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A new validated RP-HPLC method for determination of Rosuvastatin calcium in bulk and pharmaceutical dosage form

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ABSTRACT

A simple, rapid, and precise RP-HPLC method for analysis of Rosuvastatin calcium in bulk and its pharmaceutical formulations has been developed and validated. The separation was achieved on Luna C_{18}, 5µm 4.6 mm×250 mm column, using mobile phase composition of buffer (pH 4.5): Acetonitrile: methanol (45:25:35), at a flow rate of 1.0 ml/min at a detection wavelength of 248 nm. Rosuvastatin is eluted at retention time of 9.9 min. The method was validated for accuracy, precision, linearity, specificity and sensitivity in accordance with ICH guidelines. Validation revealed the method is specific, rapid, accurate, precise, reliable, and reproducible. Calibration plots were linear over the concentration ranges 25-75 µg mL^{-1}. Limits of detection and limits of quantification were 3.5µg mL^{-1} and 10.5µg mL^{-1}, respectively for both the drugs. The high recovery and low coefficients of variation confirm the suitability of the method for analysis of the rosuvastatin in formulations.

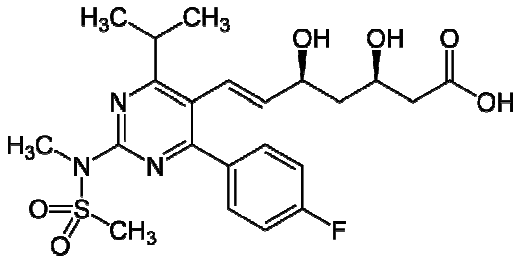
Key Words: RP-HPLC, Rosuvastatin, Crestor.

INTRODUCTION

Rosuvastatin calcium **[Fig-1],** is a class of lipid-lowering compound It is an anti cholesterenic agent. It is chemically bis[(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulfonyl)amino] pyrimidin-5-yl](3R,5S)-3,5- dihydroxyhept-6-enoic acid] calcium salt which is sparingly soluble in water and methanol, slightly soluble in ethanol, soluble in N,N-dimethyl formamide, acetone and acetonitrile. 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. Most of the dose is excreted in bile and 5-20 % excreted in urine [1-4].

Fig -1: Chemical structure of Rosuvastatin



Literature survey revealed that HPLC methods are reported for the determination of rosuvastatin [5-6]. Present study involves development and validation of new, simple RP-HPLC method for the determination of rosuvastatin in pharmaceutical formulations.

MATERIALS AND METHODS

Instrumentation:
Agilent technologies 1200 series HPLC system (LC-10 Ai, Japan) consisting of pump (LC-10 Ai), a system controller (SCL-10 AVP), an auto injector (SIL-10 ADVP) and a diode array detector (SPD-M10 AVP). Data analysis and processing were done by class ezchrome software.

Polomer-LP-139s, pH meter for pH measurement Nanopure Diamond, Barnstead thermolyne, USA

Standards and chemicals:
Rosuvastatin was gift samples obtained from MSN groups (Hyderabad, India). Purified water was prepared using a Millipore Milli-Q (Nanopure Diamond, Barnstead thermolyne, USA) water purification system. Acetonitrile of HPLC grade, Methanol of HPLC grade were purchased from Merck Ltd. (Mumbai, India), and o-phosphoric acid of A.R. grade was purchased from S.D fine chemicals Ltd (Hyderabad, India), Sodium dihydrogen phosphate of A.R. grade was purchased from Loba chemie pvt.Ltd (Mumbai, India).

Chromatographic conditions:
Chromatographic separation of Rosuvastatin was achieved at ambient room temperature using A Luna C-18 (250 x 4.6 mm, packed with 5 micron) in an isocratic mode with mobile phase Acetonitrile: 20mM potassium dihydrogen phosphate buffer (pH 4.5): methanol (25:45:35 % v/v) was used. The flow rate was 1 ml/ min and the detector set at 248 nm, injection volume was 20µl and run time was 20min. The mobile phase was filtered through 0.45 micron membrane filter under vacuum before use.

