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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ROSUVASTATIN AND EZETIMIBE IN COMBINED TABLET DOSAGE FORM

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ABSTRACT

A simple, rapid, economic, sensitive and precise HPLC method has been developed for the simultaneous determination of rosuvastatin and ezetimibe in pharmaceutical dosage form. The method was carried out using Sunfire BDS C18 250X4.6 mm ID, 5 µm column and mobile phase comprised of ammonium acetate in water as buffer, pH adjusted to 6.50±0.05 with dilute formic acid solution, acetonitrile in proportion of ratio 55:45 v/v and degassed under ultrasonication. The flow rate was 0.8 mL/min and the effluent was monitored at 230 nm. The retention time of rosuvastatin and ezetimibe were 2.74±0.5 and 4.80±0.5 respectively. The method was validated in terms of linearity, precision, accuracy, specificity, limit of detection, limit of quantitation and by performing recovery study. Linearity of rosuvastatin and ezetimibe were in the range of 98.19 to 294.56 µg/mL and 99.12 to 297.36 µg/mL respectively. The percentage recoveries of both the drugs were 99.9% and 100.9% for rosuvastatin and ezetimibe respectively from the tablet formulation. The proposed method is suitable for the routine quality control analysis of simultaneous determination of rosuvastatin and ezetimibe in bulk and pharmaceutical 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-heptenoic acid calcium[2] (Fig. 1). Ezetimibe is a 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).

Literature survey reveals that various spectrophotometric[5-8], HPLC[9-10], HPTLC[11], LC-MS[12-16] and capillary zone electrophoresis[17] methods have been reported for the determination of rosuvastatin in pure and pharmaceutical formulations and also various spectrophotometric[18-20], HPLC[21-24] and LC-MS[25-27] methods have been reported for the determination of ezetimibe in pure and pharmaceutical formulations. Few analytical methods like spectrophotometric[28-29], spectrofluorometric[30], HPLC[31-33] and HPTLC[34-35] methods have been reported for the determination of rosuvastatin and ezetimibe in combined dosage form. So an attempt was made to report a simple, rapid, sensitive, accurate and precise HPLC method for the determination of rosuvastatin and ezetimibe in combined tablet dosage form.

EXPERIMENTAL

Chemicals and reagents

Rosuvastatin calcium and ezetimibe were obtained from Rainbow Pharma Labs, Hyderabad, India. Rosuvastatin calcium and ezetimibe combined dosage form tablets were purchased from local market. HPLC grade acetonitrile and analytical grade ammonium acetate was obtained from Qualigens Fine Chemicals Ltd, Mumbai. Hydrochloric acid, sodium hydroxide, hydrogen peroxide and formic acid of analytical grade were obtained from Merck Chemicals Ltd, Mumbai. Milli-Q water was used throughout the experiment dispensed through 0.22 µ filter of the Milli-Q water purification system from Millipore, Merck KGaA, Darmstadt, Germany.

Instrumentation

The analysis of drugs was carried out on a Waters LC system equipped with 2695 pump with inbuilt degasser, 2998 photodiode array detector and an auto-injector with 100 µL sample loop. Reverse phase HPLC column Sunfire BDS C18 5µm particle size, length and internal diameter of 250X4.6 mm (Make: Agilent Technologies, USA) was used. The output of signal was monitored and integrated using Waters Empower 2 software.

Chromatographic conditions

HPLC was connected with Sunfire BDS (base deactivated silica) C18 stationery phase, 5 µm, 250X4.6 mm column. Mobile phase comprised of ammonium acetate buffer (Weigh about 1.16 grams of ammonium acetate and transfer to 1000 mL standard flask, add 400 mL of Milli-Q water mix and dilute to volume with Milli-Q water, sonicate for five minutes and cool to room temperature, measure the pH of above buffer solution and finally adjusted the pH to 6.5±0.05 with dilute formic acid solution) and acetonitrile in proportion of ratio 55:45 v/v. The mobile phase was mixed, filtered through 0.45 µ membrane filter and degassed under ultrasonication. The buffer and acetonitrile mixture in proportion of ratio 50:50 was used as diluent. Injection volume was 5 µL and flow rate was 0.8 mL/min and run time was 7.0 min. The column was maintained at ambient temperature and the eluent was monitored at 230 nm.

