IJPAR |Volume 3 | Issue 1 | Jan-Mar -2014 **ISSN: 2320-2831**

INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

Available Online at: www.ijpar.com

[Research article]

RP-HPLC Method Development and Validation for the Simultaneous Estimation of Rosuvastatin and Ezetimibe in Tablet Dosage Form

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ABSTRACT

A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous determination of Rosuvastatin and Ezetimibe in pharmaceutical tablet dosage form. Chromatographic analysis was performed on a Symmetry X-terra C8 (4.6mm x 100mm, 5µm)column at ambient temperature with a mixture of ortho phosphoric acid buffer and Acetonitrile in the ratio 40:60 v/v as mobile phase, at a flow rate of 1.0 mL min^{-1}. UV detection was performed at 237 nm.. The retention times of Rosuvastatin and Ezetimibe were 2.490 and 3.173 min, respectively. The correlation coefficient of Rosuvastatin and Ezetimibe was found to be 0.999. Calibration plots were linear over the concentration ranges 10–50 µg mL^{-1} for Rosuvastatin and Ezetimibe, respectively. The Limit of detection was 1.626 and 0.918µg mL^{-1} and the quantification limit was 4.927 µg mL^{-1} and 2.783µg mL^{-1} for Rosuvastatin and Ezetimibe, respectively. The accuracy of the proposed method was determined by recovery studies and found to be 99.59% to 100.70%.The method was validated for accuracy,linearity,sensitivity,precision,robustness,system suitability Commercial tablet formulation was successfully analyzed using the developed method and the proposed method is applicable to routine analysis of determination of Rosuvastatin and Ezetimibe in pharmaceutical tablet dosage form.

Keywords: Rosuvastatin, Ezetimibe, RP-HPLC, Validation.

INTRODUCTION

Rosuvastatin is a synthetic lipid lowering agent that blocks the production of cholesterol in the body, it is a competitive 3-hydroxy-3-methyl-glutaryl coenzyme a reductase inhibitor effective in lowering LDL cholesterol and triglycerides, developed for the treatment of dyslipidemia[1]. Chemically Rosuvastatin calcium is (3R, 5S, 6E)-7-[4-(4-fluorophenyl)-6-(1-methylehyl)-2-[methyl (methylsulphonylamino)]-5- pyrimidinyl]-3, 5-dihydroxy-6-heptanoicacidcalcium[2] (Fig.1). Ezetimibe is selective cholesterol absorption inhibitor, which potentially inhibits the intestinal absorption of cholesterol and related phytosterols by the small intestine without affecting absorption of triglycerides, fatty acids, bile acids and fat-soluble vitamins[3]. The drug is widely used in treatment of hypercholesterolemia and of sitosterolemia. Chemically Ezetimibe is 1-(4-fluorophenyl)-3(R)-[3-(4-fluorophenyl)-3(S) hydroxypropyl]-4(S)-(4-hydroxyphenyl)-2-azetidinone.[4] (Fig. 2). Numbers of reported method were already available for the individual determination of both drugs. Rosuvastatin calcium

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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 RosuvastatinCalcium and Ezetimibe from the tablet formulation.

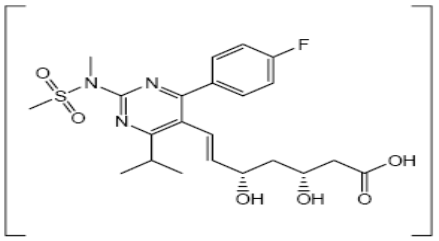


Figure-1: Molecular structure of Rosuvastatin Calcium

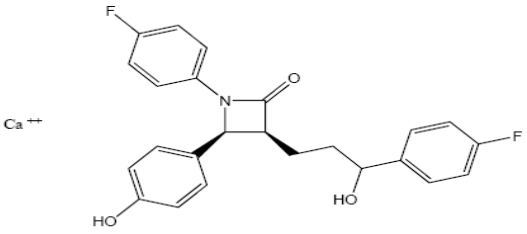


Figure-2: Molecular structure of Ezetimibe

MATERIALS AND METHODS

Chemicals/ Reagents and Solvents

Rosuvastatin-10mg(Rosuvas^{R}10) and Ezetimibe-10mg9(Ezedoc^{R})10 were obtained from, Rambaxy Laboratories Limited, .Himachal Pradesh and Hovero Labs Limited, Himachal Pradesh, respectively. Double Distilled Water (HPLC grade), Methanol(HPLC grade), Acetonitrile (HPLC grade), orthophosphoric acid and Potassium-dihydrogen phosphate were of reagent grade.

