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International Journal of Pharmaceutical Sciences and Drug Research 2014; 6(4): 283-290

Research Article

ISSN: 0975-248X
CODEN (USA): IJPSPP

New Stability-Indicating RP-HPLC Method Development and Validation for the Determination of Rosuvastatin (Calcium) In Pharmaceutical Dosage Forms

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ABSTRACT

A stability indicating, accurate, specific, linear and sensitive reverse phase-HPLC method was developed and validated for the determination of Rosuvastatin calcium (ROS) in pharmaceutical dosage form. The chromatographic separation was performed using Eclipse XDB plus C_{18} Column (4.6 × 150 mm, 5µm particle size). Mobile phase was made by using Acetonitrile: Water (60:40 v/v) at a flow rate of 1.0 ml/min with the detection wavelength at 242 nm. The retention time of ROS was found to be about 1.787 min. The linearity was obtained in the concentration range of 10-50 µg/ml with a correlation coefficient of 0.999. The percentage purity of ROS was found to be 100.06 %. The percentage recovery was determined for ROS and found to be 100.53 %. The developed analytical method was validated for linearity, precision, accuracy, ruggedness, robustness, specificity and system suitability which were within the acceptance limit according to ICH guidelines. All the degradation products obtained by stress conditions were found to be well separated from the principal peak, which means that the ROS peaks were highly pure in all chromatograms obtained. The developed method was found to be applicable for routine quality control and stability analysis of ROS in pharmaceutical dosage forms.

Keywords: Rosuvastatin Calcium, Stability-Indicating, RP-HPLC, Validation.

INTRODUCTION

((E) -7[4-(4-Fluorophenyl)-6-isopropyl-2-(Methyl (methylsulfonyl) aminopyrmidin-5yl) (3R, 5S)-3,5-dihydroxyhept-6-enoic acid) Calcium salt [Fig. 1] [1], competitively inhibits conversion of 3-Hydroxy -3-methyl glutaryl coenzyme A (HMG-CoA) to mevalonate (rate limiting step in CH synthesis) by the enzyme HMG-CoA reductase. [2-5] Its therapeutic dose reduces CH synthesis by 20-50 %. This results in compensatory increase in LDL receptor expression on liver cells which leads to increased receptor mediated uptake and catabolism of IDL and LDL. ROS is the newer and commonly used potent statin with a plasma $t_{1/2}$ of 18-24 hours. Greater LDL-CH reduction can be obtained in severe hyper cholesterolaemia. The daily dose for lowering LDL-CH by 30-35 % is 5 mg of ROS. Moreover, at its maximum recommended doses of ROS 40 mg can reduce LDL-CH up to 55 %. All statins except ROS are metabolized primarily by CYP3A4. Inhibitors and inducers of this isoenzyme respectively increase and decrease statin blood levels. The most important related compounds for ROS are anti-isomer and lactone impurity. [6]

Literature survey reveals that few Stability-indicating HPLC methods [1-15] and Spectrophotometric [16-19] methods have been reported for the estimation of ROS as a single or in combined pharmaceutical preparations.

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Received: 20 September, 2014; **Accepted:** 25 September, 2014

In the present work, we have focused on to develop and validate a stability indicating method with optimum chromatographic conditions for the determination of ROS in pharmaceutical preparations and other unknown degradation products that may be formed during stability studies. The developed method was validated as per ICH guidelines [20-22], and can be applied successfully in routine quality control of Rosuvastatin in bulk and in its dosage forms.

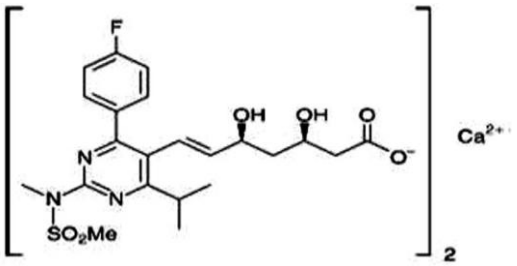

Fig. 1: Chemical Structure of Rosuvastatin Calcium

MATERIALS AND METHODS

Reagents and Chemicals

Rosuvastatin calcium (ROS) was obtained as a gift sample from Micro Labs, Bangalore. All chemicals used were of HPLC grade: Acetonitrile and Millipore water were purchased from Thermo Fisher Scientific India Pvt. Ltd., Mumbai.