Preparation of standard solution

Accurately weigh and transfer 50 mg of rosuvastatin and 50 mg of ezetimibe working standard into a 50 mL clean dry volumetric flask, add about 25 mL of diluent and sonicate to dissolve it completely, cool the solution to room temperature and dilute to volume with diluent. Further pipette 5 mL of the above standard stock solution into a 25 mL clean dry volumetric flask and dilute to volume with diluent. Standard stock dilutions were prepared in diluent over the linearity range of 98.19 to 294.56 µg/mL and 99.12 to 297.36 µg/mL for rosuvastatin and ezetimibe respectively.

Preparation of sample solution

Weigh and finely powder not fewer than 20 tablets. Accurately weigh and transfer a quantity of powder sample equivalent to 50 mg of rosuvastatin and 50 mg of ezetimibe into a 50 mL clean dry volumetric flask, add about 25 mL of diluent and sonicate to dissolve it for about 30 minutes and cool the solution to room temperature and dilute to volume with diluent. Filter about 25 mL of the above sample solution through 0.45 µ membrane filter. Pipette 5 mL of the above filtered sample solution into a 25 mL volumetric flask and dilute to volume with diluent, 5 µL of the sample solution was injected in to the HPLC system.

RESULTS AND DISCUSSION

Method development

To develop a simple and robust method for the simultaneous determination of rosuvastatin and ezetimibe in combined tablet dosage form using HPLC. Different compositions of mobile phase were pumped in binary mode to achieve the resolution of drug peaks, in initial experimental conditions column with C18 stationery phase was employed, ammonium acetate as buffer in composition with methanol/acetonitrile as

organic solvent. Finally mobile phase comprised of buffer, acetonitrile in proportion ratio of 55:45 v/v respectively, buffer strength of 15mM pH adjusted to 6.5±0.05 with dilute formic acid solution was found with better resolution of drug peaks. Diluent in different compositions were checked and finally a mixture of buffer, acetonitrile in proportion of ratio 50:50 was observed with better peak shape. Mobile phase pumped at a flow rate of 0.8 mL/min, sample injection volume of 5 µL and column maintained at ambient temperature shown reproducible results. UV detection with photodiode array detector shown best signal at a wavelength of 230 nm with no interference in blank and placebo solutions for both drug peaks in the trail injections with a runtime of 7.0 min and the results were observed to be specific, precise and fast. Further the proposed method validation was initiated. System suitability test was performed on each day prior to initiation of the validation run. The system suitability results of the method are presented in Table-1.