Instrumentation and Equipments

The HPLC analysis was accomplished on WATERS high pressure liquid chromatography outfitted with 515 reciprocating dual column HPLC pump, a manually operating Rheodyne injector with 20µL sample loop, X-terra C_8 4.6mm x 150mm analytical column reversed-phase material of 5µ size and a 2487 model UV-Visible detector. All the parameters of HPLC were controlled by N 2000 chromatographic system software. Other instruments used were TECHCOMP UV-Vis spectrophotometer of model 2310, Shimadzu electronic balance of model XEX-200, ADWA of model AD102U digital pH meter and ENERTECH of model SE60US ultrasonic bath sonicator.3.3

ANALYTICAL METHOD DEVELOPMENT

Optimization of UV conditions

Initially method development work was started by taking UV-visible spectra from 200-400 nm of rosuvastatin (10ppm) and Ezetimibe (10ppm) standard solutions. By observing the overlain spectra of standard solutions λmax 237 nm was taken for trials to develop HPLC method. The spectrum was show below

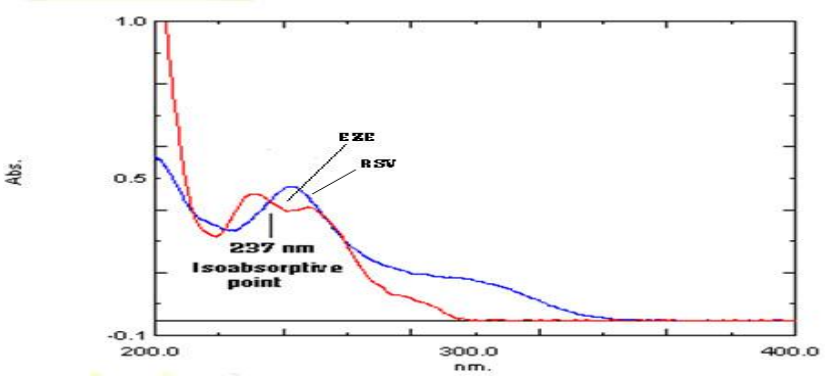


Figure-3. Isobestic point of Rosuvastatin and ezetimibe.

Optimized Method Parameters

Mobile phase	:	Acetonitrile: Phosphate buffer (pH 3.0**):** (60:40 v/v)
Column (Stationary Phase	:	Symmetry C8 (4.6mm x100mm,
5µm Make extra) or equivalent		
Flow rate	:	1.0 ml / min
Detector wavelength	:	237 nm
Retention time	:	Rosvastatin -2.490 min
Ezetimibe-3.173 min		
Column temp	:	Ambient.
Injection volume	:	20µl

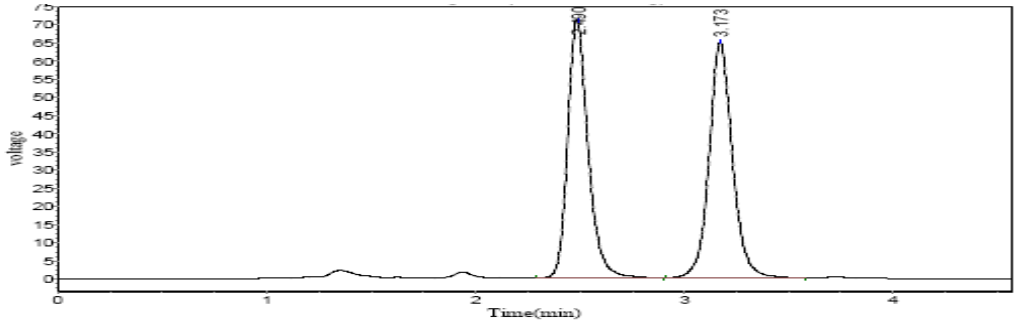


Figure- 4 Optimized chromatogram

PROCEDURE FOR PREPARATION OF SOLUTION

Preparation of buffer

Take 1000ml of HPLC grade water. Dissolve 2.72 grams of Potassium di hydrogen phosphate salt and Adjust the pH to 3.0 with orthophosphoric acid.