Equipment and Chromatographic Conditions

An Agilent model - 1220 Infinity LC (HPLC) system with Agilent openLAB CDS (EZ Chrome) software "version A.04.05" with a Variable Wavelength Detector (VWD) and Manual injector were used. It was manufactured by Agilent Technologies, USA. An Eclipse XDB plus C_{18} Column (4.6 × 150 mm, 5µm particle size) was used for analytical separation. The mobile phase consisted of mobile phase A: HPLC grade water and mobile phase B: Acetonitrile in the ratio of 40:60 v/v with isocratic elution program. The flow rate was adjusted to 1.0 ml/min, the injection volume set at 20µL with a detection wavelength of 242 nm.

Preparation of Analytical Solutions

Preparation of Diluent for sample preparation

Mobile phase A: HPLC grade water.

Mobile phase B: HPLC grade Acetonitrile

The diluent was prepared by mixing mobile phase A and B in the ratio of 40:60 v/v.

Preparation of standard solution

It was prepared by dissolving ROS standard equivalent to 25 mg of Rosuvastatin (as calcium) in 25 ml of diluent, sonicated for 15 minutes and cooled to room temperature. Then, 2.5 ml of the resulting solution was diluted to 25 ml with diluent, mixed well and filtered using 0.45µ filter to obtain 100µg/ml solution.

Preparation of sample solution

An accurately weighed quantity of tablet powder equivalent to 25 mg of ROS was transferred to 25 ml volumetric flask. 20 ml of diluent was added and shaken well by mechanical means for 15 minutes. The solution was diluted and made up to 25 ml with mobile phase. From the resulting solution 2.5 ml was taken and made up to 25 ml with the diluent, mixed well and filtered using 0.45µ filter to obtain 100µg/ml solution.

HPLC-Method Development and Validation

An analytical method was developed and validated according to ICH guidelines. Analytical variable parameters such as linearity, precision, accuracy (percent recovery), specificity, peak purity and system suitability were tested using the optimized chromatographic conditions and instruments.

Linearity

The linearity of the method was established by spiking a series of dilutions of ROS. Solutions of five different concentrations 10-50µg/ml were injected into the HPLC system. The calibration curve was constructed for the standard solutions by plotting their concentrations against their respective peak areas. Regression equation was obtained and the values of slope-a, intercept-b, and correlation coefficient (R^2) were determined as shown in Table 1 & fig. 2.

Accuracy (percent recovery)

The accuracy study was performed on 80 %, 100 % and 120 % of the analytical method target concentration of ROS. Standard and sample preparations were injected into HPLC system and three determinants for each concentration level obtained. The percentage recoveries of ROS were calculated using standard at the same concentration at each concentration level as presented in Table 2.

Precision

System precision

System precision was established by injecting five replicate preparations of the standard drug solution (30µg/ml) of Rosuvastatin. The corresponding peak areas were measured and % RSD calculated as presented in Table 3.

Method precision

The method precision study was performed for five replicate sample preparations of marketed formulation (30µg/ml) of Rosuvastatin. The corresponding peak areas were measured and % RSD calculated as exhibited in Table 4.

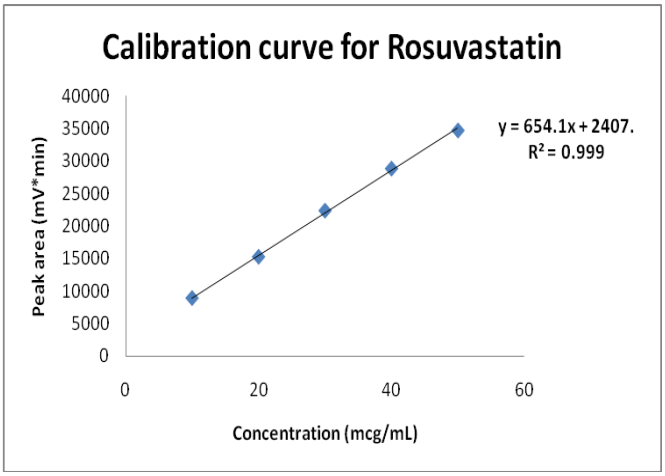

Fig. 2: Calibration curve for ROS

Table 1: Calibration of the proposed method

Concentration (µg/ml)	Peak area (mV*min)
10	8875.278
20	15253.759
30	22379.691
40	28883.311
50	34766.902
R^2	0.999

Table 2: % Recovery data of ROS

Concentration at specific level	Active drug added (mg)	Recovered amount (mg)	Mean % Recovered for all determinations
	08.0	07.98	
80%	08.0	08.10	
	08.0	08.02	
	10.0	10.12	
100%	10.0	10.05	100.53
	10.0	09.97	
	12.0	12.15	
120%	12.0	12.10	
	12.0	12.05	