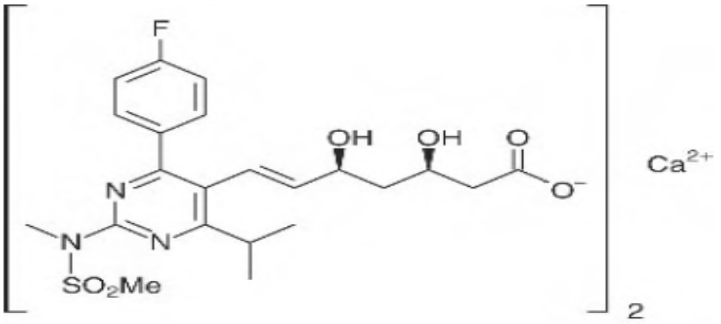

Fig.-1: Chemical structure of rosuvastatin calcium

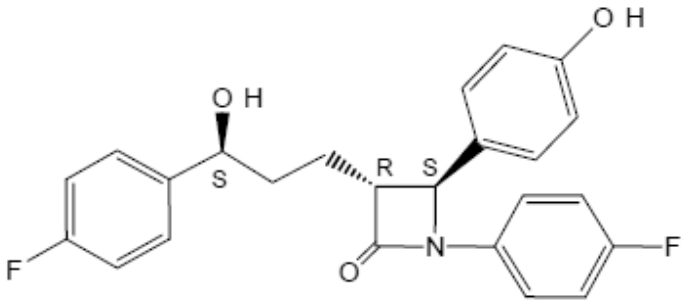

Fig.-2: Chemical structure of ezetimibe

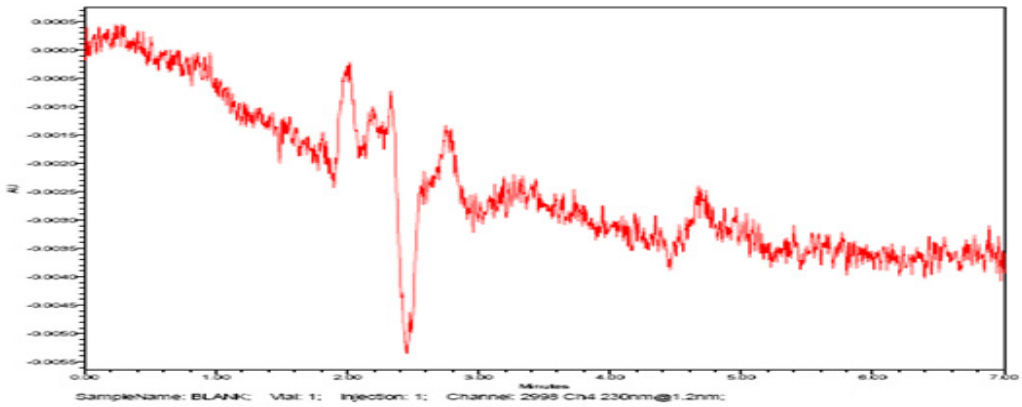

Fig.-3: Typical chromatogram showing no interference of blank for rosuvastatin and ezetimibe

Validation of the proposed method

The optimized method was validated with respect to the following parameters. The validation was performed as per the ICH guidelines[36-37].

Specificity

A study conducted to establish specificity of the proposed method involved injecting diluent and placebo using the chromatographic conditions defined for the proposed method. The blank chromatogram showed no interference peaks at the retention time of rosuvastatin and ezetimibe respectively. This indicates that diluent solution used in sample preparation do not interfere in the estimation of rosuvastatin and ezetimibe. Similarly the placebo sample chromatogram showed no interference peaks at the retention time of rosuvastatin and ezetimibe respectively, which demonstrates the specificity of the proposed method. The chromatogram of the blank and placebo using the proposed method for rosuvastatin and ezetimibe is shown in Fig. 3 and Fig.-4.

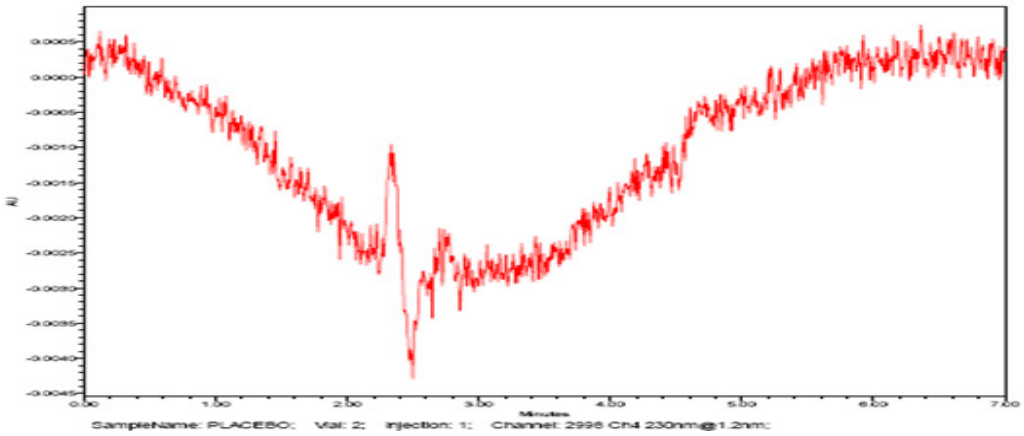

Fig.-4: Typical chromatogram showing no interference of placebo for rosuvastatin and ezetimibe

