Available online at www.ijpsdronline.com
International Journal of Pharmaceutical Sciences and Drug Research 2014; 6(2): 154-159

Research Article ISSN 0975-248X

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

Zahi Mohammad Turabi*, O'hood Atef Khatatbeh

Quality Control Department, Sama Pharmaceuticals Manufacturing Co., Nablus, Palestine

ABSTRACT

A stability indicating, accurate, specific, linear and sensitive reverse phase-HPLC method has been developed and validated for the determination of Rosuvastatin as calcium, (ROS) in pharmaceutical dosage form. The chromatographic separation was performed using end capped (Luna) C_{18} Column (250 mm × 4.6 mm, 5µm particle size). Mobile phase A was prepared by mixing 3.0g/l Ammonium dihydrogen phosphate in distilled water: Methanol: Acetonitrile: Tetrahydrofuran in the ratios (400:20:100:5v/v). To 1000 ml of the resulting solution 1 ml of triethylamine was added then the pH was adjusted to 6.3 with 5% v/v orthophosphoric acid. Mobile phase B was prepared by mixing Acetonitrile: Methanol: Tetrahydrofuran in the ratios (500:50:5v/v). Other chromatographic conditions such as flow rate set at 2.0 ml/min and 30°C column temperature with the detection wavelength at 243nm. The retention times of Rosuvastatin was found to be about 16 min. The linearity was performed in the concentration range of 40.0-60.0µg/ml with a squared correlation coefficient of 0.99998. The percentage purity of ROS was found to be >99.8%. The percentage recovery was determined for ROS and was found to be 100.067%. The developed analytical method has been validated for specificity, linearity, precision, accuracy, ruggedness and robustness 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 successfully employed for routine quality control and stability analysis of ROS in pharmaceutical dosage forms.

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

INTRODUCTION

bis((E) -7[4-(4-Fluorophenyl)-6-isopropyl-2-aminopyrmidin -5yl)(3R,5S)-3,5-dihydroxyhept-6-enoic acid) Calcium salt [Fig. 1]. [1] It belongs to the class of drugs called statins which are employed to lower hypercholesterolemia and related conditions and to prevent cardiovascular diseases. [2] It is highly effective 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor. [1, 3-8] In clinical trials, rosuvastatin achieved mark reduction in serum levels of LDL cholesterol, accompanied by modest increases in HDL cholesterol and reduction in triglyceride. [1] The most important related compounds for rosuvastatin are antiisomer and lacton impurity. [9] Literature survey reveals that few Stability-indicating HPLC methods [1-14], spectrophotometric methods [15-17]; HPTLC [18-19] methods have been reported for the estimation of ROS as a single or in combined pharmaceutical preparations.

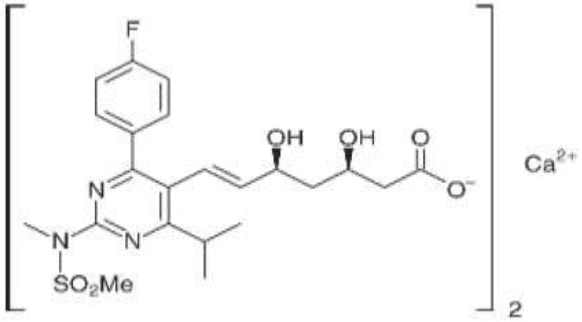

Fig. 1: Chemical Structure for Rosuvastatin Calcium

In the present work we are focused on to develop and validate a stability indicating method with optimum chromatographic conditions for the determination of ROS in pharmaceutical preparations in the presence of its related impurities (Rosuvastatin antiisomer and Lacton impurity) and other unknown degradation products that may be present during stability study. The developed method was validated

***Corresponding author: Mr. Zahi Mohammad Turabi,** Cont Pharmaceuticals Manufacturing Co. Nablus, Palestine; **Tel.:** +972598042552; **E-mail:** zahibpharm@yahoo.com

as per ICH guidelines, [20-21] and can be applied successfully to quality control purposes.

