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Validated stability indicating RP-HPLC method development for the assay of Rosuvastatin in pure and formulations

B.Koteswara Rao[1], M.Nageswara Rao[2], G.Ramu[1,3], C.Rambabu[1]*

[1]Department of Chemistry, Acharya Nagarjuna University, Andhra Pradesh, (INDIA)

[2]PVP Sidhardha College of Enginnering, Vijayawada, Andhra Pradesh, (INDIA)

[3]Department of Chemistry, Sir C.R.Reddy College P.G Courses, Andhra Pradesh, (INDIA)

E-mail: rbchintala@gmail.com

ABSTRACT

A simple, precise, accurate and rapid isocratic reverse phase liquid chromatographic method was developed for the determination of Rosuvastatin in pure and pharmaceutical formulations. Waters (Alliance) HPLC System equipped with Empower Software-2 software is adopted for the present investigation. Thermo hypersil BDS, C_{18} (150mm X 4.6mm, 5µparticle size) column maintained at a temperature of 30°C, a mobile phase of potassium di hydrogen phosphate buffer and acetonitrile in the ratio 40: 60 maintained at a flow rate of 0.8ml per minute were chosen for the analysis. The components were detected at 243nm using UV-detector. About 20 µL of standard and sample solution were injected, the chromatograms were obtained under the optimized chromatographic conditions and system suitable parameters were found to be satisfactory. Retention time, peak area and tailing factor of the standard peak were found to be 3.086 min, 4231231 and 1.17 respectively. The detector response was found to be linear over the range of concentration 5-30µg/mL. A study of forced degradation was carried out in different degradants and the percent of degradation was found be 2.62-5.62. The pharmaceutical formulations were analyzed and percentage of assay was found to be 99.88. The proposed method may be used as an alternative method in quality control in any pharmaceutical industry.

KEYWORDS

Rosuvastatin;
RP-HPLC;
Assay;
Forced degradation;
Formulations.

INTRODUCTION

Rosuvastatin (ROSV) is a member of the drug class of statins, used in combination with exercise, diet, and weight-loss to treat high cholesterol and related conditions, and to prevent cardiovascular disease. The primary use of rosuvastatin is for the treatment of dyslipidemia. ROSV is approved for the treatment of high LDL cholesterol (dyslipidemia), total cholesterol (hypercholesterolemia), and/or triglycerides (hypertriglyceridemia) and also was approved by the FDA for the primary prevention of cardiovascular events. It is a competitive inhibitor of HMG-CoA reductase, the rate-limiting enzyme that converts 3-hydroxy-3-methylglutaryl-coenzyme-A to mevalonate, a precursor of cholesterol. The systematic (IUPAC) name of the drug is given by (3*R*,5*S*,6*E*)-7-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)

pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid with molecular formula and weight $C_{22}H_{28}FN_3O_6S$ and 481.539 grams per mole respectively. The molecular structure of ROSV is presented in Figure 1. It is marketed by Astra Zeneca as Crestor; In India marketed by Zydus CND as Zyrova and is available in different dosage forms like 5, 10, 20 and 40mg per tablet. Crestor is actually rosuvastatin calcium in which calcium replaces the hydrogen in the carboxylic acid group on the right of the two structure diagrams. It is sparingly soluble in water and methanol, slightly soluble in ethanol, soluble in N, N-dimethyl formamide, acetone and acetonitrile.

Various analytical methods have been reported for determination of ROSV including UV[1-4] and Visible Spectrophotometric methods[5-7], HPTLC methods[8-10], RP-HPLC methods[11-19], solid phase extraction using tandem MS[20-21], few LC-MS methods[22-27] for the estimation of ROSU in pharmaceutical preparations and in biological fluids, LC-MS/MS[28], Two stability-indicating RP-UPLC methods for ROSV and its related impurities in pharmaceutical dosage form[29,30] were reported. Though various methods were reported in the literature, the developed method was found to be rapid and sensitive since the retention time of the ROSV is very much less than that of reported HPLC methods and detection limits are lower in concentration of the drug.