Table 3: System Precision of proposed method

	RT (min)	Peak area (mV*min)
	1.772	22243.362
	1.779	22324.448
Statistics	1.769	22511.640
	1.771	22237.107
	1.770	22422.148
Average	1.766	22347.741
Standard Deviation	0.0052	118.402
% RSD	0.295	0.52

Table 4: Method Precision

	RT (min)	Peak area (mV*min)
	1.770	22371.070
	1.773	22421.640
Statistics	1.767	22263.362
	1.763	22452.148
	1.760	22034.400
Average	1.772	22308.524
Standard Deviation	0.0035	160.871
% RSD	0.199	0.72

Table 5: Robustness of the proposed method

Parameter	Adjusted to	RT (min)	Peak Area (mV*min)
Flow rate (ml/min)	0.9	1.742	22459.961
	1.0	1.733	22324.435
	1.1	1.721	22427.354
Wavelength (nm)	237	1.741	22392.456
	242	1.725	22479.597
	247	1.735	22467.572
Change in Mobile phase B	-5%	1.850	23579.675
	No change	1.735	22379.725
	+5%	1.628	21866.902

Table 6: Method Ruggedness

	RT (min)	Peak area (mV*min)
	1.730	22437.526
	1.735	22428.739
Statistics	1.742	22503.257
	1.739	22399.795
	1.729	22417.325
Average	1.735	22437.328
Standard Deviation	0.0059	39.462
% RSD	0.34	0.175

Table 7: System suitability of the proposed method

S. No	Sample	Parameters	
		Theoretical Plates	Tailing factor
1	Rosuvastatin calcium	2877	0.98

Robustness

Robustness of the developed analytical method was tested by evaluating the affect of small variations in analytical method parameters such as change in flow rate of 1.0 ml/min by ±0.1 ml/min, change in wavelength by ± 5 nm and change in the mobile phase B ratio by ± 5 %. The results are exhibited in Table 5.

Ruggedness

Ruggedness of the proposed method was determined by injecting five independent preparations from a tablet formulation (30µg/ml) of Rosuvastatin prepared by another analyst into another HPLC system. The retention time and peak areas were obtained. The mean and % RSD were found to be within the acceptance criteria as shown in Table 6.

LOD and LOQ

Five replicates of the working standard solution were measured and analyzed. The Limit of Detection (LOD) and Limit of Quantifacation (LOQ) were then established by evaluating the signal to noise ratio of 3:1 and 10:1 respectively.

Specificity of analytical method and peak purity

The specificity and peak purity were determined to check whether there are any interference due to presence of excipients, impurities, degradation products or other components with the retention time of analytical peaks which may affect the peak purity and specificity of the analytical method. The HPLC chromatograms were recorded for the drug-matrix (mixture of the drug and excipient) which showed almost no interfering peaks within retention time ranges.

System suitability

Five replicates of working standard solution (30µg/ml) were injected and the parameters like theoretical plate number (N) and tailing factor (K) of samples were calculated to check the system suitability. The results are presented in Table 7.

Procedure for forced degradation studies

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 h at room temperature. The results showed that the retention time and peak area of ROS remained almost similar (% R.S.D less than 2.0) with no significant degradation within the specified period indicating that both solutions were stable for at least 24 h, which was sufficient to complete the whole analytical procedure. Further forced degradation studies were conducted to indicate the stability of the method developed.

Acid degradation studies

Twenty tablets were taken and made into a fine powder. A quantity of the powder equivalent to 25 mg of ROS was accurately weighed and dissolved in 15 ml of the mobile phase in a 25 ml volumetric flask. The solution was shaken well and allowed to stand for 15 min with intermittent sonication to ensure the complete solubility of drug. 5 ml of 1N Hydrochloric acid was then added, refluxed for 6 hrs at 60°C and cooled to

room temperature. The resulting solution was neutralized with 1 N NaOH, diluted to volume with mobile phase and filtered through a 0.45µ membrane filter. From this 0.25 ml of the sample solution was pipetted out into a 25 ml volumetric flask and made up to volume with mobile phase resulting in 10µg/ml solution. 20µl of the sample was injected into the column. Typical chromatogram for acid degradation of ROS shown in Fig. 6.

Base degradation studies

Twenty tablets were taken and made into a fine powder. A quantity of the powder equivalent to 25 mg of ROS was accurately weighed and dissolved in 15 ml of the mobile phase in a 25 ml volumetric flask. The solution was shaken well and allowed to stand for 15 min with intermittent sonication to ensure the complete solubility of drug. 5 ml of 1N NaOH was added, refluxed for 6 hrs at 60°C and cooled to room temperature. It was neutralized with 1 N Hydrochloric acid, diluted to volume with mobile phase and filtered through a 0.45µ membrane filter. From this, 0.25 ml of the sample solution was taken into a 25 ml volumetric flask and made up to volume with mobile phase resulting in 10µg/ml solution. Typical chromatogram for base degradation of ROS shown in Fig. 7.