MATERIALS AND METHODS

Reagents and Chemicals

Rosuvastatin, Rosuvastatin antisomer and Rosuvastatin lacton impurity were purchased from MSN Laboratories Limited, India. All chemicals used were of HPLC grade: Acetonitrile, Tetrahydrofuran, Methanol, Orthophosphoric acid were purchased from J.T. Baker, Triethylamine was purchased from Mallinckrodt Chemicals, and Ammonium dihydrogen phosphate was purchased from Merck. Water used was freshly prepared by Sama Pharmaceuticals Manufacturing Co.

Equipment and Chromatographic Conditions

A Dionex UltiMate 3000 HPLC system with Chromeleon software "version 6.8", Photodiode Array Detector and Autosampler was used. It was manufactured by Dionex Corporation Company, USA. An end capped (Luna) C_{18} Column (250 mm × 4.6 mm, 5µm particle size) was used for analytical separation. The mobile phase consisted of mobile phase A: (3.0 g/l Ammonium dihydrogen phosphate: Methanol, Acetonitrile: Trtrahydrofuran) in the ratios of (400:20:100:5v/v). To 1000 ml of the resulting solution 1ml of triethylamine was added then the pH was adjusted to 6.3 with 5% v/v orthophosphoric acid. Mobile phase B: (Acetonitrile: Methanol: Trtrahydrofuran) in the ratios (500:50:5v/v) with gradient elution program as presented in Table 1. The flow rate was adjusted to 2.0 ml/min, the injection volume was set at 20µL, the column compartment was operated at 30°C and the UV detection was set at 243nm. The purity analysis was performed over a wavelength range of 200-400nm.

Preparation of Analytical Solutions

Preparation of Ammonium Dihydrogen Phosphate

It was prepared by dissolving 3.0 g of ammonium dihydrogen phosphate in 1000 ml of distilled water.

Preparation of Diluent for samples preparation

It was prepared by mixing 500 ml of Acetonitrile and 500 ml of Distilled Water.

Preparation of mobile phase A

It was prepared by mixing 1200 ml of ammonium dihydrogen phosphate, 60 ml of Methanol, 300 ml of acetonitrile and 15 ml of tetrahydrofuran, to 1000 ml of the resulting solution 1.0 ml of triethylamine was added and the pH was adjusted to 6.3 with 5% orthophosphoric acid. Degassed in ultrasonic water bath for 2 minutes and filtered through 0.45µ filter under vacuum filtration.

Preparation of mobile phase B

It was prepared by mixing 500 ml Acetonitrile, 50 ml of Methanol and 5 ml of tetrahydrofuran. Degassed in ultrasonic water bath for 2 minutes and filtered through 0.45µ filter under vacuum filtration.

Preparation of stock system suitability solution

It was prepared by dissolving 1.0mg of each of Rosuvastatin antiisomer and Lacton impurity in 100 ml of diluent, sonicated for 5 minutes, allowed to cool to room temperature and filtered using 0.45µ filter to obtain a solution having a concentration of 0.01mg/ml of each.

Preparation of stock standard solution for preparation of system suitability solution

It was prepared by dissolving an accurately weighed quantity of Rosuvastatin calcium equivalent to 25.4 mg of Rosuvastatin (as calcium) in 50.0 ml of diluent, sonicated for 5 minutes, allowed to cool and filtered using 0.45µ filter to obtain a solution having a concentration of 0.508 mg/ml.

Preparation of system suitability solution

It was prepared by transferring 5.0 ml of each of stock standard solution and stock system suitability solution to 50 ml volumetric flask and completed to volume with diluent. Mixed and filtered using 0.45µ filter to obtain a solution having a concentration of 0.001 mg/ml of each of Rosuvastatin antiisomer and Lacton impurity and 0.0508 mg/ml of Rosuvastatin.

Preparation of standard solution

It was prepared by dissolving ROS standard equivalent to 25 mg of Rosuvastatin (as calcium) in 50 ml of diluent, sonicated for 5 minutes, cooled to room temperature then 5.0 ml of the resulting solution was diluted to 50 ml with diluent, mixed well and filtered using 0.45µ filter to obtain a solution having a concentration of 0.05 mg/ml.

Preparation of sample solution

An accurately weighed portion of powdered tablets equivalent to 50 mg of ROS was transferred to 100 ml volumetric flask. 70 ml of diluent were added, shook by mechanical means for 15 minutes, cooled to room temperature and diluted with diluent to volume. 5.0 ml of the resulting solution were diluted to 50 ml with diluent, mixed well and filtered using 0.45µ filter to obtain a solution having a concentration of 0.05mg/ml.