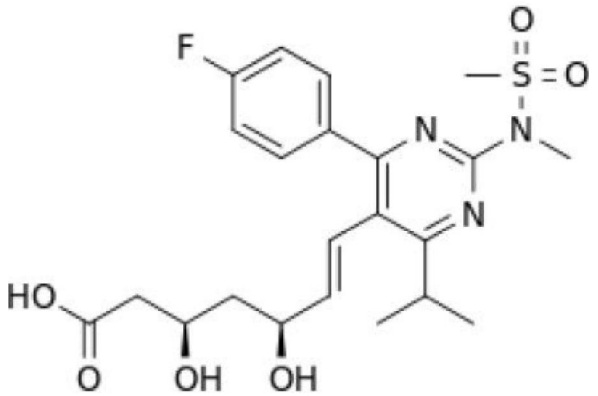

Figure 1 : Chemical structure of Rosuvastatin

MATERIALS AND METHODS

Instrumentation

Waters HPLC 2 2695 series consisting 4 pump, auto sampler with 5 racks, each has 24 vials holding capacity with temperature control. Auto injector has capacity to inject 5µL to 500µL. UV-Vis Detector with PDA. Thermostat column compartment connected it has a capacity to maintain 5°C to 60°C column temperature. Waters (alliance) HPLC System is equipped with Empower software-2 software.

Chemicals and reagents

HPLC grade Acetonitrile (MeCN) and AR grade potassium dihydrogen orthophosphate were procured from Merck (India). HPLC grade water obtained from Millipore system was used throughout the analysis. The investigated sample, Rosuvastatin was obtained as a gift sample from Dr. Reddy's Laboratory, Hyderabad, India. The pharmaceutical formulations were purchased from the local pharmacy.

Preparation of solutions

(a) Buffer preparation

About 2.60 g of KH_2PO_4 and 0.75 grams of K_2HPO_4 were accurately weighed and transferred into 1000ml volumetric flask and dissolved in water and sonicated for five minutes and filtered through 0.40 micron membrane filter.

(b) Mobile phase preparation

Mobile phase was prepared by mixing buffer and acetonitrile in the ratio 40: 60, sonicated the resulting solution and degased using vacuum filtration through 0.4 micron membrane filter.

(c) Standard stock solution (1mg/ml) preparation

Accurately weighed and transferred an amount of 100 mg of Rosuvastatin working standard into 100 mL volumetric flask added 50 mL of diluent and sonicated to dissolve and diluted to volume with diluent.

(d) Standard (20µg/ml) preparation

About 1 mL of standard stock solution is accurately transferred into 50 mL volumetric flask and diluted to volume with diluent.

(e) Sample preparation

Average weight of five tablets was found, grind them in mortar using piston and an amount of fine powder equivalent to 100mg of Rosuvastatin standard (API) was weighed and transferred into a 100 mL volumetric

flask and added 50 mL of diluent, sonicated to dissolve for 10 minutes and diluted to volume with diluent. Further filtered the solution through 0.4 micron membrane filter, and then diluted 1 ml of filtrate to 50 ml with mobile phase.

Chromatographic procedure

Various trials were performed so as to obtain optimized chromatographic conditions. About 20 µL of standard or sample solution was injected into the Thermo Hypersil BDS, C_{18}, 150mm X 4.6mm, 5µ particle size HPLC column maintained at a constant temperature of 30°C. Mobile phase of potassium di hydrogen phosphate buffer and actonitrile in the ratio 40:60 was allowed to flow at a rate of 0.8 ml/min for the elution of the components and were detected at 243nm using a UV-detector. Mobile phase was used for the dilutions and water: acetonitrile in the ration 90:10 was used for needle wash.

RESULTS AND DISCUSSION

Specificity

Specificity of the present investigation was determined by injecting about 20µL of blank, working standard and sample solutions separately and chromatograms were recorded. No additional peaks were found in the blank and sample except the drug peak. Retention time and peak area of standard (or sample) were found to be 3.806 (or 3.801) and 4231231 (or 4222542) respectively. The RP-HPLC chromatograms of blank, standard and sample were represented in Figure 2 – Figure 4 respectively.

