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Der Pharma Chemica, 2010, 2(1): 371-377
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X

Micellar Liquid Chromatographic Method Development for Determination of Rosuvastatin Calcium and Ezetimibe in Pharmaceutical Combination Dosage Form

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Abstract

This paper described validated, rapid, simple and sensitive liquid chromatographic procedure that use micellar mobile phase containing only Tween-20 and n-Butanol, is reported for the determination of 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 Tween-20 and n-Butanol Phosphate buffer, pH 5.1 (60:20:20 v/v) at flow rate of 1.0 ml/min at 25^{o}C temperature. Quantitation was achieved by UV detection at 314 nm over the concentration range 5-10 mg/ml for both the drugs with mean recoveries of 99.91% $\pm$ 0.12 and 100.11% $\pm$ 0.29 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.

Keywords: Rosuvastatin Calcium, Ezetimibe Micellar liquid chromatography, Tween-20.

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 agent. 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] (EZE), (3R, 4S)-1-(4-fluorophenyl)-3-[(3S)-(4-fluorophenyl)-3hydroxypropyl]-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]. Micellar liquid chromatography has been reported as a suitable technique for pharmaceuticals and intermediate for drug and cosmetics interest [8]. Micellar solution can replace conventional aqueous organic mobile phase with good results. Micellar liquid chromatography (MLC) is a reversed phase liquid chromatographic (RPLC) mode with mobile phases containing a surfactant (Ionic or Non ionic) above its critical concentration (CMC) [9]. In these conditions the stationary phase is modified with a approximately constant amount of surfactants monomers, and solubilizing capability of mobile phase is altered by the presence of micelles, giving rise to diverse interactions (Hydrophobic, ionic and satiric) with major implications and selectivity.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 Micellar liquid chromatography method 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. Micellar mobile phases have been used with different bonded stationary phases (mostly C8, C18 and cyanopropyle). The most common surfactant are the anionic sodium dodecyl sulphate (SDS) cationic cetytri methyl ammonium bromide (CTAB), and non-ionic Tween-20, several organic solvents have been used as modifiers, short/medium chain alcohols and acetonitrile being the most suitable. The presence of micellar contributes well above their solubility in water. Also, the risk of evaporation is diminished.

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 Tween-20, n-Butanol Phosphate buffer (60:20:20 v/v) adjusted to pH 5.5 $\pm$ 0.01.Quantitation was achieved with UV detection at 238nm based on peak area. Complete resolution of the peaks with clear baseline separation was obtained (Fig.3). The system suitability test parameters are shown in(Table 1).

Validation of the proposed method

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 Table3.
Accuracy- The recovery experiments were performed by standard addition method. The recoveries obtained were 100.77 $\pm$ 0.13 % and 99.99 $\pm$ 0.02% for ROS and EZE respectively (Table 4).
Method precision- The RSD values for ROS and EZE were found to be 0.095 % and 0.124 % respectively (Table 4).
Intermediate precision- The RSD values were found to be < 2%, which indicates that the proposed method is reproducible (Table 4).

LOD and LOQ – LOD values for ROS and EZE were found to be 0.02 and 0.008µg/ml respectively. LOQ values for ROS and EZE were found to be 0.05 and 0.07µg/ml respectively. (Table 4).

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).

Materials and Methods

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.

Reagent and Material

ROS and EZE pure powder were procured as gifts sample from Sun Pharmaceutical Industries Silvassa Dadra Nager Hawali India. Rozavel EZ tablets (Sun Pharmaceuticals Industries Dadra Nager Hawali India) were procured from local market. Label claim of Rozavel EZ tablet for ROS and EZE were 10 mg and 10 mg respectively. Tween-20, n-Butanol and water were obtained from Merck. All reagents were of HPLC grade unless otherwise specified. 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.

Chromatographic condition of method

The Licrosphere C_{18} column was used 25^{o}C temperature. The mobile phase considered 5% n-Butanol in 0.05 molL^{-1} Tween-20 pH adjusted to 5.5 $\pm$ 0.01 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 314 nm and the injection volume was 20 µl.

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 n-Butanol. 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. The two main advantages of micellar procedure are the elimination of organic solvents and simplification of sample preparation step. The seven point's calibration graphs were constructed covering a concentration range. 0.5 to 5 mg/ml. linear relationship was obtained between the peak area ratio of ROS and EZE in the concentration range 25 ppm to 125 ppm. The correlation coefficient was found 0.9999. According to International Conference on Harmonization (ICH) guidelines the following expression is used to evaluate LOD and LOQ.

Preparation of sample solution

Twenty 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 n-Butanol with vigorous shaking for 5-10 minutes. The supernatant liquid was transferred to 50ml of volumetric flask through whattman 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.

Method Validation

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 n-Butanol. Aliquots (20µl) of each solution were injected under the operating chromatographic condition described above [Number of replicates (n=6)].

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 pre-analyzed sample. Each determination was performed in triplicate. The result of recovery study is presented in table 2.

Method precision (repeatability)

The precision of the instrument was checked by repeatedly injecting (n = 6) mixed standard solution of ROS and EZE.The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by assaying samples, at same concentration and during the same day. The intermediate precision was studied by comparing the assays on different days. Five sample solutions were prepared and assayed.

