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Original Article

VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF ROSUVASTATIN CALCIUM AND EZETIMIBE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: To develop and validate a rapid, sensitive and specific reverse phase high performance liquid chromatography (RP-HPLC) method for simultaneous determination of Rosuvastatin Calcium and Ezetimibe in a combined dosage form.

Methods: The chromatographic separation of the two drugs were achieved using Enable C18G (5 µm, 250 mm x 4.6 mm i.d. column). The drugs were separated in isocratic elution mode with a mobile phase consists of Acetonitrile-Water (75:25, v/v) at a flow rate of 0.6 ml/min and a detection wavelength of 252 nm using a UV detector.

Results: The linearity and range for both Rosuvastatin Calcium and Ezetimibe were 5-40 µg/ml (R^2= 0.9995) and 5-40 µg/ml (R^2= 0.9992), respectively. Accuracy of the method was determined through recovery studies by adding known quantities of standard drug to the preanalyzed test solution and was found to be 99.6-100.3 % and 99.5-99.9% for Rosuvastatin Calcium and Ezetimibe respectively. The % RSD values for both interday and intraday precision were found to be<2%.

Conclusion: A rapid, sensitive and specific RP-HPLC method was developed and validated for simultaneous determination of Rosuvastatin Calcium and Ezetimibe in a combined dosage form and hence, it can be used in the quality control analysis of an active pharmaceutical ingredient and pharmaceutical dosage form.

Keywords: Rosuvastatin Calcium, Ezetimibe, Method development, Validation.

INTRODUCTION

Rosuvastatin calcium, a new member of a class of cholesterol lowering drugs commonly referred to as "statins", was approved for the treatment of dys lipidemia [1-3]. Rosuvastatin calcium (ROS) is chemically bis [(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl-(methylsulfonyl) amino] pyrimidin-5-yl] (3R, 5S)-3, 5-dihydroxy hept-6-enoicacid] calcium salt (fig.1). It is a synthetic lipid lowering agent, selective and competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, the key rate-limiting enzyme of cholesterol biosynthesis in liver. ROS is used to reduce the amounts of LDL cholesterol, total cholesterol, triglycerides and lipoprotein B in the blood. ROS also modestly increases the level of HDL cholesterol in the blood. These actions are important in reducing the risk of atherosclerosis, which in turn can lead to several cardiovascular complications such as heart attack, stroke and peripheral vascular disease. ROS peak plasma concentrations were reached by 3–5 hrs following oral administration in humans [4].

Ezetimibe [5-6] (EZE) (3R, 4S)-1-(4-flourophenyl)-3-[(3S)-3-(4-flourophenyl)-3-hydoxypropyl]-4-(4-hydroxypenyl) azetidine-2-one(fig. 2), is an anti-hyperlipidemic medication, acts by decreasing cholesterol absorption in the intestine. Both drugs are used in combination to treat Hyperlipidemia, Hypercholesterolemia and to prevent cardiovascular disease including atherosclerosis. Numbers of reported method were already available for the individual determination of both drugs. Rosuvastatin Calcium alone has been determined by Spectrophotometric methods [7-9], Stability indicating method [10], HPTLC [11] and RP-HPLC [12-14]. Ezetimibe was also estimated using UV method [15-17], Derivative Spectroscopy [18-19] and LC-MS/MS [20]. As per literature survey only three HPLC methods [21-23] has been developed for the simultaneous determination of both the drugs in tablets. The present research work describes the simple, rapid, accurate, sensitive and reproducible RP-HPLC method for simultaneous estimation of Rosuvastatin Calcium and Ezetimibe from the tablet formulation.