System precision

Working standard solution of concentration 20µg/ml was prepared and about 20 µL of it was injected into the HPLC system six times, chromatograms were obtained under the suitable chromatographic conditions. Mean, standard deviation and percent of standard deviation were calculated on area of the chromatographic peaks of six replicates and found to be 4226402, 3001.04 and 0.071 respectively. The results were presented in TABLE 1.

Method precision

Prepared six solutions of concentration 20µg/ml by transferring 1ml of stock solution in to six 100ml volumetric flasks and diluted up to the mark and chromatograms were obtained under identical conditions. Statistical methods were applied to determine mean, standard deviation and percent of standard deviation of area of the chromatographic peaks and found to be 4262427, 18724.64 and 0.439 respectively. The results of method precision were given in TABLE 2.

Linearity

The study of linearity between detector response against concentration of Rosuvastatin is demonstrated by preparing standard solutions over the range of concentration 25 to 150% with respect to the precision concentration. Each solution was injected into the system; chromatograms were obtained under the optimized conditions. A calibration plot was constructed and presented in Figure 5. Slope, intercept and correlation co-

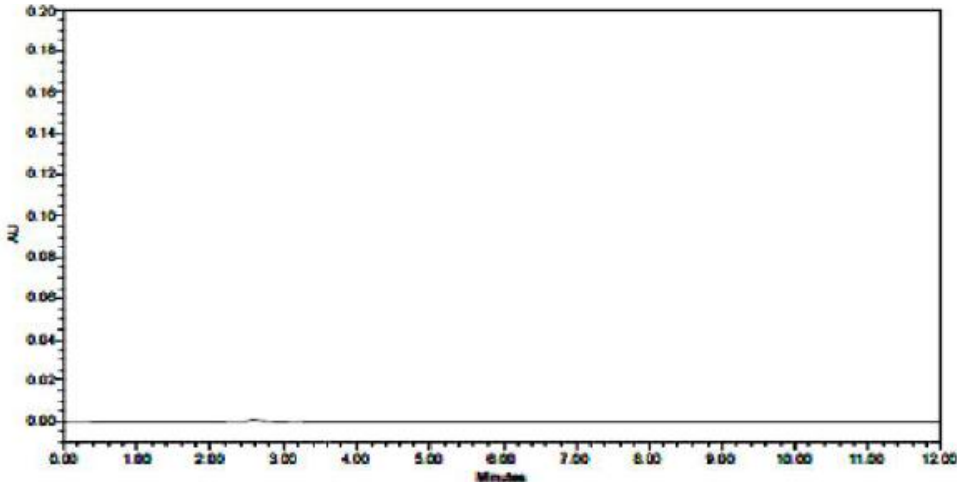

Figure 2 : Chromatogram of blank solution

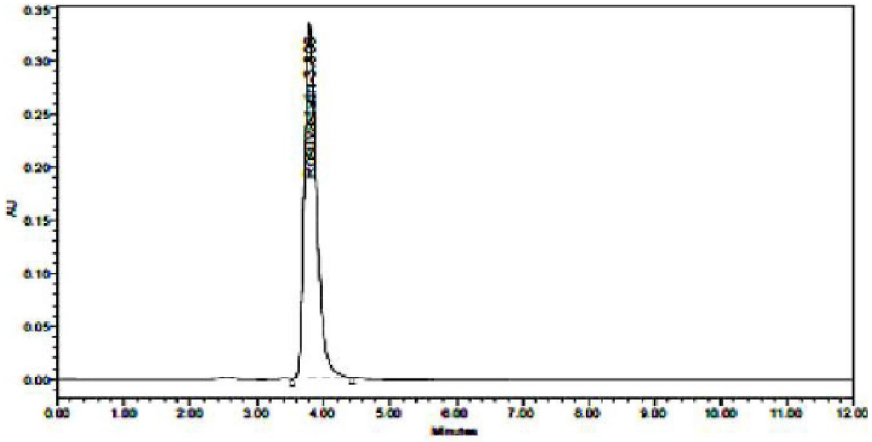

Figure 3 : A typical RP-HPLC chromatogram of working standard of Rosuvastatin

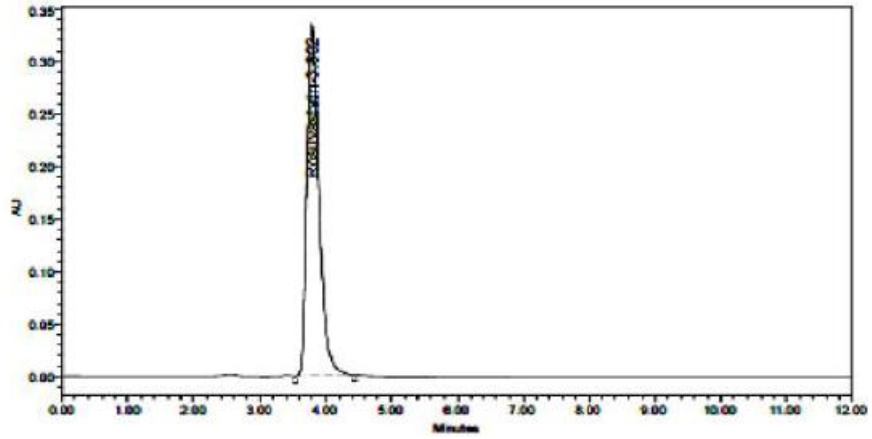