Oxidative degradation studies

Twenty tablets were taken and made into a fine powder. A quantity of the powder equivalent to 25 mg of ROS was accurately weighed and dissolved in 15 ml of the mobile phase in a 25 ml volumetric flask. The solution was shaken well and allowed to stand for 15 min with intermittent sonication to ensure the complete solubility of drug. 5 ml of 30 % Hydrogen peroxide was added, refluxed for 1 hr at 60°C and cooled to room temperature. The resulting solution was diluted to volume with mobile phase and filtered through a 0.45µ membrane filter. From this, 0.25 ml of the sample solution was pipetted out into a 25 ml volumetric flask and made up to volume with mobile phase resulting in 10µg/ml solution. Typical chromatogram for oxidative degradation of ROS shown in Fig. 8.

Thermal degradation studies

Twenty tablets were taken and made into a fine powder. This powdered drug was exposed to dry heat at 80°C for 2 days. A quantity of the powder equivalent to 25 mg of ROS was accurately weighed and dissolved in 15 ml of the mobile phase in a 25 ml volumetric flask. The solution was shaken well, allowed to stand for 15 min with intermittent sonication to ensure the complete solubility of drug and finally made up to volume with the diluent. The solution was then filtered through a 0.45µ membrane filter. From this, 0.25 ml of the sample solution was pipetted out into a 25 ml volumetric flask and made up to volume with mobile phase resulting in 10µg/ml solution. Typical chromatogram for thermal degradation of ROS shown in Fig. 9.

Photolytic degradation

Twenty tablets were taken and made into a fine powder. This powdered drug was subjected to sunlight and UV light exposure for 8 days (1.2 million Lux hours). A quantity of the powder equivalent to 25 mg of ROS was accurately weighed and dissolved in 15 ml of the mobile phase in a 25 ml volumetric flask. The solution was shaken well, allowed to stand for 15 min with intermittent sonication to ensure the complete solubility of drug and finally made up to volume with the diluent. The resulting solution was filtered through a 0.45µ membrane filter. From this, 0.25 ml of the sample solution was pipetted out into a 25 ml volumetric flask and made up to volume with the mobile phase resulting in 10µg/ml solution. Typical chromatogram for photolytic degradation of ROS shown in Fig. 10.

RESULTS AND DISCUSSION

The study was aimed to develop a sensitive, precise and accurate stability-indicating method for the analysis of ROS in tablet dosage forms by RP-HPLC. An Eclipse XDB plus C18 Column (4.6 × 150 mm, 5 µm particle size) was chosen as the stationary phase for the separation and determination of ROS. For the optimization of the mobile phase, various mixtures consisting of acetonitrile, water and methanol were examined at different ratios. The choice of the optimum composition is based on chromatographic response factor. A composition of 40:60 v/v of water and acetonitrile provided an efficient separation of ROS with sufficient retention time. The injection volume was set to 20µL and the drug was detected using a Variable Wavelength Detector at 242 nm.