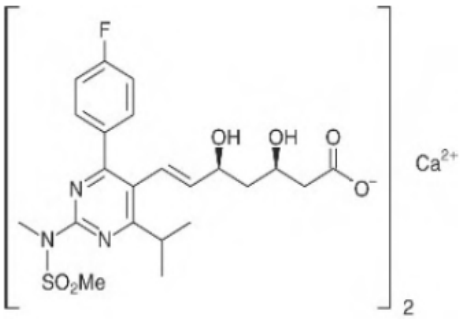

Fig. 1: structure of Rosuvastatin calcium

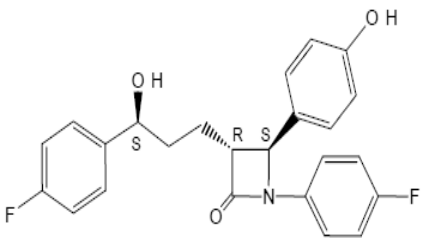

Fig. 2: structure of Ezetimibe

MATERIALS AND METHODS

Instrumentation

Shimadzu HPLC comprising of LC-20AD binary gradient pump, a variable wavelength programmable SPD-20A detector and an SCL 20A system controller. A Rheodyne injector fitted with a 20 µL loop

was used and data were recorded and evaluated by use of LC solutions software.

Chemicals and reagents

ROS and EZE pure samples were procured as gift samples. "ROZAVEL-EZ" tablets (Sun Pharmaceuticals Ltd) were procured from the local market. Label claim of Rozavel EZ tablet for ROS and EZE was 10 mg and 10 mg respectively. Acetonitrile HPLC grade was purchased from Merck, India. Milli-Q (Qualigens) water HPLC grade was used throughout the experiment.

Chromatographic conditions

Chromatographic analysis was performed on a Enable C18G (250 x 4.6 mm i.d., 5µ) column. The mobile phase consisted of acetonitrile: water (75: 25 % v/v). The mobile phase was degassed and filtered through 0.2 µm membrane filter before pumping into HPLC system.

- Mobile phase: Acetonitrile: water (75:25 % v/v).
- Detection wavelength: 252 nm.
- Flow rate: 0.6 ml/min.
- Injection volume: 20 µl.
- Column temperature: ambient.
- Runtime: 8 mins.
- Run mode: isocratic.

Preparation of standard stock solution

10 mg each of ROS and EZE was accurately weighed and transferred in 100 ml volumetric flasks separately and dissolved in 25 ml of Acetonitrile. After the immediate dissolution, the volume was made up to the mark with same solvent. These standard stock solutions were diluted with mobile phase to get 100 µg/ml of ROS and EZE respectively.

Preparation of sample solution

Twenty tablets were taken and their average weight was determined and crushed to fine powder. Powder equivalent to 10 mg of ROS and 10 mg EZE was taken in 100 ml volumetric flask and dissolved in 75 ml of acetonitrile with shaking for 5-10 minutes and then centrifuged. The supernatant liquid was sonicated for 10 min and filtered through 0.2 µm membrane filter. The filtrate was transferred to 100 ml volumetric flask and volume were made up with acetonitrile. From this 10 ml of the above solution was diluted up to 100 ml with mobile phase.

Method validation [24]

The developed chromatographic method was validated for system suitability, linearity & range, specificity, accuracy, precision, ruggedness and robustness as per ICH guidelines.

RESULTS AND DISCUSSION

Method development and optimization

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 acetonitrile: water (75: 25 % v/v) at a flow rate of 0.6 ml/min. The iso-absorptive point of the combined spectrum of both drugs at 252 nm was chosen for the detection drugs. Complete resolution of the peaks with clear baseline separation was obtained. Under these conditions ROS and EZE were eluted at 2.931 min and 6.631 min respectively with a run time of 5 min. A chromatogram of ROS and EZE was shown in fig. 3.

Method validation

System suitability

System suitability is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from six replicate injections of standards solutions of both ROS and EZE. The parameters like retention time, number of theoretical plates, tailing factor, HETP were investigated and the results are given in table 1. From the results it was observed that all the values are present within the limits indicating good performance of the system.