Figure 4 : A typical RP-HPLC chromatogram of of Rosuvastatin formulation

TABLE 1 : Study of system precision of the proposed method

S.No.	Retention Time	Peak Area
1	3.806	4228472
2	3.809	4224768
3	3.802	4229734
4	3.810	4222734
5	3.808	4228951
6	3.803	4223752
Mean	3.806	4226402
SD	0.0033	3001.0400
%RSD	0.086	0.071

TABLE 2 : Results of method precision of the proposed method

S.No.	Retention Time	Peak Area
1	3.810	4258705
2	3.811	4237452
3	3.807	4287581
4	3.812	4264783
5	3.818	4247586
6	3.814	4278456
Mean	3.812	4262427.167
SD	0.0037	18724.6494
%RSD	0.098	0.439

efficient of the developed method were determined and found to be 211032.63, 7856.89 and 0.9998 respectively. The LOD and LOQ of the proposed method were calculated by using standard deviation of the intercept (σ) and slope (s) of the calibration curve. These were calculated by using the formulae LOD=3σ/s and LOD=10σ/s, and found to be 0.0441 and 0.1434 respectively. The experimental results of linearity studies were presented in TABLE 3.

Accuracy

The accuracy of the test method is demonstrated by finding percent of recovery. The sample preparations are prepared at three concentration levels (Like 80% 100% and 120% with respect to precision con-

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TABLE 3 : Study of linearity between peak area and concentration

Linear solutions	Volume of Stock solution taken in (mL)	Final Dilution	Concentration	Peak Area
25%	0.25	50	5	1075919
50%	0.50	50	10	2149288
75%	0.75	50	15	3157429
100%	1.00	50	20	4161077
125%	1.25	50	25	5324518
150%	1.50	50	30	6345194
	Slope			211032.63
	Intercept			7856.89
	Correlation coefficient			0.9998

