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Research Article

METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ROSUVASTATIN CALCIUM AND EZETIMIBE IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT

A simple, precise and efficient high performance liquid chromatographic method was developed for simultaneous determination of Rosuvastatin calcium and Ezetimibe in tablets. A symmetry C_{18} column 75×4.6mm i.d. 3.5µ in isocratic mode with mobile phase containing Acetonitrile: methanol: buffer (0.01M sod.dihydrogen phosphate) (30:20:50 v/v) pH adjusted to 3.0 using ortho phosphoric acid (v/v) .The flow rate was 1.0 ml/min and effluent was monitored at 263 nm. The retention time and linearity range for Rosuvastatin calcium and Ezetimibe were (3.88, 8.16 min) and (10-60, 10-60 µg/ml), respectively. The validation of the proposed method was carried out for its specificity, linearity, accuracy, precision, limit of detection and quantification for both Rosuvastatin calcium and Ezetimibe. The developed method can be used for simultaneous determination of Rosuvastatin calcium and Ezetimibe in tablet dosage form.

KEYWORDS: Rosuvastatin calcium, Ezetimibe, RP- HPLC, Optimization and Validation.

INTRODUCTION

Rosuvastatin, new member of a class of cholesterol lowering drugs commonly referred to as "statins", was approved for the treatment of dyslipidemia ([1–3]).

Rosuvastatin (RST) 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. RST, a

synthetic lipid lowering agent, is a selective and competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, the key rate-limiting enzyme of cholesterol biosynthesis in liver. RST is used to reduce the amounts of LDL cholesterol, total cholesterol, triglycerides and a lipoprotein B in the blood. RST 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. RST peak plasma concentrations were reached by 3–5 hrs following oral administration in humans[4]. Ezetimibe [5-6] (3*R*,4*S*)-1-(4-flourophenyl)-3-[(3*S*)-3-(4-flourophenyl)-3-hydoxypropyl]-4-(4-hydroxypenyl)azetidine-2-one, is an anti-hyperlipidemic medication, acts by decreasing cholesterol absorption in the intestine. Both drugs are used in combination to treat dyslipidemia, 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]. To the best of knowledge, 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 rapid, accurate, sensitive and reproducible RP-HPLC method for simultaneous estimation of Rosuvastatin Calcium and Ezetimibe from the tablet formulation.

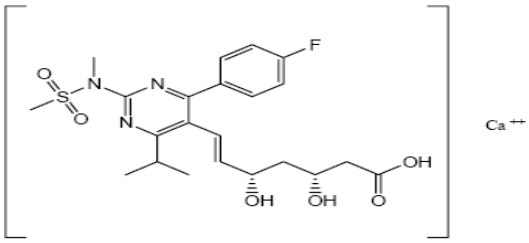

Figure1: Molecular structure of Rosuvastatin Calcium

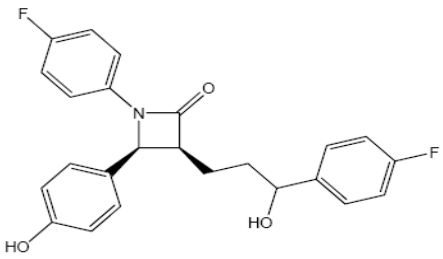

Figure2: Molecular structure of Ezetimibe

EXPERIMENTAL WORK

Instruments, Reagents and Materials.

High performance liquid chromatograph, Water model e2695 pump LC-10AT VP equipped with Rheodyne inject with 20µl fixed loop. Empower software was used. ROS and EZE pure samples were procured as gifts sample from Dr.Reddys labs, Hyderabad. "ROZAVEL- EZ" tablets (Sun Pharmaceuticals Ltd) were procured from local market. Label claim of Rozavel EZ tablet for ROS and EZE were10 mg and 10 mg respectively. Methanol HPLC grade, Acetonitrile HPLC grade were purchased from E.Merck (Mumbai, India), sodium Dihydrogen Phosphate and o-phosphoric acid were purchased from SD fine chemical Ltd (Ahmadabad, India) and were of analytical grade Water of HPLC grade was used.

Chromatographic condition of method

The symmetry C18 column was used at ambient temperature. The mobile phase considered Acetonitrile: Methanol: Sodium dihydrogen phosphate buffer (30:20:50 v/v) pH adjusted to 3.0± 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 monitored ROS and EZ at 263 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 methanol and 25 ml of Acetonitrile. 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.