HPLC-Method Development and Validation

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

Specificity of analytical method and peak purity

The specificity and peak purity were carried out to determine whether there are any interference due to presence of impurities, degradation products or other components that may be present in retention time of analytical peaks and affect the peak purity and specificity of the analytical method. Forced degradation studies were carried out by using 0.5M HCl (at 90ºC for 20 minutes), 2M NaOH (at 90ºC for 60 minutes), thermal degradation (at 105ºC for 16 Hours), 33% Hydrogen peroxide (at 90ºC for 30 minutes) and Photo degradation (for 20 Hours).

Linearity

The linearity of the method was established by spiking a series of sample of ROS, the solutions of five different concentrations 40-60µg/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 curve was obtained and slope-a, intercept-b, and correlation coefficient-R^2 were determined.

Precision and ruggedness

Precision was determined by injecting six independent preparations from a single lot of formulation (50µg/ml) of ROS into HPLC system, while ruggedness was determined by injecting six independent preparations prepared by another analyst into another HPLC system. The retention time and peak area were obtained and the mean and %RSD were found to be within the acceptance criteria.

Accuracy (per cent 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 were obtained. The percentage recoveries of ROS were calculated using standard at the same concentration at each concentration level.

Table 1: Gradient elution program

Time (min)	Mobile Phase A%	Mobile Phase B%	Flow Rate (ml/min)
0	100	0	2.0
19	80	20	2.0
29	25	75	2.0
29.1	100	0	2.0
32	100	0	2.0

Table 2: Linearity results for ROS

Conc. (µg/ml)	Peak area (mAU*min)
40	17.282
45	19.47
50	21.703
55	23.884
60	26.061
R^2	0.99998
Slope-a	0.43423
y-intercept	-0.02607

Table 3: System precision for ROS

	RT (min)	peak area(mAU*min)
Statistics	16.12	21.741
	16.13	21.754
	16.14	21.782
	16.14	21.806
	16.14	21.815
	16.16	21.518
Average	16.138	21.736
St. Dev.	0.0133	0.1105
% RSD	0.08	0.509

Table 4: Method precision for ROS

	RT (min)	peak area(mAU*min)
Statistics	16.13	21.771
	16.14	21.769
	16.14	21.772
	16.13	21.746
	16.14	21.765
	16.14	21.688
Average	16.136	21.7518
St. Dev.	0.052	0.0327
% RSD	0.032	0.15

Table 5: Method Ruggedness for ROS

	RT (min)	peak area(mAU*min)
Statistics	16.23	21.792
	16.22	21.823
	16.22	21.839
	16.22	21.864
	16.23	21.900
	16.22	21.932
Average	16.223	21.858
St. Dev.	0.0052	0.051
% RSD	0.032	0.235

Robustness

Robustness of the developed analytical method was tested by evaluating the effect of small variations in analytical method parameters such as changing in flow rate from 2.0ml/min to 2.1ml/min and 1.9ml/min (±5%), changing in column temperature (± 5°C), changing in wavelength (±5nm) and changing in the mobile phase B ratio (± 5%).

System suitability

System suitability test was carried out on freshly prepared system suitability solution. System suitability parameters were calculated by injecting system suitability solution and the values of theoretical plates, tailing factor and resolution were recorded.