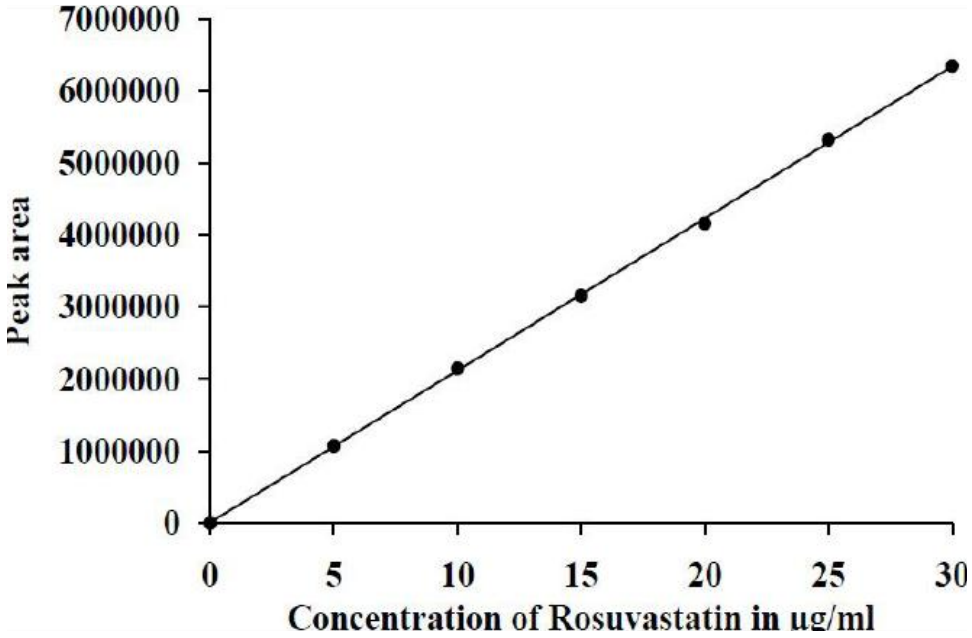

Figure 5 : Linearity plot of Rosuvastatin

TABLE 4 : Study of accuracy of the proposed method

S.No.	Concentration level	Peak area	Concentration level	Peak area	Concentration level	Peak area
1		3356445		4221745		5112116
2	80%	3345425	100%	4235652	120%	5112415
3		3367432		4223512		5113518
Mean value		3356434		4226970		5112683
S.D.		79.34		99.92		120.86
Amount added		16.0		20.0		24.0
Amount Recovered		15.87		19.98		24.17
% recovery		99.18		99.92		100.71

TABLE 5 : The results of ruggedness of the proposed method

S.No.	Instrument-1	Instrumrnt-2	Column-1	Column-2	Day-1	Day-2
Mean	4230344	4215402	4221344	4218344	4226402	4240344
SD	1963.07	4171.08	1263.07	1463.07	3001.04	14963.07
RSD	0.453	0.271	0.553	0.323	0.071	0.353

TABLE 6 : Study of robustness of the proposed method

S.No.	Parameter	Retention Time	Peak Area
1	Standard	3.806	4228472
2	Robustness-Flow-1	5.677	6374994
3	Robustness-Flow-2	2.891	3193489
4	Robustness-Oven Temp-1	3.782	4241504
5	Robustness-Oven Temp-2	3.848	4260989

columns and different days under the same experimental conditions.

Robustness

The robustness of test method is demonstrated by carrying out intentional method variations like mobile phase flow changes and column oven temperature variations. The % of RSD of areas & RTs from repeated injections should not be more than 2.0 % and the results were given in TABLE 6.

STUDY OF DEGRADATION

Acid hydrolysis

Transferred quantitatively 100 mg of Rosuvastatin API in to 200 mL RB flask, added 100 ml of freshly prepared 0.1 N HCl. Left it for 10 hrs, then filtered the solution through filter paper and neutralized the solution with suitable base. Diluted 1 ml of filtrate to 50 ml with mobile phase.

centration) and each solution was injected three times, chromatograms were recorded and mean percent of recovery was determined at each concentration and the results were given in TABLE 4.

Ruggedness

Ruggedness was experimentally determined by performing analysis using different instruments, different

TABLE 7 : Study of forced degradation of Rosuvastatin

	Peak area	Found assay	% Of Assay	%Degradation
Acid-Hydrolysis				
Degraded API	4172666			
Standard	4298221	97.18	99.8	2.62
Base-Hydrolysis				
Degraded API	4137790			
Standard	4298221	96.27	99.88	3.61
Heat-Exposure				
Degraded API	4185122			
Standard	4298221	97.08	99.88	2.80
Oxidation-Peroxide				
Degraded API	4067666			
Standard	4298221	94.26	99.88	5.62
UV-Exposure				
Degraded API	4148861			
Standard	4298221	96.52	99.88	3.36

Base hydrolysis

About 100 mg of Rosuvastatin was transfered quantitatively in to 200 ml RB flask, 100 ml of freshly prepared 0.1 N NaOH was added. After 10 hrs, filtered the solution through filter paper neutralize the solution with suitable acid. Dilute 1 ml of filtrate to 50 ml with mobile phase.