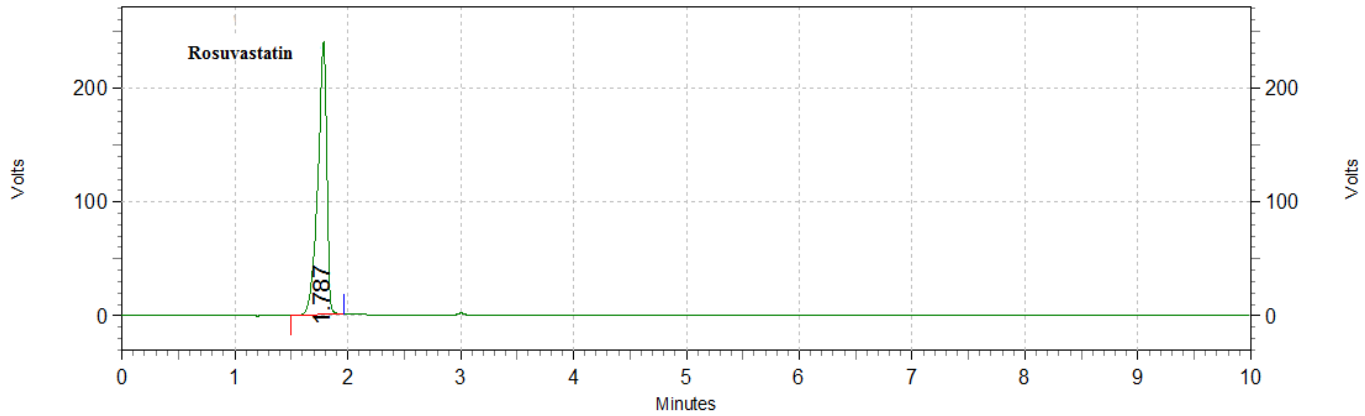

Fig. 3: Chromatogram for Standard Solution

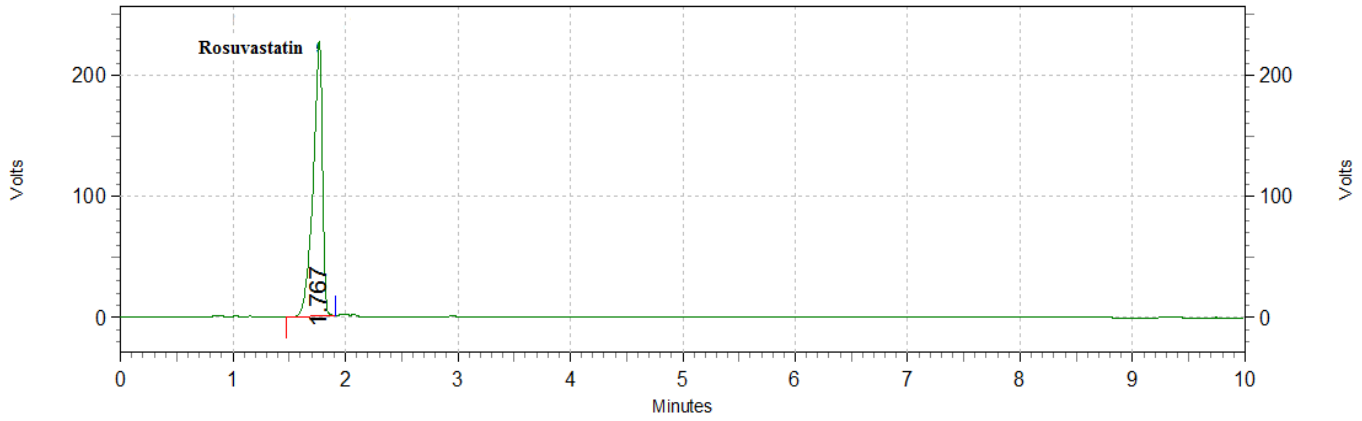

Fig. 4: Chromatogram for Test Solution

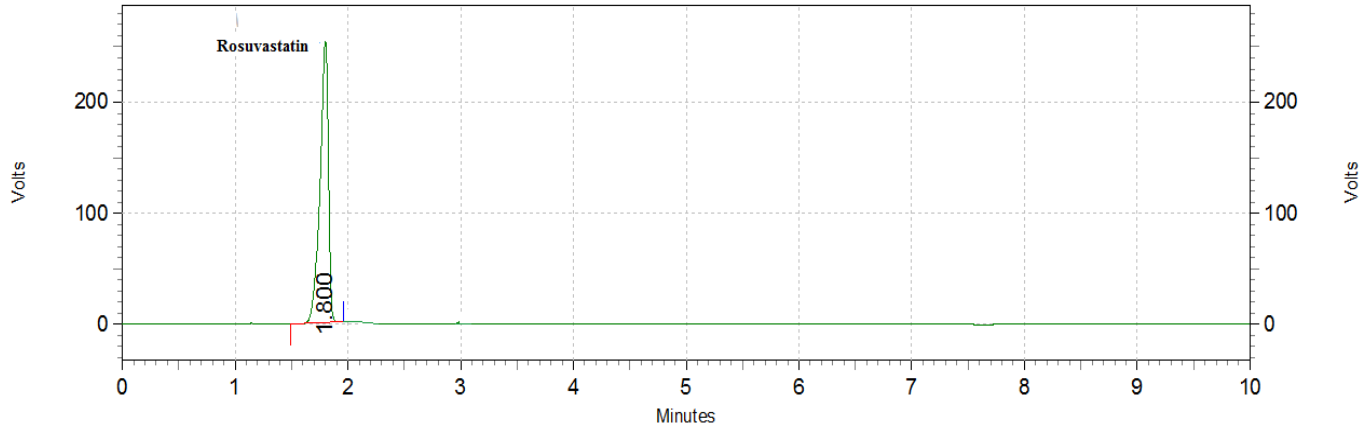

Fig. 5: Chromatogram for System Suitability

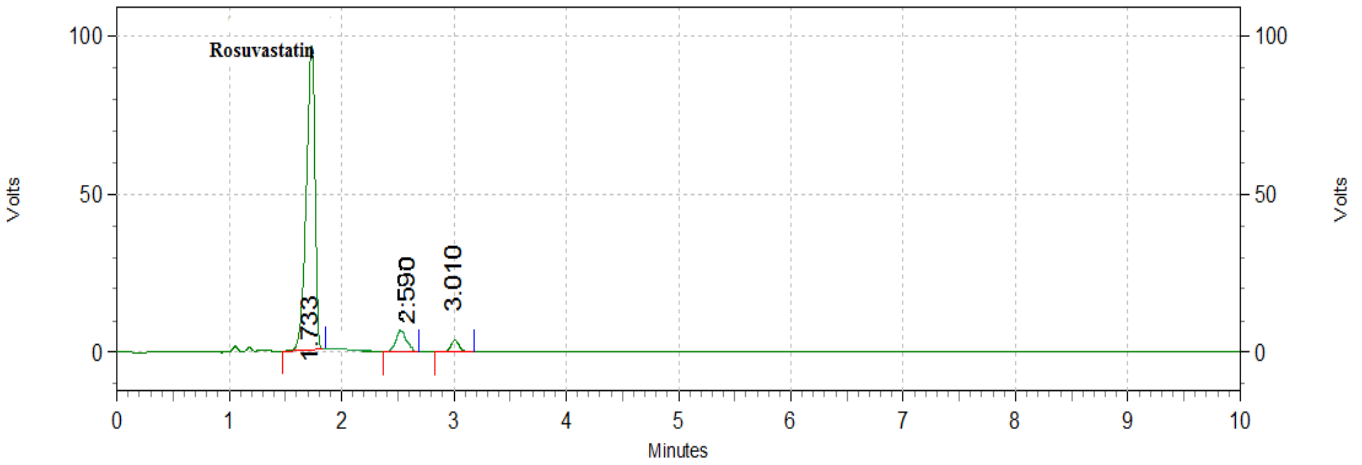

Fig. 6: Chromatogram for Acid Degradation

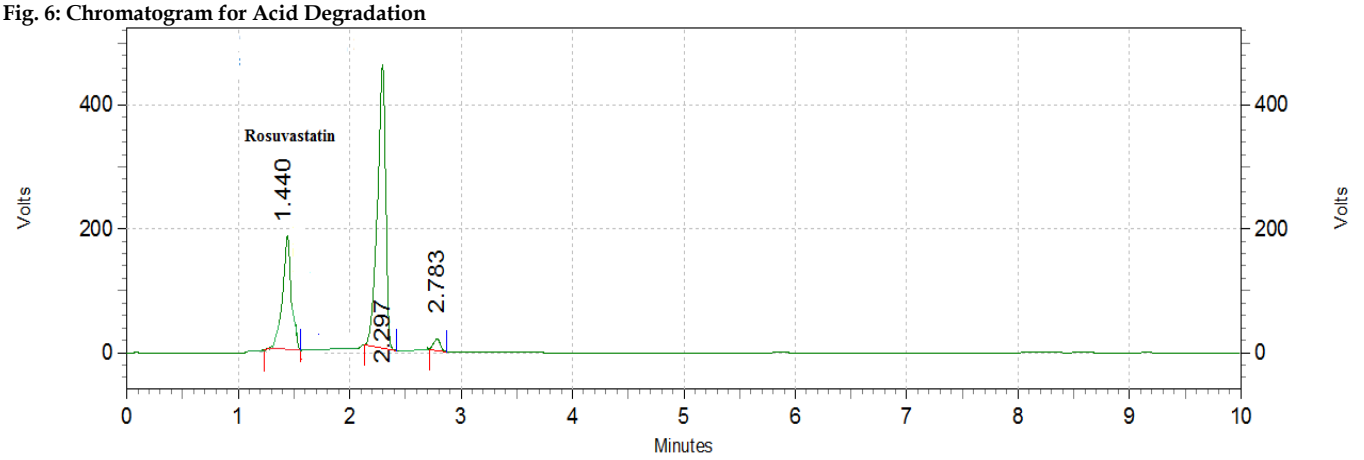

Fig. 7: Chromatogram for Base Degradation

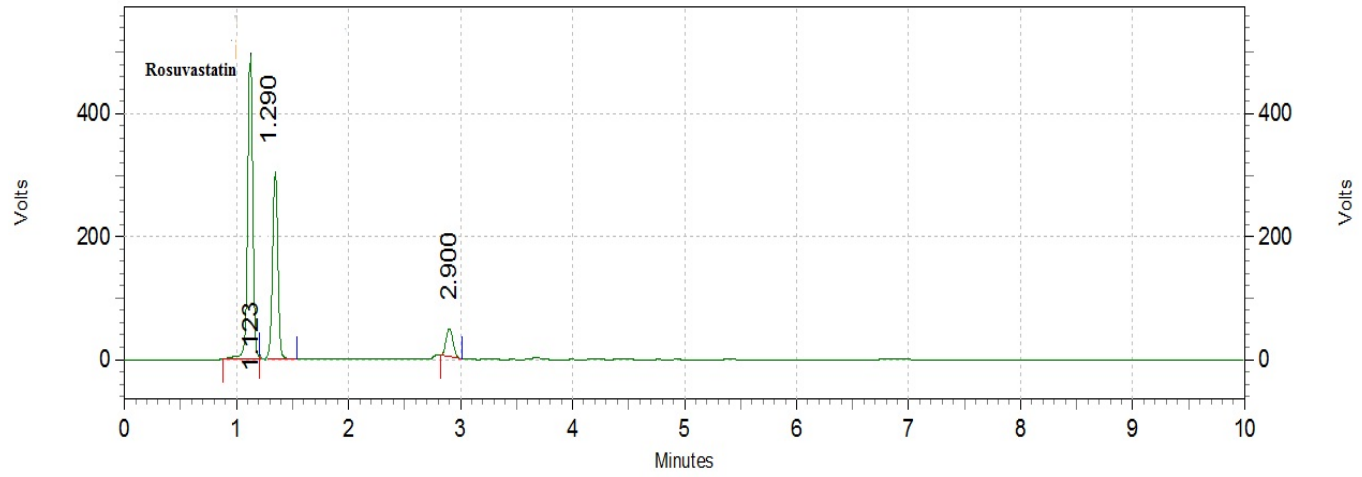

Fig. 8: Chromatogram for Oxidative Degradation

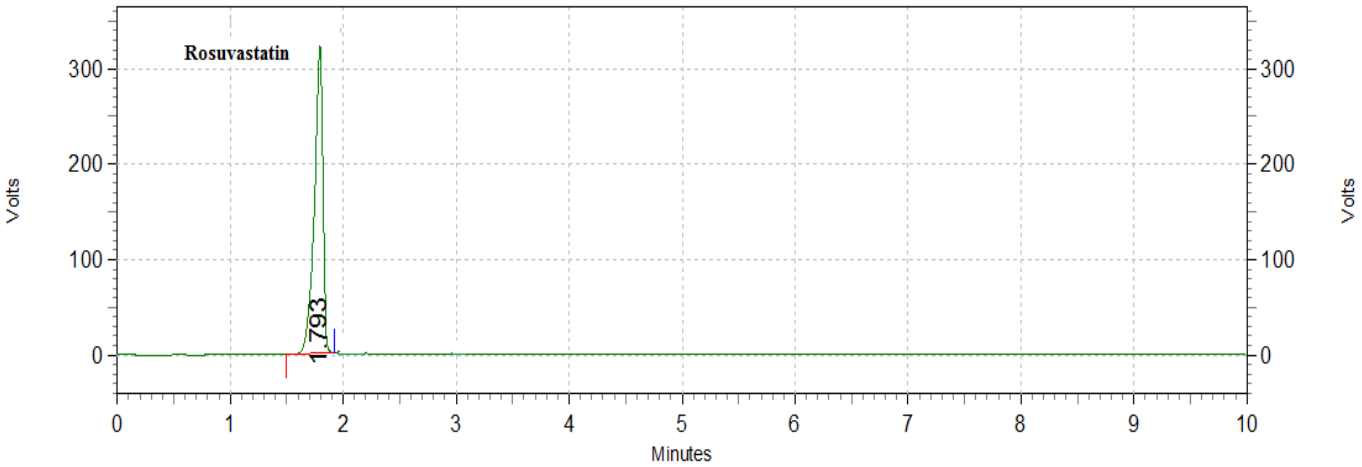

Fig. 9: Chromatogram for Thermal Degradation

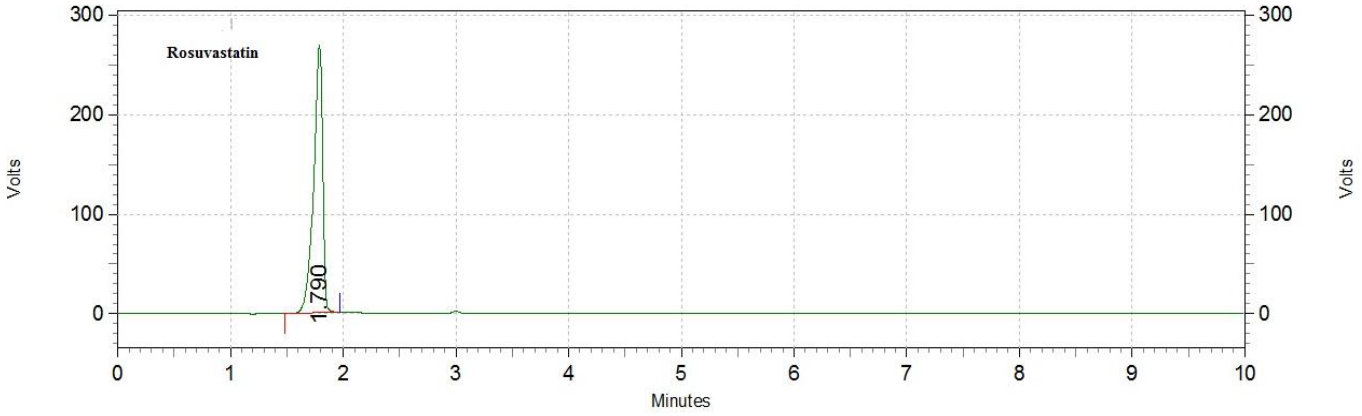

Fig. 10: Chromatogram for Photolytic Degradation

The run time was 10 min. A flow rate of 1.0 ml/min was found to be optimum from the studied range 0.5-2.0 ml/min, which gave optimum retention time of 1.787 min, base line stability and noise.

A good linear relationship (R^2 = 0.999) was observed between the concentrations of ROS and the corresponding peak areas. The regression of ROS concentration over its peak area was found to be y = 654.1x+2407 (where y is the peak area and x is the concentration of ROS). The recovery study was