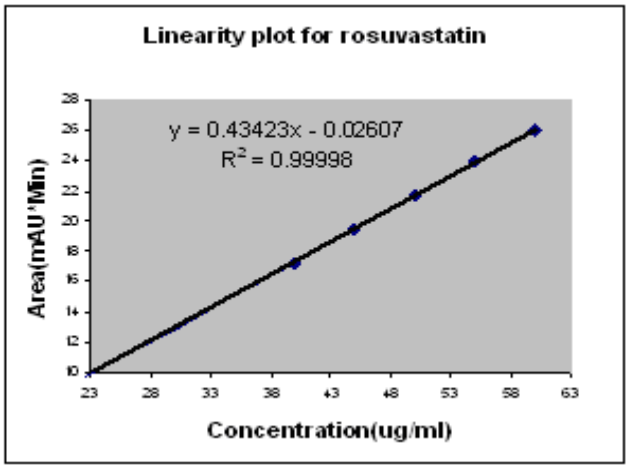

Fig. 2: Linearity plot for ROS

RESULTS AND DISCUSSION

The developed analytical method is a new stability indicating RP-HPLC method for the estimation of ROS in pharmaceutical dosage forms. Various mobile phases and columns were used for the development and validation of the analytical method. The final method was optimized with the following conditions: The mobile phase consisted of: mobile phase A: (3.0 g/l Ammonium dihydrogen phosphate: Methanol, Acetonitrile: Tetrahydrofuran), (400:20:100:5v/v), to 1000 ml of the resulting solution 1 ml of triethylamine was added then the pH was adjusted to 6.3 with 5% v/v orthophosphoric acid and mobile phase B: Acetonitrile: Methanol: Tetrahydrofuran (500:50:5v/v) with gradient elution system as presented in Table 1 . An end capped (Luna) C_{18} Column (250 mm × 4.6 mm, 5µm particle size) was used for chromatographic separation. The flow rate was adjusted to 2.0 ml/min and the column oven was operated at 30°C. The injection volume was set to 20µL and the photodiode array detector was set at 243nm. The specificity and peak purity were carried out to determine whether there was any interference due to presence of impurities, degradation products or other components that may be present at the retention time of analytical peak and affect the peak purity and specificity of the analytical method. The purity analysis was performed over a wavelength range of 200-400nm. The linearity was determined as linearity regression of the analyte concentration of the range 40-60µg/ml (ROS). The calibration curve obtained by plotting concentration versus peak area (presented in Table 2 and Fig2**)** was linear and the squared correlation coefficient was found to be 0.99998 for ROS.

The precision of the method was determined from the peak areas of six determinants of homogeneous sample preparation. The % Relative Standard Deviation for system precision exhibited in Table 3 was found to be 0.509, the % Relative Standard Deviation for method precision exhibited in Table 4 was found to be 0.15 and the % Relative Standard Deviation for ruggedness exhibited in Table 5 was found to be 0.235. The accuracy study was performed on 80%, 100% and 120% of the target concentration of ROS. The percentage recovery was determined for ROS and was found to be 100.067% as a mean % recovery of all determinants at the three concentration levels as shown in Table 6.