Preparation of mobile phase

A mixture of above prepared buffer 400 ml (40%), and 600 ml of HPLC grade Acetonitrile (60%) were mixed and degassed in ultrasonic water bath for 5 minutes. The mobile phase was filterred through 0.45 µ filter under vacuum.

Diluent Preparation

Use the Mobile phase as Diluent.

ASSAY

Preparation of Standard Solution

Accurately weighed and transferred 10mg of rosuvastatin and 10 mg of Ezetimibe working standard into a 100 ml clean dry volumetric flask and added about 70 ml of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent. (Stock solution)

From the above stock solution, 1 ml of the a solution was pipetted into a 10 ml volumetric flask and diluted up to the mark with diluent.

Sample Solution Preparation

Accurately weighed and transferred tablet powder equivalent to 10mg of rosuvastatin and 10 mg of Ezetimibe into a 100 ml clean dry volumetric flask and added about 70ml of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent. (Stock solution)

From the above stock solution, 1ml of the solution was pipetted into a 10 ml volumetric flask and diluted up to the mark with diluent.

Procedure

20 µL of the standard and sample solutions were injected into the chromatographic system and areas for the Rosuvastatin and Ezetimibe peaks were measured. %Assay was calculated by using the formulae.

Calculation

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.\ Wt}{Label\ Claim} \times 100$$

Where:

AT = Average area counts of sample preparation.
AS = Average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P = Percentage purity of working standard
LC = Label Claim mg/ml.

ANALYTICAL METHOD VALIDATION

The HPLC method was validated in accordance with ICH guidelines.

Accuracy

Accuracy was carried out by % recovery studies at three different concentration levels. To the pre-analyzed sample solution of Rosuvastatin and Ezetimibe a known amount of standard drug powder of Rosuvastatin and Ezetimibe were added at 50%, 100% and 150 % level.

Precision

The system precision of the method was verified by five replicate injections of standard solution containing Rosuvastatin and Ezetimibe. The method precision was carried out the analyte five times using the proposed method. Repeatability was measured by multiple injections of a homogenous sample of Rosuvastatin and Ezetimibe.

Linearity

The linearity was determined separately for Rosuvastatin and Ezetimibe Linearity of the method was studied by injecting 5 concentrations of both drugs prepared in methanol and calibration curves were constructed by plotting peak area against the respective concentrations.

Limit of detection and Limit of quantitation

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD = 3.3 x ASD/S and LOQ = 10 x ASD/S, Where, 'ASD' is the average standard deviation and 'S' is the slope of the line.

Robustness

Robustness was evaluated by making deliberate variations in method parameters such as variation of wave length; flow rate and change in mobile phase composition. The robustness of the method was studied for Rosuvastatin and Ezetimibe

RESULTS

Selection of Chromatographic Conditions and Optimization of Mobile Phase

Mobile phase was optimized to separate Rosuvastatin and Ezetimibe using Symmetry C8 column (100 mm x 4.6 mm i.d., 5µm). Initially, Acetonitrile and phosphate buffer and methanol in the Equal proportions were tried as mobile phase but the splitting of the peaks for both these drugs was observed. Therefore, after adjustment of pH of mixed phosphate buffer to 3.0 with ortho-phosphoric acid, and mobile phase composition (phosphate buffer, ACN in 40:60 % v/v*)* was tried for resolution of both drugs. Good resolution and symmetric peaks were obtained for both drugs when the pH of the mobile phase (buffer) was adjusted to 3.0. The flow rate of the mobile phase was 1.0 ml/ $min^{-1.}$ Under optimum chromate graphic conditions, the retention time for Rosuvastatin and Ezetimibe was found to be 2.49 and 3.17 min, respectively when the detection was carried out at 237nm. A typical chromatogram of two drugs is shown in (Figure -4).

