ tablet for ROS and EZE were 10 mg and 10 mg respectively. Methanol HPLC grade, Acetonitrile HPLC grade were purchased from E.Merck (Mumbai, India), Potassium Dihydrogen Phosphate and o-phosphoric acid were purchased from SD fine chemical Ltd (Ahmedabad, India) and were of analytical grade Water of HPLC grade was used.

2.3 Chromatographic condition of method

The Licrosphere C_{18} column was used 25^{o}C temperature. The mobile phase considered Methanol: acetonitrile: phosphate buffer (60:20:20 v/v) pH adjusted to 3.5 $\pm$ 0.1 with o-phosphoric acid. It was pumped at flow rate of 1ml/min. the mobile phase was passed through nylon 0.45 µm membrane filters and degassed before use.

The elution was moni ROS at 279 nm and the injection volume was 20 µl.

2.4 Preparation of standard stock solution

The equivalent of 10 mg each of ROS and EZE were accurately weighed in 100 ml volumetric flasks separately and dissolve in 25 ml of methanol. After the immediate dissolution, the volume was made up to the mark with solvent. These standard stock solutions were observed to contain 100 µg/ml of ROS and EZE.

2.5 Preparation of sample solution

20 tablets were taken and their average weight was determined, they were crushed to fine powder. Then powder equivalent to 10 mg of ROS and 10 mg EZE was taken in 25ml volumetric flask and dissolved in 75ml of methanol with vigorous shaking for 5-10 minutes. The supernatant liquid was transferred to 50ml of volumetric flask through a.whatman no 41 filter paper. The residue was washed twice with solvent and the combined filtrate was made up to 100ml mark. After that 10 ml of the above solution was diluted up to 100 ml with solvent.

3. Method validation

3.1 Linearity

Calibration graphs were constructed by plotting peak area Vs concentration of ROS and EZE and the regression equation were calculated. The calibration graphs were plotted over 5 different concentrations in the range of 5-25µg/ml for both drugs. Accurately measured mixed standard solution aliquots of ROS and EZE (0.5, 1.0, 1.5, 2.0, 2.5 ml) were transferred to series of 10 ml volumetric flasks and diluted to mark with methanol. Aliquots (20µl) of each solution were injected under the operating chromatographic condition described above [Number of replicates (n=6)].

3.2 Accuracy

The accuracy of the method was established using recovery technique i.e external standard addition method. The known amount of standard was added at three different levels to preanalysed sample. Each determination was performed in triplicate. The result of recovery study is presented in Table 2.

Table 2. Recovery study.

ROS				EZE			
Label claimed	%Amount added	Found in(µg/ml)	%recovery	Label claimed	%Amount added	Found in(µg/ml)	%recovery
10	85	9.15	99.93	10	85	9.10	99.00
	95	9.96	97.73		95	9.97	99.75
	105	11.5	98.40		105	9.99	99.97

3.3 Method precision (repeatability)

The precision of the instrument was checked by repeatedly injecting (n = 6) mixed standard solution of ROS and EZE.

3.4 Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing mixed standard solution of ROS and EZE at concentration 5µg/ml and 25µg/ml 3 times on the same day and on 3 different days. The results are reported in terms of relative standard deviation.

3.5 Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD with signal to noise (S/N) ratio of 3:1 and LOQ with (S/N) ratio of 10:1 were calculated for both drugs using the following equations according to International Conference on Harmonization guidelines [8]

$$LOD = 3.3 \times \sigma/S$$
$$LOQ = 10 \times \sigma/S$$

where σ = the standard deviation (SD) of the response and S = the SD of the y-intercept of the regression line.

3.6 Analysis of ROS and EZE in tablet dosage form

The response of sample solutions were measured at 279 nm for quantitation of ROS and EZE by the method described above. The amount of ROS and EZE present in the sample solution were determined by applying values of peak area to regression equation of the calibration graph.

4. Results and discussion

To optimize the HPLC parameters, several mobile phase compositions were tried. A satisfactory separation of ROS and EZE with good peak symmetry and steady baseline was obtained with mobile phase Methanol: Acetonitrile: Phosphate buffer (60:20:20 v/v) adjusted to

pH 3.5.Quantitation was achieved with UV detection at 238nm based on peak area. Complete resolution of the peaks with clear baseline separation was obtained (Fig. 1).

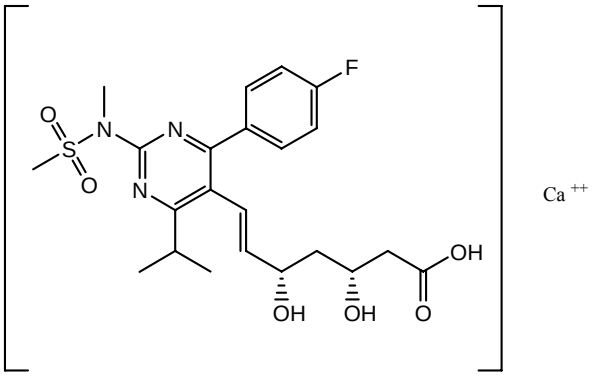

Fig. 1. Rosuvastatin calcium.