performed on 80 %, 100 % and 120 % of the expected analyte concentration of ROS. The percentage recovery for ROS was found to be 100.53 % as a mean % recovery of all determinants at the three concentration levels as shown in Table 2, which indicates that the method was accurate and the commonly used excipients and additives present in the tablet formulation did not interfere with the retention time of the proposed method. The precision of the method was determined from the peak areas of five homogeneous sample preparations. The % RSD for system precision and method precision were found to be 0.52 % and 0.72

% respectively as shown in Table 3 and 4, indicating that the method was quite precise. The lower limit of detection and the limit of quantification were found to be 0.08µg/ml and 0.25µg/ml respectively.

The optimum HPLC conditions like detection wavelength, ratio of mobile phase and flow rate of mobile phase set for the proposed method have been slightly modified as a means to evaluate the robustness of the method. The results indicated that the selected factors remained unaffected by small variations in these parameters, as shown in Table 5. The % RSD obtained in ruggedness studies was found to be 0.175 % as shown in Table 6, indicating that the method is quite rugged. System suitability results such as theoretical plates and tailing factor were observed and found to be 2877 (theoretical plates) and 0.98 (tailing factor) respectively for ROS peak as exhibited in Table 7.

The forced degradation studies revealed significant degradation by acid hydrolysis with 1 M HCl, base hydrolysis with 1 M NaOH and also oxidative degradation with 30 % Hydrogen peroxide as shown in figures 3-10. On treatment with 1 N HCl the peak area decreased and the retention time changed from 1.787 to 1.733 min. The percentage recovery after acid treatment was found to be 97.4 %. On treatment with 1 N NaOH, it was found that there is increase in peak resolution and decrease in peak area. The retention time changed from 1.787 to 1.440 min and additional peaks were observed due to degradation products. The percentage purity of ROS after alkali treatment was found to be 89.74 %. A decrease in peak area was noticed when the drug sample was treated with 30 % hydrogen peroxide with a change in retention time from 1.787 to 1.107 min along with additional peaks due to degradation products. The percentage recovery after oxidative degradation was found to be 89.10 %. Thermal degradation and photo degradation studies showed no significant degradation products. All the degradation products obtained by stress conditions discussed above were found to be well separated from the principal peak, which means that the ROS peaks were highly pure in all chromatograms obtained.

The proposed analytical method was developed and validated for system suitability, linearity, specificity, accuracy, robustness and ruggedness. All parameters tested were found to be within limits of ICH guidelines. The study indicates that the method has significant advantages in indicating stability and purity of active drug with accuracy and precision. Hence, this method can easily be adopted for the routine and stability analysis of ROS in tablet dosage forms.

ACKNOWLEDGMENTS

The author expresses sincere thanks to Micro Labs, Bangalore for providing us gift samples and Maharajah's college of Pharmacy for providing necessary facilities for the study.

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Source of Support: Nil, Conflict of Interest: None declared.