Table-1 Accuracy data for Rosuvastatin And Ezetimibe

	Rosuvastatin			**Ezetimibe**		
Injection	50%	100%	150%	50%	100%	150%
Inj-1	9208872	1371282	1695389	1068344	1566080	1931607
Inj-2	9200584	1397934	1685300	1063819	1577201	1951677
Inj-3	9205366	1383795	1687584	1062311	1585054	1943746
AVG	9204940	1384337	1689425	1064825	1576112	1942343
S.D	4160.3339	13334.26	5290.11	3139.713	95331.79	10108.26
%R.S.D	0.045	0.963	0.313	0.294	0.604	0.520

Table-2 Accuracy (Recovery) result for Rosuvastatin and Ezetimibe

Drug Name	Spike level	Area	Amount Added(mg)	Amount Found(mg)	% Recovery	% of mean recovery
	50%	9204947	45	44.99	99.93	
Rosuvastatin	100%	1384337	60	60	100.00	
	150%	1689425	75	74.96	98.84	99.59
Ezetimibe	50%	1064825	45	45.09	100.6	
	100%	1576112	60	60.45	101.5	
	150%	1942343	75	75.01	100.02	100.70

Table-3 System precision for Rosuvastatin and Ezetimibe

S.No	Injections	Area of rosuvastatin	Area of Ezetimibe
1	Injection-1	603934	702684
2	Injection-2	600822	705354
3	Injection-3	618066	715784
4	Injection-4	626154	728094
5	Injection-5	619942	716584
	Average	**613783**	**713699**
	Standard deviation	**788.981**	**10134.685**
	%RSD	**0.1284**	**1.420**

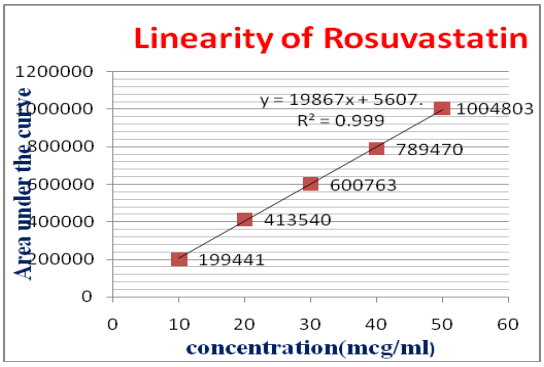
Table-4 Intermediate precision result for Rosuvastatin and Ezetimibe

S.No	Injections	Area of rosuvastatin	Area of Ezetimibe
1	Injection-1	628225	735595
2	Injection-2	649686	756979
3	Injection-3	647830	748467
4	Injection-4	630358	730877
5	Injection-5	627171	734043
	Average	**636654**	**741191.6**
	Standard deviation	**11128.24**	**11079.133**
	%RSD	**1.74**	**1.49**

Table-5 Linearity Results Of Rosuvastatin and Ezetimibe

S.No	Concentration(µg/ml)	Area of Rosuvastatin	Area of Ezetimibe
1	10	199441	236255
2	20	413540	477534
3	30	600763	693188
4	40	789470	920806
5	50	1004803	1152005

Figure-5: Linearity Graphs Of Rosuvastatin and ezetimibe



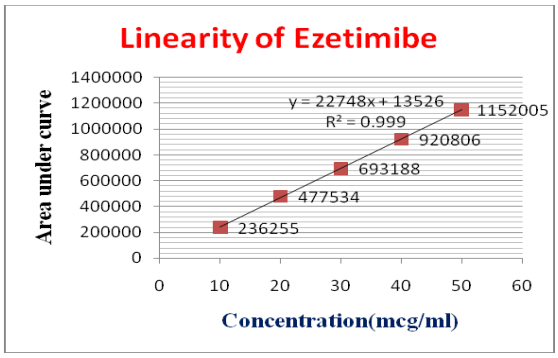


Table-6 Result of LOD and LOQ

S.No	Drug name	Standard deviation	Slope	LOD	LOQ
1	Rosuvastatin	9789.619	19687	1.626	4.927
2	Ezetimibe	6332.167	22748	0.918	2.783