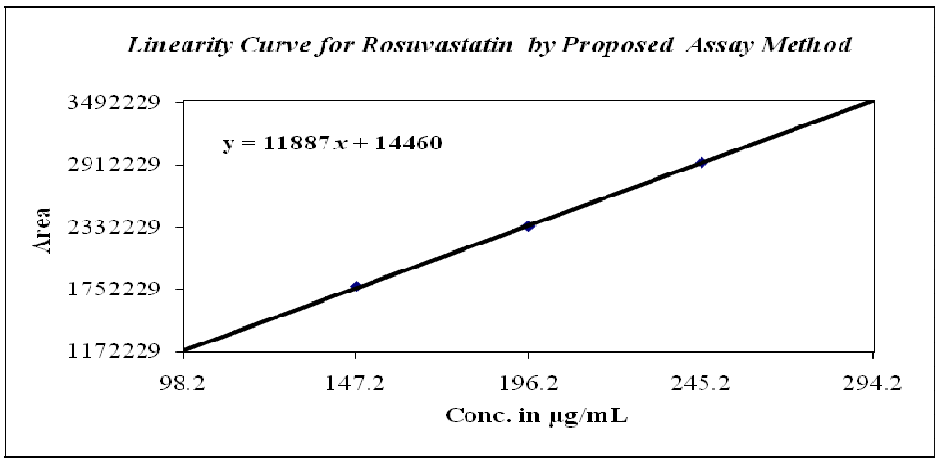

Fig.-5: Linearity curve for rosuvastatin

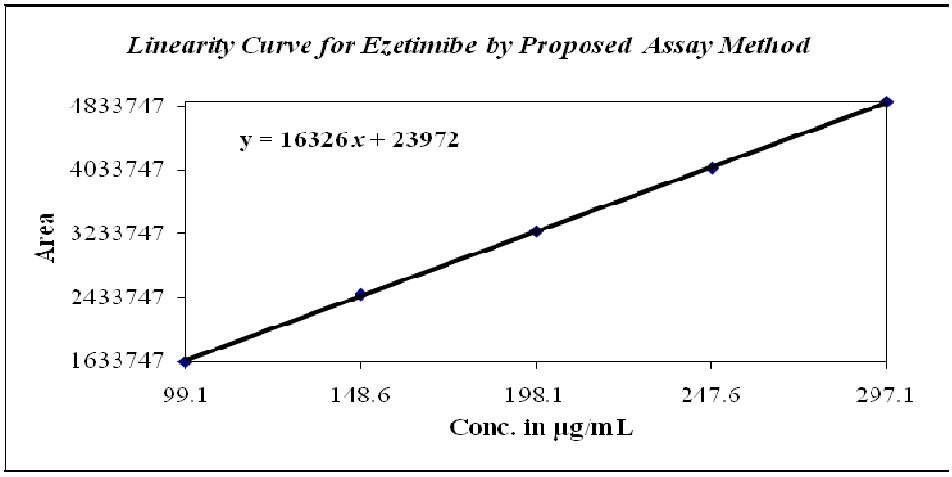

Fig.-6: Linearity curve for ezetimibe

Linearity

Detector response for the proposed method determined to be linear over the range of five concentration levels prepared and injected, 98.19 to 294.56 µg/mL for rosuvastatin and 99.12 to 297.36 µg/mL for ezetimibe. The calibration curve was plotted as concentration of the respective drug versus the obtained peak area at each concentration level. The linearity of the method was evaluated by linear regression analysis. The linear regression equation of proposed method representing slope and intercept for rosuvastatin and ezetimibe were given in Fig.-5 and Fig.-6. The statistical data calculated for rosuvastatin and ezetimibe found to be accurate and was given in Table-2.

Table-1: System suitability parameters for rosuvastatin and ezetimibe by proposed method

Parameter	Rosuvastatin	Ezetimibe
Theoretical Plates	4727	4096
Tailing Factor	1.0	1.0
%RSD	0.9	0.7
Retention Time (min)	2.74±0.5	4.80±0.5

Table-2: Linearity study for rosuvastatin and ezetimibe by proposed method

% Level (Approx.)	Rosuvastatin		Ezetimibe	
	Conc.(µg/mL)	Peak Area	Conc.(µg/mL)	Peak Area
50	98.19	1172229	99.12	1633747
75	147.28	1782476	148.68	2469721
100	196.38	2342922	198.24	3260683
125	245.47	2930543	247.80	4047961
150	294.56	3516218	297.36	4890253
Slope		11887.0		16326.0
Intercept		14460.0		23972.0
% Y-Intercept		121.6		146.8
Residual Sum of Squares		11892.0		18354.0
CC(r)		0.9999		0.9999

RSQ(r^2)		0.9999		0.9998
LOD		3.30		3.71
LOQ		10.00		11.24

Accuracy

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out on blend collected from twenty tablets of rosuvastatin and ezetimibe and analyzed as per the proposed method. The percentage recoveries found are in the range of 98.5 to 100.9 and 99.2 to 101.8 for rosuvastatin and ezetimibe respectively. From the data obtained, the proposed method found to be accurate. The results are summarized in Table-3.