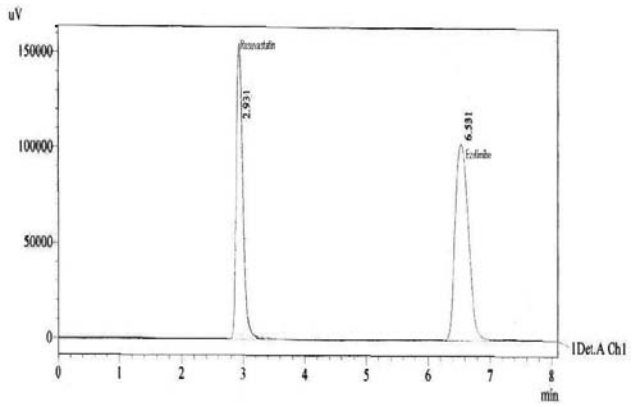

Fig. 3: Chromatogram of ROS and EZE

Table 1: System suitability parameters of ROS and EZE

Parameters	Rosuvastatin calcium	Ezetimibe
Retention time	2.931 (mins)	6.531 (mins)
Theoretical plates	3366	4465
Tailing factor	1.3	1.2
HETP	44.56	33.59
Resolution	12.1	

Specificity

Specificity was checked for the interference of excipients in the analysis of injecting sample solution under optimized chromatographic conditions to demonstrate separation of both ROS and EZE from excipients. Peak purity values were good for the both drugs, which show that the analyte peaks were pure and there were no interferences from excipients in the analyte peaks. Therefore, it could be said that the developed method was highly specific.

Linearity and range

Various concentrations from standard solutions of ROS & EZE were prepared and the calibration graph was plotted by the values of the peak area versus concentration (µg/ml) which were found to be linear over the concentration ranges of 5-40 µg/ml. Calibration curves of ROS and EZE were shown in fig. 4 & 5**.** The linearity results were shown in table 2.

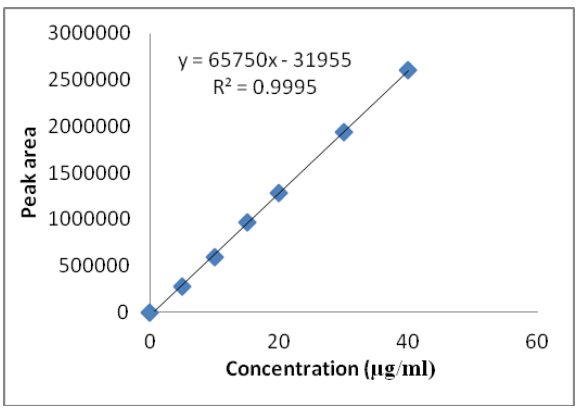

Fig. 4: Calibration curve of Rosuvastatin Calcium

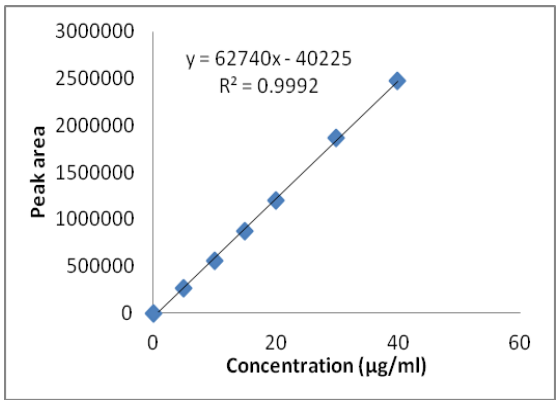

Fig. 5: Calibration curve of Ezetimibe

Precision

The precision of the developed method was studied by method precision, interday precision and intraday precision. The precision of the method was checked by repeatedly injecting six times the mixed standard solution of Rosuvastatin and Ezetimibe. The results were shown in table 3. Intra-day precision was estimated by assaying the quality control sample of the tablet formulation containing 20 µg/ml of ROS and 20 µg/ml of EZE, six times (results averaged for statistical evaluation) in the same analytical run. The statistical validation data for intraday precision is summarized in table 4**.** Inter-day precision was evaluated by analyzing a set of quality control samples of the tablet formulation containing 20 µg/ml of ROS and 20 µg/ml of EZE, six levels analyzed on three consecutive days in the same analytical runs. The statistical validation data results averaged for statistical evaluation) for inter day precision is summarized in table 5**.** The % RSD value of <2 % indicates the high precision of the developed method.