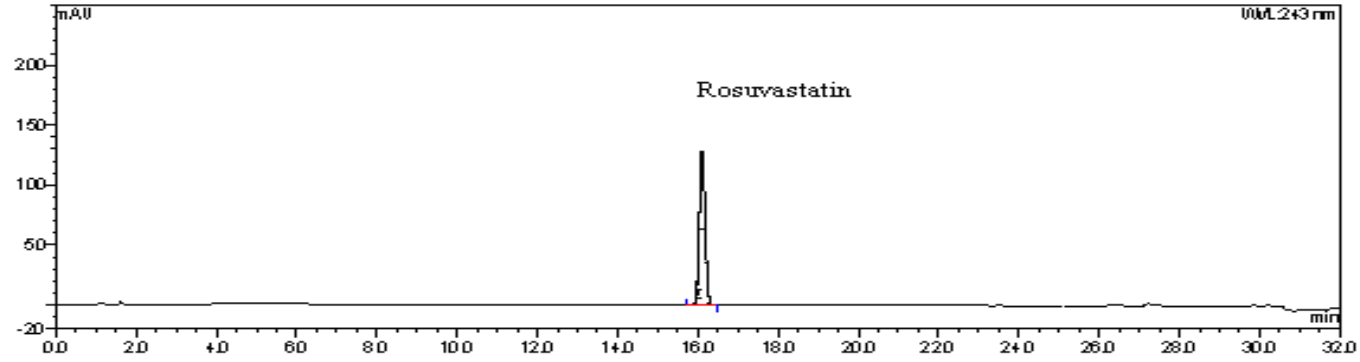

Fig. 3: Chromatogram for Standard Solution

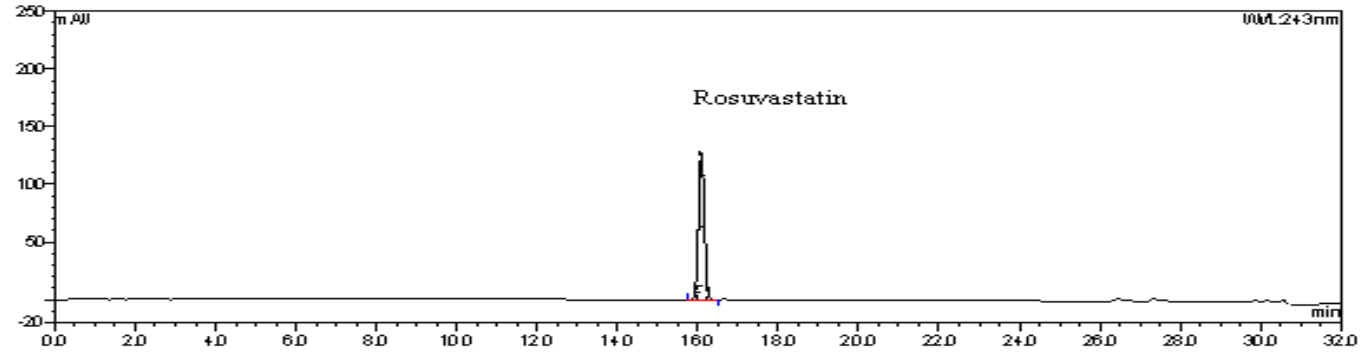

Fig. 4: Chromatogram for Test Solution

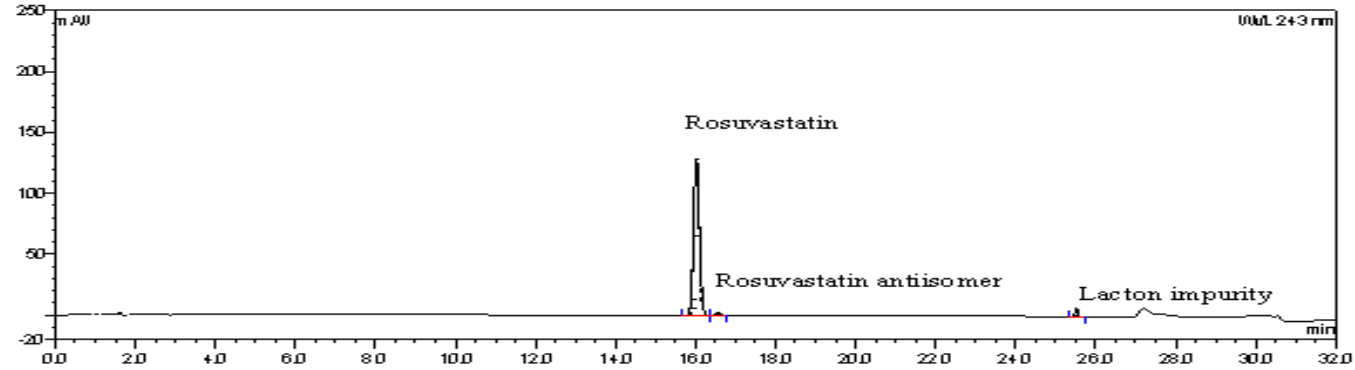

Fig. 5: Chromatogram for System Suitability

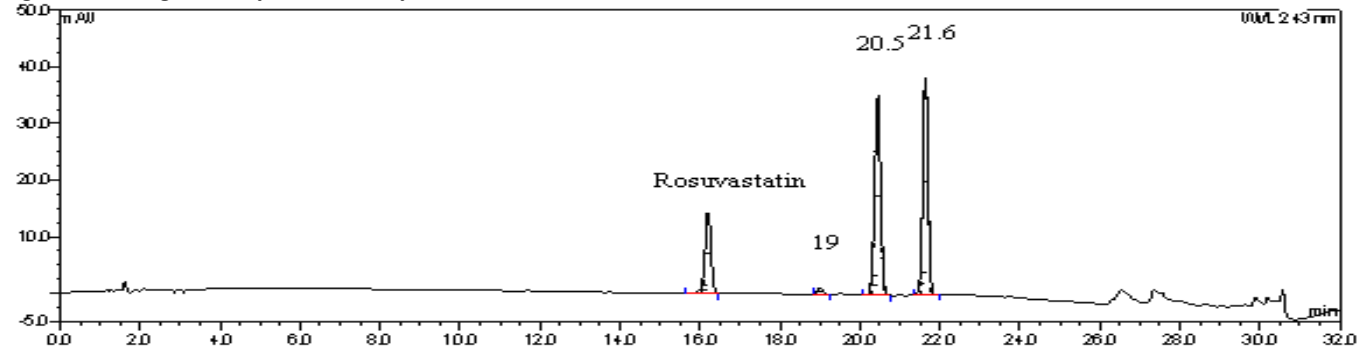

Fig. 6: Chromatogram for Photo degradation

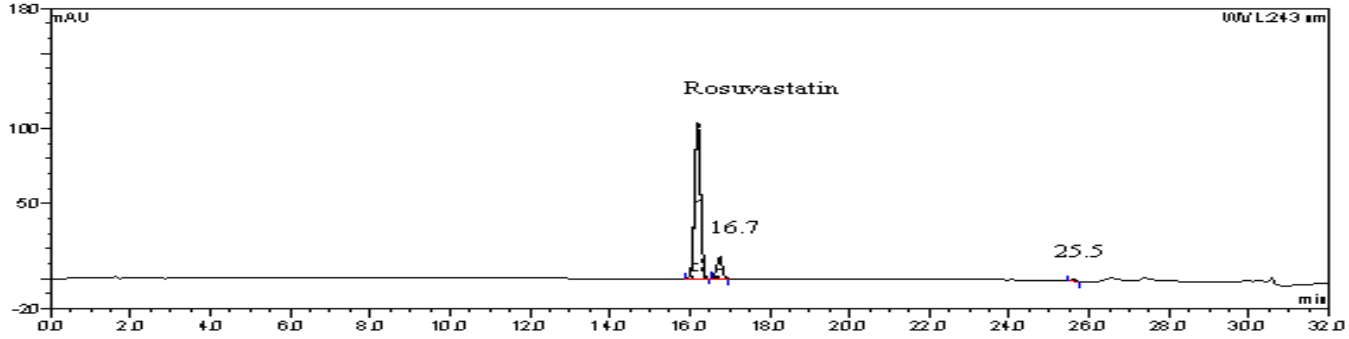