Precision

In the study of the instrumental system precision where, a RSD of 0.4% and 0.5% was obtained for the standard area obtained corresponding to the first day for rosuvastatin and ezetimibe respectively, being 0.3% and 0.8% for the second day, respectively for rosuvastatin and ezetimibe. The method precision study for six sample preparations in marketed samples showed a RSD of 1.0% and 0.6%, the assay range of 98.7-101.1 and 98.5-100.3, respectively for rosuvastatin and ezetimibe. For the intermediate precision, a study carried out by the same analyst working on different day. The results calculated as inter-day RSD (For Standard) corresponded to 0.3% and 0.8% for rosuvastatin and ezetimibe respectively. The same study was carried out for different analysts (n=6 number of samples per analyst) obtaining a RSD of 1.1% and 1.0% (Intermediate Precision) and the assay range of 98.8-101.6 and 99.3-101.8, respectively for rosuvastatin and ezetimibe. The overall %RSD for n=12 is 1.0 for rosuvastatin and 1.1 for ezetimibe. Both results together with the individual results are showing that the proposed analytical technique has a good intermediate precision. Results are summarized in Table 4. Robustness of the method was determined by small deliberate changes in flow rate, mobile phase pH and mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust.

Quantification limit

The LOD is the lowest concentration of the analyte that can be detected and LOQ is the lowest concentration that can be quantified with acceptable precision and accuracy. The limit of detection (LOD) and limit of quantification (LOQ) by proposed method for rosuvastatin were 3.30 µg/mL and 10.00 µg/mL respectively and for ezetimibe were 3.71 µg/mL and 11.24 µg/mL respectively.

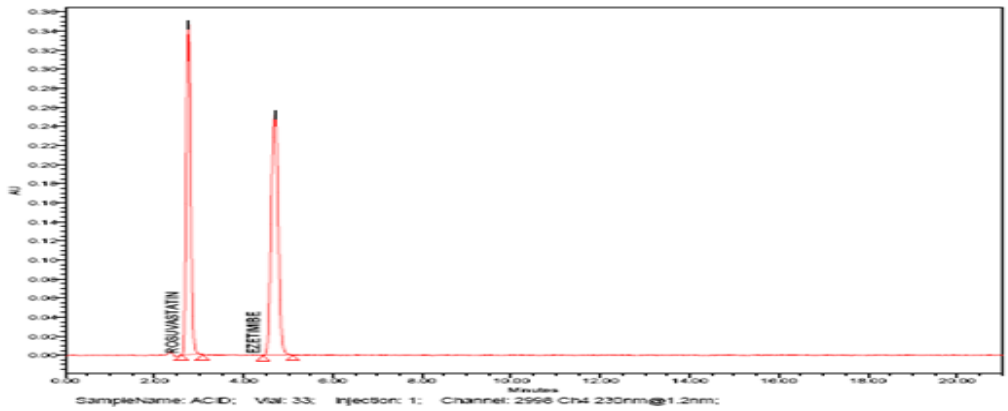

Fig.-7: Typical chromatogram of acid degradation showing rosuvastatin and ezetimibe

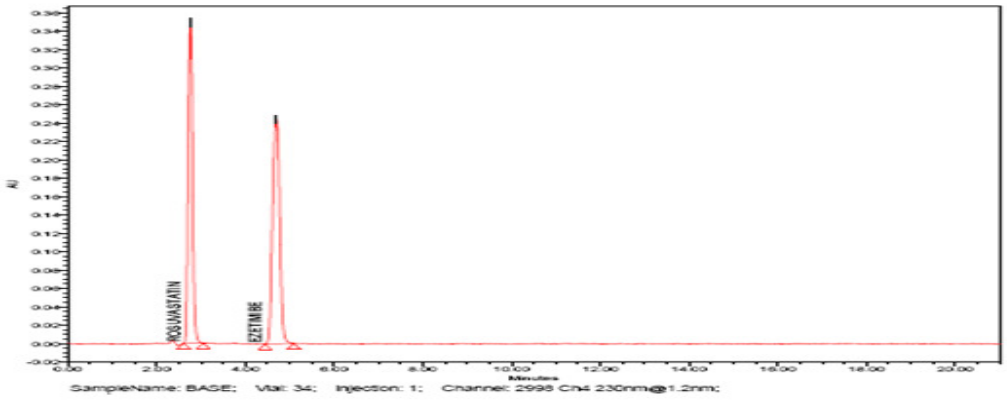

Fig.-8: Typical chromatogram of base degradation showing rosuvastatin and ezetimibe