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 100 ml 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 what´s man 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.

Uv Spectras of Rosuvastatin and Ezetimibe

Absorbance maxima of Rosuvastatin Calcium and Ezetimibe were detected at 244.40 nm (λ2) and 234.00 nm (λ1), respectively. Both the spectra's were

overlapped at 263 nm. Both the drugs showed linearity with absorbance in the range 10-60 µg/ml, when measured at 234.00 nm and 244.40 nm. Calibration curves were plotted from the absorbance values at these wavelengths.

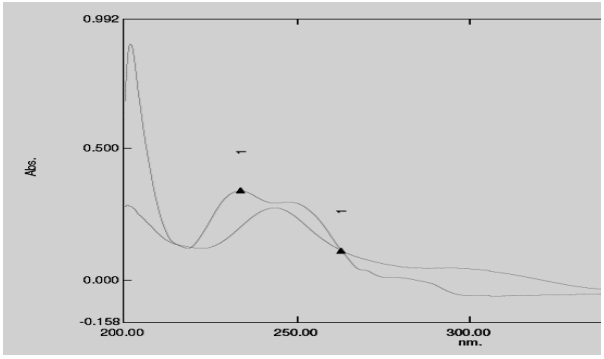

Figure-3: UV Spectra of Rosuvastatin and Ezetimibe

3. METHOD VALIDATION

a) 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 6 different concentrations in the range of 10- 60µg/ml for both drugs. Accurately measured mixed standard solution aliquots of ROS and EZE (1, 2, 3,4,5,6 ml) were transferred to series of 10 ml volumetric flasks and diluted to mark with mobile phase. Aliquots (20µl) of each solution were injected under the operating chromatographic condition described above [Number of replicates (n=6)].

b) 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 (Table2).

c) Method precision (repeatability)

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

d) 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[2].

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

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

Where σ = the standard deviation (SD) of the response and

S = the SD of the y-intercept of the regression line.

e). Analysis of ROS and EZE in tablet dosage form

The response of sample solutions were measured at 263 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 Acetonitrile: Methanol: SodiumdihydrogenPhosphate buffer (30:20:50 v/v) adjusted to pH 3.0.Quantitation was achieved with UV detection at 263nm based on peak area. Complete resolution of the peaks with clear baseline separation was obtained (Fig.4)