Fig. 7: Chromatogram for 0.5M HCl Degradation

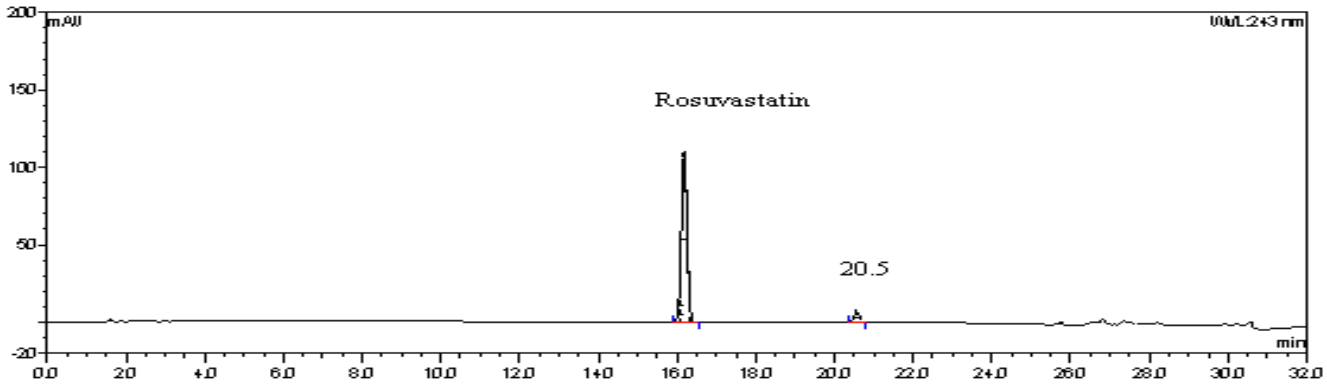

Fig. 8: Chromatogram for Thermal degradation

Table 6: % Recovery for ROS

Concentration at specific level	Active drug added (mg)	Recovered amount (mg)	Mean % Recovered for all determinations
80.0%	20.0	20.19	100.067%
	20.0	20.27	
	20.0	20.24	
100.0%	25.0	24.91	
	25.0	24.94	
	25.0	24.92	
120.0%	30.0	29.94	
	30.0	29.85	
	30.0	29.89	