Fig.-9: Typical chromatogram of oxidative degradation showing rosuvastatin and ezetimibe

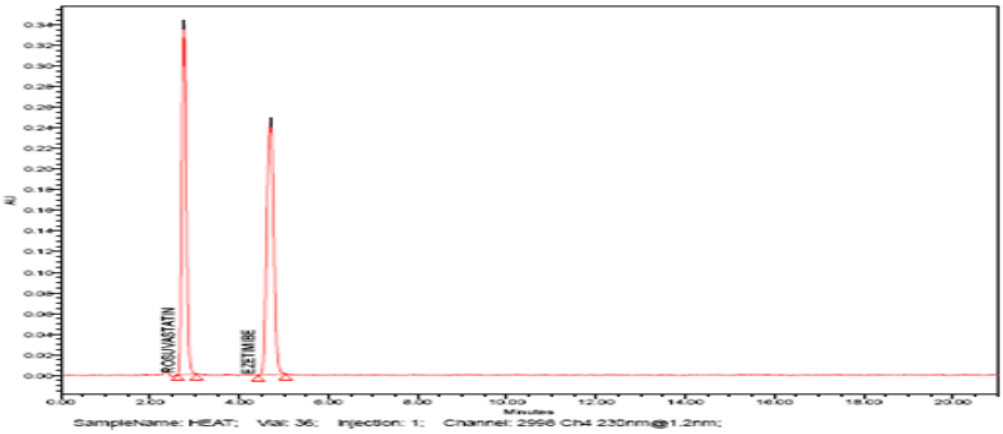

Fig.-10: Typical chromatogram of thermal degradation showing rosuvastatin and ezetimibe

Stability studies

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 hr at room temperature. The results show that for both solutions, the retention time and peak area of rosuvastatin and ezetimibe remained almost similar (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 hr, which was sufficient to complete the whole analytical process. Further forced

degradation studies were conducted indicating the stability of proposed method. The results of the degradation studies are presented in Table-5.

Table-3: Recovery studies by proposed method

% Level	Rosuvastatin			Ezetimibe		
	% Recovery	Mean % Recovery at each level	%RSD at each level	% Recovery	Mean % Recovery at each level	%RSD at each level
50	99.3	99.2	0.6	101.8	101.1	0.4
50	99.5			100.8		
50	98.6			101.2		
50	98.5			100.6		
50	100.2			101.1		
50	99.3			101.2		
100	99.9	99.7	0.2	99.8	100.0	0.9
100	99.6			100.9		
100	99.5			99.2		
150	100.8	100.6	0.3	101.8	101.2	0.4
150	100.1			100.8		
150	100.9			101.6		
150	100.8			101.3		
150	100.4			100.7		
150	100.5			101.0		
Mean Recovery	99.9			100.9		
SD	0.76			0.70		
%RSD	0.8			0.7		

Table-4: Inter-day and Intra-day precision summary for rosuvastatin and ezetimibe by proposed method

Rosuvastatin		Ezetimibe	
Inter-day	Intra-day	Inter-day	Intra-day
99.1	100.6	101.3	99.0
99.4	100.4	99.5	98.5
101.6	99.6	99.3	99.5
99.4	101.1	101.1	100.3
100.8	99.0	101.8	98.8
98.8	98.7	99.9	99.0
Overall Avg.	99.9		99.8
Average Std Dev.	0.98		1.07
Overall %RSD	1.0		1.1

Table-5: Forced degradation study results for rosuvastatin and ezetimibe by proposed method

Stress Conditions	Degradation Time (Hrs)	Rosuvastatin		Ezetimibe	
		Assay %	Degradation %	Assay %	Degradation %
Control	--	99.9	--	100.5	--
Acid	1	95.7	-4.2	91.9	-8.6
Base	1	93.3	-6.6	91.7	-8.8
Peroxide	1	96.6	-3.3	88.3	-12.2
Thermal	48	94.1	-5.8	89.2	-11.3

Control sample
Weigh and finely powder not fewer than 20 tablets. Accurately weigh and transfer a quantity of powder sample equivalent to 50 mg of rosuvastatin and 50 mg of ezetimibe into a 50 mL clean dry volumetric flask, add about 25 mL of diluent and sonicate to dissolve it for about 30 minutes with intermittent shaking at controlled temperature then cooled to room temperature and dilute to volume with diluent. Filter about 25 mL of the above sample solution through 0.45 µ membrane filter. Pipette 5 mL of the above filtered sample solution into a 25 mL volumetric flask and dilute to volume with diluent.