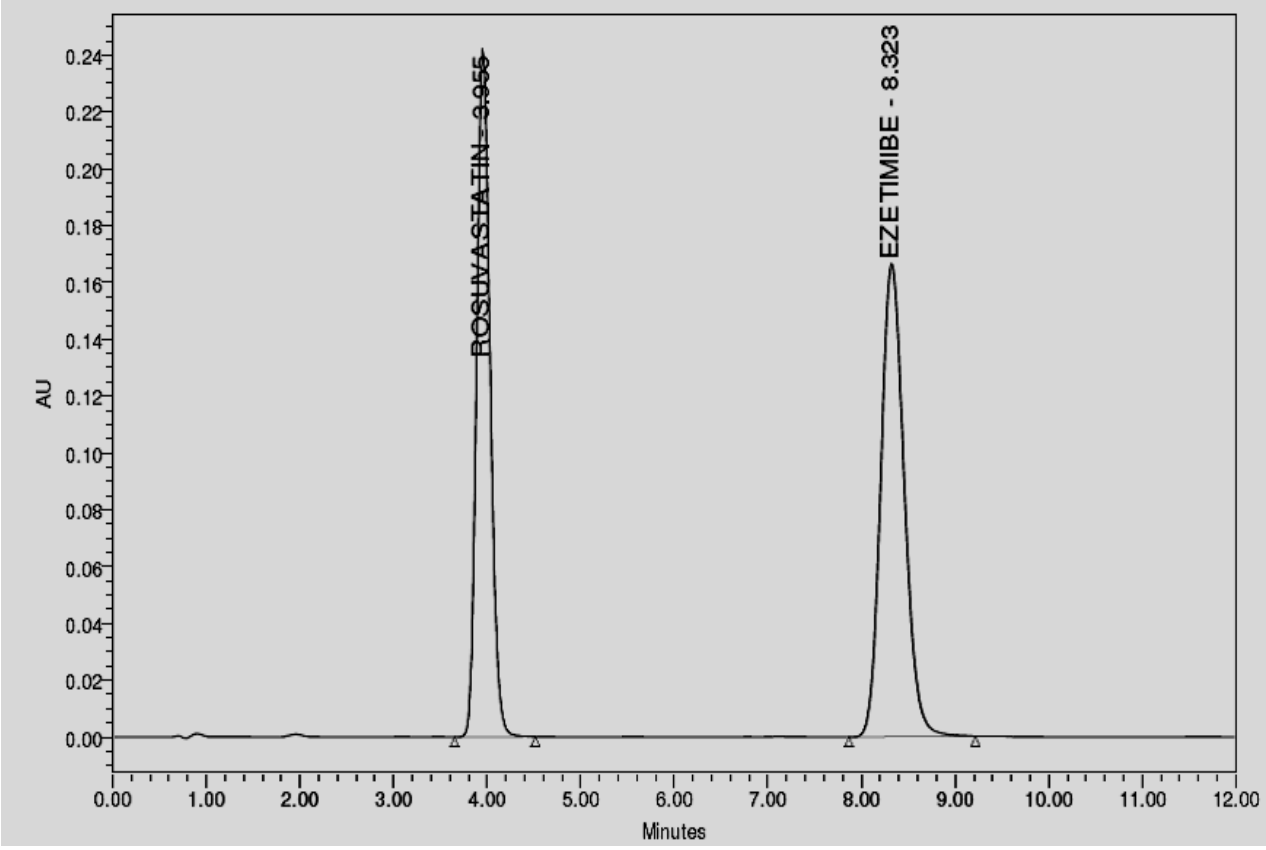

Fig. 4. High performance liquid chromatogram of ROS and EZE with detection at 263 nm.

VALIDATION OF THE PROPOSED METHOD

a). Linearity

Linear correlation was obtained between peak areas and concentration of ROS and EZE in the range of 10-60µg/ml for both the drugs, respectively. Data of the regression analysis are summarized in Table 1

Rosuvastatin calcium: **Table.1.1**

S.No	% of Test	Concentration(µg/ml)	Area
1	25	10	480809
2	50	20	951395
3	75	30	1437366
4	100	40	1935934
5	125	50	2432360
6	150	60	2932530

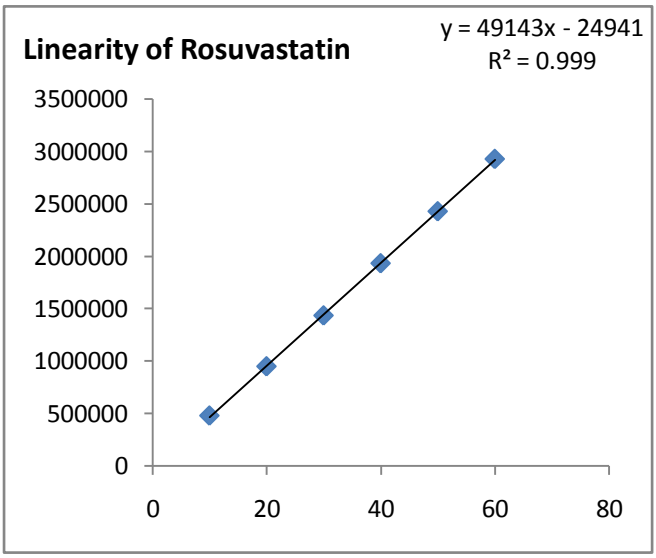

Fig. 4. Linearity graph of rosuvastatin calcium

S.NO	% of test	Concentration(µg/ml)	Area

1	25	10	571993
2	50	20	1132355
3	75	30	1712233
4	100	40	2306834
5	125	50	2895886
6	150	60	3487158