Oxidation (Peroxide)

The study of oxidation degradation was performed by transferring quantitatively 100 mg of Rosuvastatin in to 200 mL RB flask; about 100 ml of freshly prepared 1.0% Hydrogen Peroxide was added. After 10 hrs filtered the solution through filter paper and diluted 1 ml of filtrate to 50 ml with mobile phase.

Heat exposure

Transferred about 100 mg of Rosuvastatin API on to clean and dry petri dish spread it throughout the plate. Placed the petri dish in oven which is maintain-

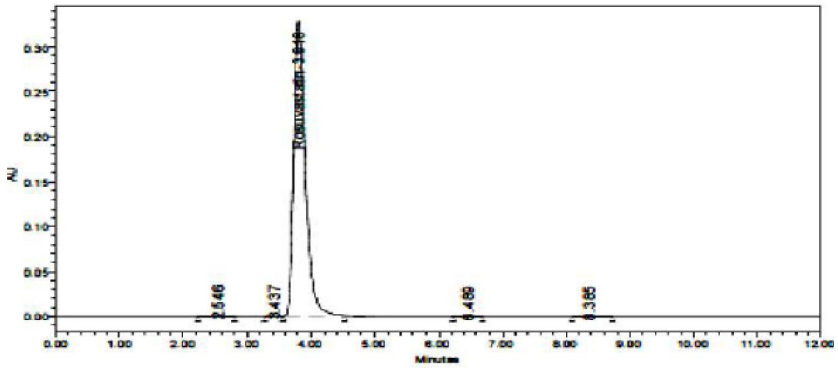

Figure 6 : A typical RP-HPLC chromatogram of Rosuvastatin in acid degradation studies

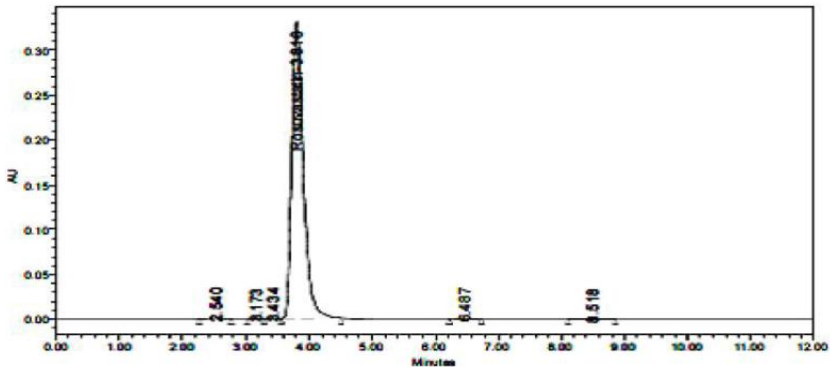

Figure 7 : A typical RP-HPLC chromatogram of Rosuvastatin in base degradation studies

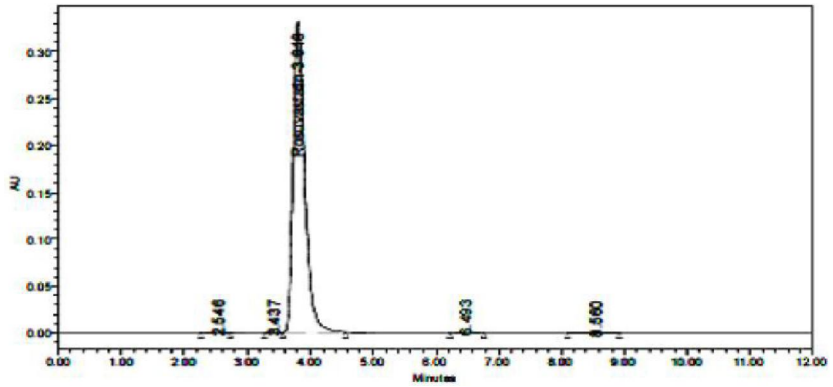

Figure 8 : A typical RP-HPLC chromatogram of Rosuvastatin inthermal degradation studies