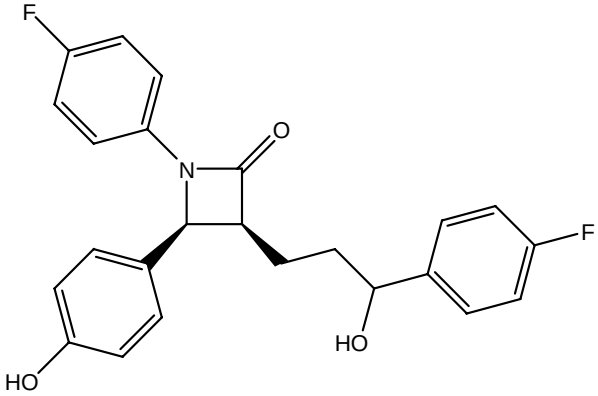

Fig. 2. Ezetimibe molecule.

The system suitability test parameters are shown in Table 1.

Table 1. System suitability test parameter for ROS and EZE.

Property (n*=6)	ROS	EZE
Retention time(min)	3.245	4.577
Tailing factor ROS	1.36	1.30
Capacity factor ROS	1.70	1.20
Theoretical plates number	1905	2372
Resolution	1.25	1.23

* n = Number of determination

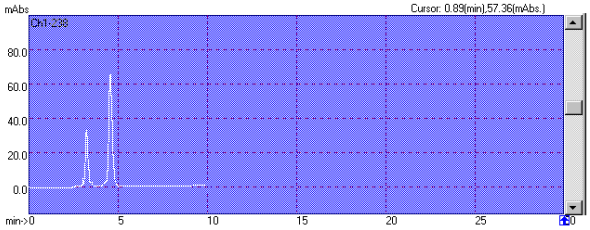

Fig. 3. High performance liquid chromatogram of ROS and EZE with detection at 279 nm.

4.1 Validation of the proposed method

4.1.1 Linearity

Linear correlation was obtained between peak areas and concentration of ROS and EZE in the range of 5-25µg/ml for both the drugs, respectively. Data of the regression analysis are summarized in Table 3.

Table 3. Regression analysis of calibration graph for ROS and EZE.

Parameter	ROS	EZE
Concentration range	5-25 µg/ml	5-25 µg/ml
Slope	24739	25904
SD[$] of the slope	19.35	20.56
Intercept	419240	384347
SD[a] of the intercept	164.47	214.85
Correlation coefficient	0.9932	0.9994

[$] SD = Standard Deviation

4.1.2 Accuracy

The recovery experiments were performed by standard addition method. The recoveries obtained were 98.77 $\pm$ 0.12 % and 99.78 $\pm$ 0.21% for ROS and EZE respectively (Table 4).

Table 4. Summary of validation parameter.

Parameter	ROS	EZE
LOD[a]	0.06µg/ml	0.09µg/ml
LOQ[b]	0.07µg/ml	0.04µg/ml
Accuracy, %	99.73 $\pm$ 0.43	99.88 $\pm$ 0.31%
Repeatability(RSD[c], %, n =6)	0.831	0.341
Precision (RSD, %)		
Intraday(n =3)	0.061	0.054
Interday(n = 3)	0.085	0.073

4.1.3 Method precision

The RSD values for ROS and EZE were found to be 0.095 % and 0.124 % respectively (Table 4).

4.1.4 Intermediate precision

The RSD values were found to be < 2%, which indicates that the proposed method is reproducible. (Table 4).

4.1.5 LOD and LOQ

LOD values for ROS and EZE were found to be 0.01 and 0.004µg/ml respectively. LOQ values for ROS and EZE were found to be 0.03 and 0.01µg/ml respectively. (Table 4).

4.2 Assay of the tablet dosage form (ROS 10mg/tablet and EZE 10mg/tablet)

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 5)

Table 5. Result of assay of tablet formulation.

ROS		EZE	
Amount claimed (mg/tablet)	Amount found (mg/tablet)	Amount claimed (mg/tablet)	Amount found (mg/tablet)
10	9.89	10	9.75
	10.05		9.96
	10.1		10.27
	9.91		9.98
	9.98		10.01
	9.91		10.01
Mean	6.873	Mean	8.032
±SD	0.0643	±SD	0.490

5. Conclusions

The proposed method has advantage of simplicity and convenience for the separation and quantitation of ROS 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.

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