Ezetimibe: Table.1.2

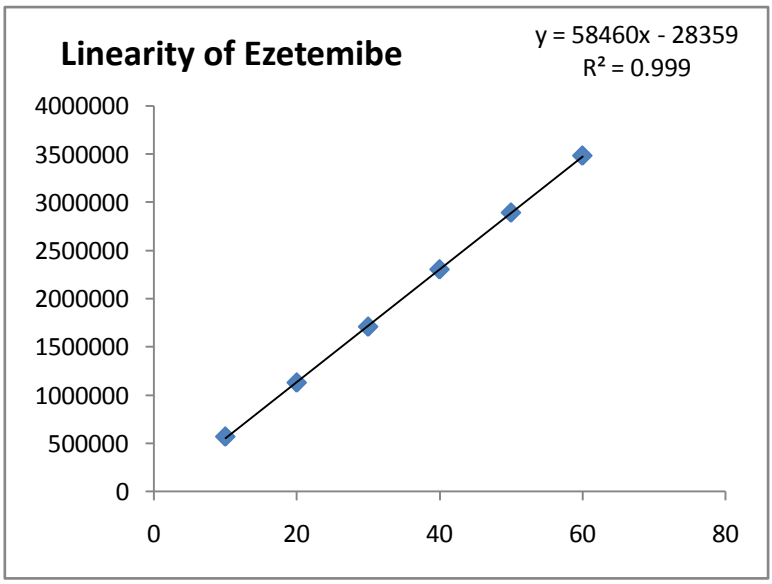

Fig. 5. Linearity graph of Ezetimibe

b).Accuracy:

The recovery experiments were performed by standard addition method. The recoveries obtained were 98.81 + 0.19 % and 99.97 + 03% for ROS and EZE respectively. The result of recovery study is presented in (Table2).

Rosuvastatin calcium: Table. 2.1

S.No	Spike Level	µg/ml added	µg/ml found	% recovery	mean % recovery
1	50%	20	19.99	99.99	100.00%
2	50%	20	20.00	100.01	
3	50%	20	1999	99.99	
1	100%	40	39.85	99.64	99.44%
2	100%	40	39.53	98.84	
3	100%	40	39.94	99.86	
1	150%	60	60.00	100.00	99.99%
2	150%	60	59.99	99.99	
3	150%	60	59.99	99.99	

Ezetimibe: **Table 2.2**

S.No	Spike Level % recovery	µg/ml found	µg/ml found	mean %recovery	mean %recovery
1	50%	20	39.98	99.95%	99.95%
2	50%	20	39.982	99.95%	
3	50%	20	39.98	99.95%	
1	100%	40	19.99	99.99%	100.01%
2	100%	40	20.00	100.00%	
3	100%	40	20.01	100.05%	
1	150%	60	59.98	99.97%	99.96%

2	150%	60	59.98	99.97%	
3	150%	60	59.96	99.97%	

c). Method precision

The RSD values for ROS and EZE were found to be 0.31 % and 0.31% respectively (Table3)

Rosuvastatin calcium: **Table. 3.1**

S.No	Retention Time(min)	Area
1	3.875	1959547
2	3.877	1963087
3	3.88	1961306
4	3.874	1957401
5	3.897	1970034
6	3.928	1972717
Average	3.8885	1964015
S.D	0.021116	6064.009
%R.S.D	**0.54**	**0.31**

Ezetimibe: **Table.3.2**

S.No	Retention Time(min)	Area
1	8.128	2332336
2	8.131	2334714
3	8.133	2333381
4	8.123	2329574
5	8.189	2343311
6	8.276	2348093
Average	8.1633	2336902
S.D	0.060321	7183.961
%R.S.D	0.74	0.31

d). LOD and LOQ

LOD values for ROS and EZE were found to be 0.407 and 0.405µg/ml respectively. LOQ values for ROS and EZE were found to be 1.27 and 1.26µg/ml respectively. (Table 4).

Table. 4

Parameter	ROS	EZE
LOD	0.4072µg/ml	0.4055µg/ml
LOQ	1.27090 µg/ml	1.265 µg/ml

e). 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

Drug	**%Assay**	**Amount Present**
Rosuvastatin calcium	99.81	9.98mg/tab
Ezetemibe	99.92	9.92mg/tab

f).System suitability parameters: Table.6

Parameter	**Acceptance criteria**	**Observed value**
1.Theoritical plates	(not less than 3000)	
Rosuvastatin calcium		3808
Ezetimibe		5938
2.Tailing factor	(not more than 2)	
Rosuvastatin calcium		1.2
Ezetimibe		1.1
3.Repeatability	(RSD <1% for N>5)	
Rosuvastatin calcium		1.2
Ezetimibe		1.1

4.Resolution(Rs)	(Rs>2)	12.54

CONCLUSION

The proposed method has 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, 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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