Table -7 Robustness Result For Rosuvastatin And Ezetimibe At Different Condition

S.No	Parameter	Rosuvastatin			Ezetimibe		
		Theoretical plates per column	Tailing factor	Resolution	Theoretical plates per column	Tailing factor	Resolution
1	Less flow(0.9ml/min)	3238	1.225	-	5463	1.042	6.516
2	Standard flow rate(1.0 ml/min)	33 38	1.255	-	5384	1.042	6.399
3	More flow(1.1 ml/min)	3299	1.216	-	5501	1.029	6.654
4	%10 Less organic	3289	1.244	-	5294	1.033	6.591
5	Standard (100% organic)	3338	1.244	-	5384	1.042	6.399
6	%10 More organic	3300	1.22	-	5500	1.032	6.514
	Avarage	3300.333	1.232	-	5419.333	1.036	6.4955
	S.D	37.0225	0.013	-	84.729	0.005	0.0943
	%RSD	1.121	1.07	-	1.56	0.57	1.462

RESULTS AND DISCUSSION

Accuracy

The accuracy of the method studied at three different concentration levels i.e. 50%, 100 % and 150 % showed acceptable % recoveries in the range of 99.59% for Rosuvastatin and 100.70% for Ezetimibe . The results are shown in Table 1&2

Precision

The precision study was evaluated on the basis of % RSD value was found to be The RSD values for ROS and EZE were found to be 0.128% and 1.42% respectively Table -3

Linearity

The linearity was determined separately for Rosuvastatin and Ezetimibe. Linearity of the method was studied by injecting 5 concentrations of both drugs prepared in mobile phase and calibration curves were constructed by plotting peak area against the respective concentrations. The Roauvastatin and Ezetimibe followed linearity in the concentration range of 10-50 µg ml^{-1} and 10-50 µg ml^{-1}; respectively. The results are shown in Table 5.and Fig no 5.

Limit of detection and Limit of quantitation

The LOD for Rosuvastatin and Ezetimibe was found to be 1.626 and 0.918 µg/ml, respectively. The LOQ for Rosuvastatin and Ezetimibe was found to be 4.927 and 2.783 µg/ml respectively. The low values of LOD and LOQ indicates high sensitivity of the method. The results are shown in Table 6.

Robustness study

Robustness of the method was studied by making deliberate changes in the chromatographic conditions and the effects on the results were examined. The low value changes of theoretical plates, tailing factor indicating robustness of the method. The results are shown in Table 7.

Analysis of marketed tablet formulation

3 replicates of the samples solutions (20 µL) were injected for quantitative analysis. The amounts of Rosuvastatin and Ezetimibe estimated were found to 99.35 % and 100.77%, respectively. A good separation and resolution of both drugs indicates that there was no interference from the excipients commonly present in pharmaceutical formulations. The results are shown in Table 8.

System Suitability Test

The system suitability parameters such as resolution, number of theoretical plates and tailing factor were studied and were summarized in Table 9.

Table- 8 ASSAY RESULTS

Assay Results Drug	Amount present/tablet	% of Assay
Rosuvastatin	10mg	99.35
Ezetimibe	10mg	100.77

Table-9 System Suitability parameter

System suitability parameters	Rosuvastatin	Ezetimibe
Retention time(min	2.490	3.173
Tailing factor	1.25	1.08
Theoretical plates number	3216	4218
Resolution	-	6.3

CONCLUSION

The developed RP-HPLC method is simple, precise, accurate, selective and reproducible. The method has been found to be adequately rugged and robust and can be used for simultaneous determination of Rosuvastatin and Ezetimibe in tablet formulation. The method was validated as per ICH guidelines.

ACKNOWLEDGEMENTS

I like thankful to Pharmatech research labotatories., Hyderabad, India for providing the gift samples of rosuvastatin calcium and ezetimibe. And also to the principal Dr.k.Rajeswar Dutt Smt. Sarojini Ramulamma College of Pharmacy, Mahabubnagar , Andhrapradesh, India and special thanks for Ms. Ramathilagam madam as well as my friends who helped during the project work

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