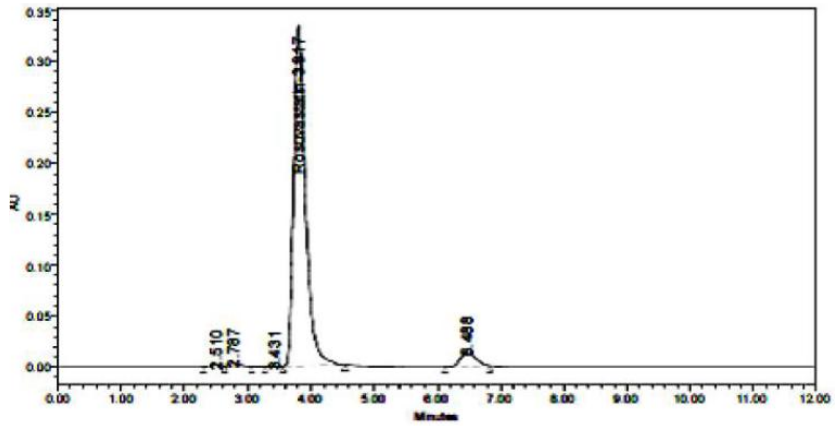

Figure 9 : A typical RP-HPLC chromatogram of Rosuvastatin in oxidation degradation studies

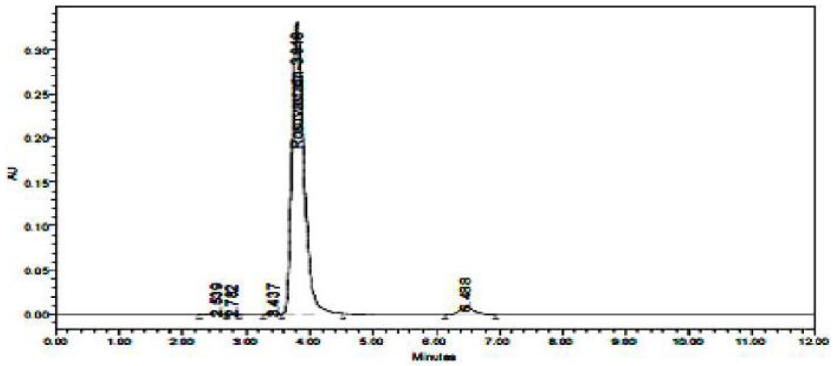

Figure 10 : A typical RP-HPLC chromatogram of Rosuvastatin in UV- degradation studies

TABLE 8: Study of pharmaceutical formulations

S.No.	Formulation	Dosage	Found	%Assay
1	Zyrova	5 mg	4.982	99.64
2	Zyrova	10 mg	10.088	100.88
3	Zyrova	20 mg	20.160	100.80
4	Zyrova	40 mg	39.970	99.92

ing at 100°C for 10 hrs. After 10 hrs transferred the contents in to 100 mL volumetric flasks added 50 mL of diluent and sonicated it for 10 minutes and dilute volume with diluent. Further filtered the solution through filter paper and diluted 1 ml of filtrate to 50 ml with mobile phase.

UV exposure

About 100 mg of Rosuvastatin was transfer quantitatively on to clean and dry petri plate and placed in UV Chamber for 10 hrs. After 10 hrs the contents were transferred in to 100 mL volumetric flask, added 50 mL of diluent and sonicated it for 10 minutes and dilute to volume with diluent. Further filtered the solution through filter paper, and then diluted 1 ml of filtrate to 50 ml with mobile phase.

About 20 µL of each of the above solution was injected into the system, chromatogramed (Figure 6-Figure 10), the percent of degradation was calculated in each case and the results of were presented in TABLE 7.

ASSAY OF ROSUVASTATIN

Injected about 20 µL of blank, standard and sample solutions into the system and recorded the chromatograms. The average percentage of assay was calculated and found to be 100.31

CONCLUSIONS

A simple isocratic RP-HPLC method was developed for the determination of Rosuvastatin in pharmaceutical formulations. The proposed method was found to be simple, precise, accurate, robust and rugged. Therefore the method can be used for routine analysis in quality control.

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