Table 7: Robustness results

Parameter	Adjusted to	RT (min)	peak area(mAU*min)
Column Temp. (ºC)	25	16.49	22.043
	30	16.09	21.626
	35	15.79	22.041
Flow rate (ml/min)	1.9	16.6	23.14
	2.0	16.09	21.626
	2.1	15.85	20.899
Wavelength (nm)	238	16.09	20.869
	243	16.09	21.626
	248	16.09	20.188
Change in Mobile phase B	-5%	16.54	22.087
	No change	16.09	21.626
	+5%	15.8	22.080

Table 8: System suitability values

ROS		ROS Antiisomer		Lacton Impurity	
Theoretical plates	Tailing factor	Theoretical plates	Tailing factor	Theoretical plates	Tailing factor
56595	1.06	63151	0.98	590665	1.02
Resolution between ROS and ROS Antiisomer				2.06	

The robustness was carried out by changing in analytical parameters (detection wavelength, column temperature, ratio of mobile phase B and flow rate) and the results were exhibited in Table 7. System suitability results such as theoretical plates and tailing factor were observed and were found to be 56595 (theoretical plates) and 1.06 (tailing factor) for ROS peak, while the resolution was found to be 2.06 between ROS peak and Rosuvastatin antiisomer peak exhibited in Table 8. And the Relative Standard Deviation in retention time was found to be 0.095 for ROS peak, 0.092 for Rosuvastatin antiisomer peak and 0.0277 for Lacton impurity peak. Significant degradation was obtained by 0.5M HCl, Thermal degradation, Photo degradation, and Hydrogen peroxide degradation. Chromatograms for standard solution, Test solution, system suitability solution, Photo degradation solution, HCl degradation and thermal degradation exhibited in Figures [3-8] respectively.

Chromatogram for photo degradation Figure 6 exhibited three degradation products with retention times at 19minute, 20.5minute and 21.6 minute which were found to be well separated from each others and not affecting the ROS peak purity. Chromatogram for 0.5M HCl degradation Figure 7 exhibited two degradation products at retention times 16.7minute and 25.5 minute which were the same retention times of rosuvastatin antiisomer and lacton impurity respectively. Finally Chromatogram for thermal degradation Figure 8 exhibited a single well separated degradation product with retention time at 25.5minute. 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 prescribed 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 guideline. The study indicates that the method has significant advantages in term of stability indicating (good resolution between active drugs and Rosuvastatin antiisomer or other degradation products), high purity of active drug, accuracy and precision. The developed analytical method was successfully employed for routine and stability analysis of ROS in pharmaceutical dosage forms.

ACKNOWLEDGMENTS

Authors are thankful to Sama Pharmaceuticals Manufacturing Co. for providing facilities to execute the research work. Authors are thankful to Ala' Dmaidi for electronic treatment and work.

REFERENCES

1. Mostafa NM, Badawey AM, Lamie NT, Abd El-Aleem AB. Stability-indicating methods for the determination of rosuvastatin calcium in the presence of its oxidative degradation products. Int J Pharm Biomed Sci. 2012; 3: 193-202.
2. Kaila HO, Ambasana MA, Thakkar RS, Saravaia HT, Shan AK. A new improved RP-HPLC Method for assay of rosuvastatin calcium in tablets. Indian J of Pharmaceutical Sciences 2010; 72: 592-598.
3. Thriveni J, Rambabu R, Rao JV, Vidyadhara S. Development and validation of RP-HPLC method for simultaneous estimation of rosuvastatin calcium and fenofibrate in bulk and pharmaceutical dosage forms. International J of Research in Pharmacyand chemistry 2013; 3: 208-212.
4. Moinuddin M, Rahman SA, Yadav BR, Battu R. Development and validation of a reverse phase HPLC method for simultaneous estimation of rosuvastatin calcium and fenofibrate in tablet dosage

form. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4: 150-154.
5. Tajan D, Raurale AM, Bharande PD, Mali AN, Gadkari AV, Bhosale VR. Development and validation of a RP-HPLC-PDA method for the simultaneous determination of rosuvastatin calcium and amlodipine besilate in pharmaceutical dosage form. J of Chemical and Pharmaceutical Research 2012; 4: 2789-2794.
6. Pandia CB, Channabasavaraj KP, Chudasama JD, Mani TT. Development and validation of RP-HPLC method for determination of rosuvastatin calcium in bulk and pharmaceutical dosage form. J of Pharmaceutical Sciences Review and Research 2010; 5: 82-86.
7. Godavariya Vijay D, Prajapati Pintu B, Morolia Bhavin P, Shah Sailesh A. Development and validation of RP-HPLC method for the simultaneous estimation of rosuvastatin calcium and aspirin in marketed formulation. International Research J of Pharmacy 2012; 3: 173-175.
8. Donthula S, Kumar MK, Teja GS, Kumar YM, Krishana JY, Ramesh D. A new validated RP-HPLC method for determination of rosuvastatin calcium in bulk and pharmaceutical dosage form. Der Pharmacia Lettre 2011; 3:350-356.
9. Trivedi HK, Patel MC. Development and validation of a stability-indicating RP-UPLC method for determination of rosuvastatin and related substancesin pharmaceutical dosage form. Scientia Pharmaceutica. 2012; 80:393-406.
10. Thukabai S, V. Rao VUM, Shaik MR. Development and validation of RP-HPLC method for simultaneous estimation of rosuvastatin and fenofibrate in bulk and tablet dosage form. International J of Pharmacy 2013; 3: 607-612.
11. Rao AL, Suneetha D. Development and validation of RP-HPLC method for the estimation of rosovastatin in bulk and pharmaceutical dosage form. Int. J. Cem. Sci. 2010; 8: 2010.
12. Shah Y, Iqbal Z, Ahmad L, Khan A, Khan Ml, Nazir S, Nazir F. Simultaneous determination of rosuvastatin and atorvastatin in human serum using RP-HPLC/UV detiction: method development , validation and optimization of various experimental parameters. J Chromatogr B Analyt Technol Biomed Life Sci. 2011; 879: 557-563.
13. Joshi H, Kumar S, Yadav YC, Seth AK. New analytical method development and validation of rosuvastatin and Ezetimibe in tablet dosage form. International J of Drug Discovery and Medical Research 2012; 1:22-29.
14. Varma DPSRChNP, Rao AL, Dinda SC. Development and validation of stability indicating RP-HPLC method for simultaneous estimation of rosuvastatin and ezetimibe in combined tablet dosage form. Rasayan J Chem. 2012; 5: 269-279.
15. Gupta A, Mishra P, Shah K. Simple UV. Spectrophotometric determination of rosuvastatin calcium in pure form and in pharmaceutical formulations. E. J Chem. 2009; 6: 89-92.
16. Krishna MV, Sankar DG. Extractive spectrophotometric methods for the determination of rosuvastatin calcium in pure form and in pharmaceutical formulations by using safranin O and methylen blue. E. J Chem. 2007; 4: 46-49.
17. Singh RM, Ansari TA, Jamil S, Kumar Y. Spectrophotometric estimation of rosuvastatin calcium in tablet formulation. India Drugs 2005; 42: 244-245.
18. Sane RT, Kamat SS, Menon SN, Inamdar SR. Determination of rosuvastatin calcium in its bulk drug and pharmaceutical preparations by high-performance thin-layer chromatography. J Planer. Chromatogr. Mod. TLC. 2005; 18: 194-198
19. Chaudhari B, Patel N, Shah P. Determination of simvastatin, pravastatin sodium and rosuvastatin calcium in tablet dosage forms by HPTLC. Indian J Pharm. Sci. 2007; 69: 130-132.
20. Code Q2A-Text on Validation of Analytical Procedure Step-3 Consensus Guideline, 1994, ICH Harmonized Tripartite Guideline.
21. Code Q2B- Validation of Analytical Procedure Methodology Step-4 Consensus Guideline, 1994, ICH Harmonized Tripartite Guideline.