Table 2: Calibration curve data of ROS and EZE

S. No.	Concentration (µg/ml)	Peak area	
		Rosuvastatin calcium	Ezetimibe
1	5	282595	268538
2	10	591671	557966
3	15	965607	876362
4	20	1282481	1204391
5	30	1936825	1867657
6	40	2607083	2472329

Table 3: Precision data of ROS and EZE

S. No.	Concentration (µg/ml)	Rosuvastatin calcium	Ezetimibe
1	20	1282481	1203983
2	20	1287925	1204706
3	20	1286725	1200468
4	20	1283221	1203589
5	20	1282913	1205614
6	20	1280535	1204191
Mean		1283967	1203759
S. D		2790.218	1756.941
%RSD		0.21	0.14

Table 4: Intraday precision

Drug interpolated concentration(µg/ml)±SD*	%RSD
ROS(20 µg/ml) 19.65±0.090	0.46
EZE(20 µg/ml) 19.88±0.101	0.51

*six determinations

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 % recovery for Rosuvastatin Calcium and Ezetimibe was found to be 99.6-100.3 % and 99.5-99.9% respectively. The results of recovery study were presented in table 6.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as change in wavelength, composition of mobile phase and flow rate. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust. The % RSD values for all the three changed conditions were<2%. The results were shown in table 7.

Table 5: Interday precision

Drug interpolated concentration(µg/ml)±SD*	%RSD
ROS(20 µg/ml) 19.45±0.147	0.76
EZE(20 µg/ml) 19.68±0.179	0.91

*six determinations

Table 6: Recovery studies of ROS and EZE

Drug	Spiked level (%)	Amount taken (µg/ml)	Amount found (µg/ml)	Percent recovery (% w/w)±%RSD
	80	8	7.97	99.6±0.76
ROS	100	10	10.03	100.3±0.8
	120	12	11.96	99.6±0.98
	80	8	7.96	99.5±0.45
EZE	100	10	9.99	99.9±0.87
	120	12	12.06	100.6±0.92

Table 7: Robustness studies of ROS and EZE

Condition	Modification	Peak area		mean % RSD	
		ROS	EZE	ROS	EZE
Mobile phase composition	80: 20	281495	264838	0.27	0.11
Flow rate (ml/min)	0.5	289215	265412	0.28	0.21
Wavelength (nm)	257	283261	264738	0.15	0.15

Sensitivity

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision and variability. The LOD and LOQ were calculated from the linear curve using formulae

LOD= 3.3 * σ / S

LOQ= 10 * σ / S

(Where σ = the standard deviation of the response and S = Slope of calibration curve).

The results were shown in table 8.

Table 8: LOD and LOQ data for ROS and EZE

Drug	LOD (µg/ml)	LOQ (µg/ml)
ROS	0.76	2.3
EZE	0.91	2.7

Application of method to dosage form

The developed method was used for the quantitative estimation of ROS and EZE in commercial dosage form ROZAVEL-EZ tablets. Each sample was analyzed in triplicate after extracting the drugs. None of the tablet ingredients was interfered with the analyte peak. Results are shown in table 9.

Table 9: Assay results of tablet formulation (ROZAVEL-EZ)

Drug	Label claim (mg)	Assay (% label claim)	%RSD
ROS	10	99.98	0.26
EZE	10	99.87	0.15

CONCLUSION

The proposed method has the advantage of simplicity and convenience for the separation and quantitation of ROS and EZE in the combination and can be used for the assay of their dosage form. Also, low solvent consumption and short analytical run time lead to environmentally friendly chromatographic procedure. The method is accurate, precise, rapid and specific for simultaneous estimation of Rosuvastatin calcium and Ezetimibe in tablet dosage form. Hence it can be applied in routine analysis of Rosuvastatin Calcium and Ezetimibe in formulation.

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CONFLICT OF INTERESTS

Declared None

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