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.

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 [10]

$LOD = 5.23 \times \sigma/S$

$LOQ = 3.1 \times \sigma/S$

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

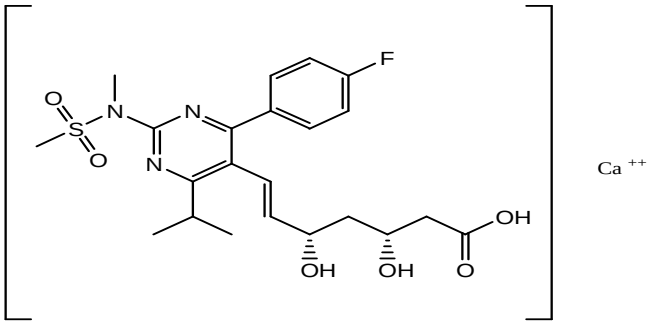

Figure-1 Rosuvastatin Calcium

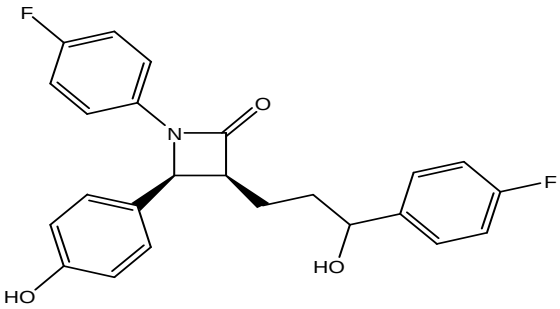

Figure-2 Ezetimibe Molecule

Table 1.System suitability test parameter for Rosuvastatin Calcium and Ezetimibe

Property (n*=6)	**ROS**	**EZE**
Retention time(min)	8.943	11.756
Tailing factor ROS	5.85	6.53
Capacity factor ROS	0.938	1.12
Theoretical plates number	2431	4352
Resolution	2.43	4.84

ROS- Rosuvastatin Calcium**,** EZE- Ezetimibe * n = Number of determination

Table 2. Recovery Studies Rosuvastatin Calcium and Ezetimibe

ROS				**EZE**			
Label claimed	% Amount added	Found in(µg/ml)	% recovery	Label claimed	% Amount added	Found in(µg/ml)	% recovery
10	85	10.21	100.21	10	85	10.05	100.09
	95	10.02	100.03		95	10.12	100.01
	105	9.99	99.98		105	100.06	100.32

ROS- Rosuvastatin Calcium**,** EZE- Ezetimibe

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	32417	45632
SD[$] of the slope	32.75	54.85
Intercept	54738	45328
SD[a] of the intercept	21.76	68.08
Correlation coefficient	0.9987	0.9999

ROS- Rosuvastatin Calcium**,** EZE- Ezetimibe, [$] SD = Standard Deviation

Table 4. Summary of validation parameter

Parameter	ROS	EZE
LOD[a]	0.01µg/ml	0.03µg/ml
LOQ[b]	0.09µg/ml	0.07µg/ml
Accuracy, %	100.73 ± 0.12	99.98 ± 0.01%
Repeatability(RSD[c], %, n =6)	1.121	1.847
Precision (RSD, %)		
Intraday(n =3)	0.0321	0.0438
Interday(n = 3)	0.0123	0.0123

ROS- Rosuvastatin Calcium**,** EZE- Ezetimibe

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	10.11	10	10.53
	9.98		10.03
	9.99		10.16
	10.03		9.99
	10.21		10.05
	9.97		9.98
Mean	2.643	Mean	4.065
±SD	0.0453	±SD	0.0654

ROS- Rosuvastatin Calcium**,** EZE- Ezetimibe

Analysis of ROS and EZE in tablet dosage form

The response of sample solutions were measured at 314 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.

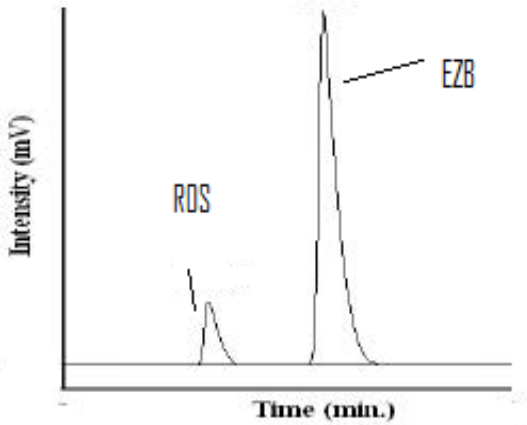

Figure.3. High Performance Liquid Chromatogram of ROS and EZE with Detection at 314 nm

Conclusion

The proposed miceller chromatographic method has been evaluated over the linearity, precision, accuracy, specificity and proved to be conveninant and effective for the quality control The proposed method has advantage of simplicity and convenience for the separation and quantization 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.

Acknowledgements

We are grateful to Head School of Pharmacy, Devi Ahilya Vishwavidyalaya,Indore (M.P) 452001,India, for given valuable suggestion and provided facility and Sun Pharmaceutical Industries Mumbai India for the gifts sample of Pure Rosuvastatin Calcium and Ezetimibe.

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