Acid degradation sample
Weigh and finely powder not fewer than 20 tablets. Accurately weigh and transfer a quantity of powder sample equivalent to 50 mg of rosuvastatin and 50 mg of ezetimibe into a 50 mL clean dry volumetric flask, add about 25 mL of diluent and sonicate to dissolve it for about 30 minutes with intermittent shaking at controlled temperature. Then add 5 mL of 5N acid (Hydrochloric acid), refluxed for 60 minutes at 60°C, then cooled to room temperature, neutralize with 5N base (Sodium hydroxide) and dilute to volume with diluent. Filter about 25 mL of the above sample solution through 0.45 µ membrane filter. Pipette 5 mL of the above filtered sample solution into a 25 mL volumetric flask and dilute to volume with diluent. Typical chromatogram of acid degradation for rosuvastatin and ezetimibe is shown in Fig.-7.

Base degradation sample
Weigh and finely powder not fewer than 20 tablets. Accurately weigh and transfer a quantity of powder sample equivalent to 50 mg of rosuvastatin and 50 mg of ezetimibe into a 50 mL clean dry volumetric flask, add about 25 mL of diluent and sonicate to dissolve it for about 30 minutes with intermittent shaking at controlled temperature. Then add 5 mL of 5N base (Sodium hydroxide), refluxed for 60 minutes at 60°C, then cooled to room temperature, neutralize with 5N acid (Hydrochloric acid) and dilute to volume with diluent. Filter about 25 mL of the above sample solution through 0.45 µ membrane filter. Pipette 5 mL of the above filtered sample solution into a 25 mL volumetric flask and dilute to volume with diluent. Typical chromatogram of base degradation for rosuvastatin and ezetimibe is shown in Fig.-8.

Peroxide degradation sample
Weigh and finely powder not fewer than 20 tablets. Accurately weigh and transfer a quantity of powder sample equivalent to 50 mg of rosuvastatin and 50 mg of ezetimibe into a 50 mL clean dry volumetric flask add about 25 mL of diluent and sonicate to dissolve it for about 30 minutes with intermittent shaking at controlled temperature. Then add 2 mL of 30% peroxide, refluxed for 60 minutes at 60°C, then cooled to room temperature and dilute to volume with diluent. Filter about 25 mL of the above sample solution through 0.45 µ membrane filter. Pipette 5 mL of the above filtered sample solution into a 25 mL

volumetric flask and dilute to volume with diluent. Typical chromatogram of peroxide degradation for rosuvastatin and ezetimibe is shown in Fig.-9.

Thermal degradation sample

Weigh and finely powder not fewer than 20 tablets, this powder is exposed to heat at 105°C for about 2 days. Accurately weigh and transfer a quantity of powder sample equivalent to 50 mg of rosuvastatin and 50 mg of ezetimibe into a 50 mL clean dry volumetric flask, add about 25 mL of diluent and sonicate to dissolve it completely, cool the solution to room temperature and dilute to volume with diluent. Filter about 25 mL of the above sample solution through 0.45 µ membrane filter. Pipette 5 mL of the above filtered sample solution into a 25 mL volumetric flask and dilute to volume with diluent. Typical chromatogram of heat degradation for rosuvastatin and ezetimibe is shown in Fig.-10.

Similarly UV light exposure, sunlight exposure and water hydrolysis stress samples are prepared and checked for their purity by proposed method. From the above data of degradation profile it can be conclude that there is no interference found for rosuvastatin and ezetimibe peak.

CONCLUSION

Thus the proposed stability indicating RP-HPLC method for the simultaneous determination of rosuvastatin and ezetimibe in tablet dosage form was accurate, precise, linear, reliable, simple, economic and robust. The method has several advantages, including simple mobile phase, low solvent consumption, rapid analysis, simple sample preparation and improved selectivity as well as sensitivity. The method can be used for routine analysis of marketed products of rosuvastatin and ezetimibe in combined tablet formulation.

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[RJC-964/2012]