Preparation of standard drug solutions:
An accurately weighed 10 mg of each of Rosuvastatin was dissolved in 10 ml of methanol to obtain a concentration of 1 mg/mL each. From 1 mg/mL solution 1 ml was taken and made to 10

ml with methanol to obtain a concentration of 100µg/mL each. Daily working standard solutions of Rosuvastatin was prepared by suitable dilution of the stock solution with methanol. Similarly stock solution and working standard solutions of internal standard was also prepared.

Sample preparation of Pharmaceutical Dosage forms:

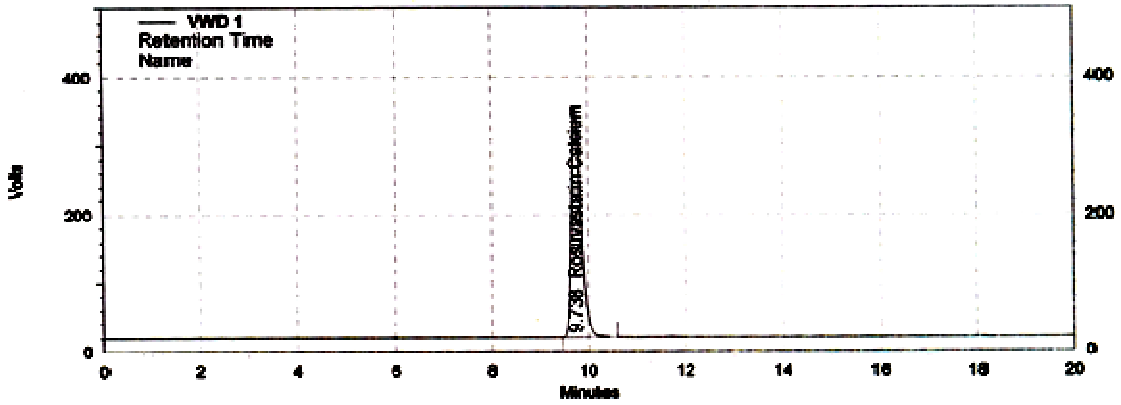
20 Tablets (Brand Name: Crestor) were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 10 mg of Rosuvastatin [equivalent to one tablet] was extracted with Methanol in a 50ml volumetric flask using ultra sonicator. This solution was filtered through Whatmann No.1 filter paper. The solution obtained was diluted with the Methanol so as to obtain a concentration in the range of linearity previously determined. All determinations were carried out in six replicates. The amount of drug recovered was calculated from the linearity graph. The represented data and chromatogram was shown in **Table – IV, [Fig-4].**

Table – IV : Recovery study from formulation (n=3) of Rosuvastatin

Brand Name	Labeled amount(mg)	Calculated amount (mg)		Assay (%)
		mean± SD	%RSD	
Crestor	10	9.98±0.10	0.1	99.8

Values are expressed in Mean ±SD

Fig-4: Chromatogram of rosuvastatin from formulation



RESULTS AND DISCUSSION

Method Development and Optimization:

During the method development, top priority was given for the complete separation of rosuvastatin from solvent peaks. Rosuvastatin is hydrophobic, almost insoluble in aqueous solutions and are freely soluble in methanol. The chromatographic method was optimized by changing various parameters, such as pH of the mobile phase, organic modifier and buffer used in the mobile phase and composition of mobile phase. Several mobile phases were tested until good resolution obtained between two drugs. Mixture of 20mM potassium dihydrogen phosphate buffer (pH 4.5) and Acetonitrile (ACN) in the proportions of 40:60, 45:55, 50:50, 60:40 and 65:35(v/v) were tested as a mobile phase with luna C-18 column. Increasing the composition of organic modifier decrease in retention time, the peak shape of drugs was poor and shoulder peak was observed along with rosuvastatin peak (40:60 v/v Buffer: ACN). Decreasing the composition of

organic modifier increase in retention time (60:40 v/v Buffer: ACN). The mobile phase composition of 25:35:45 v/v ACN: methanol: buffer, resolution, retention time was good. The method was optimized with the mobile phase composition of Acetonitrile, methanol and phosphate buffer 25:35:45 (v/v).

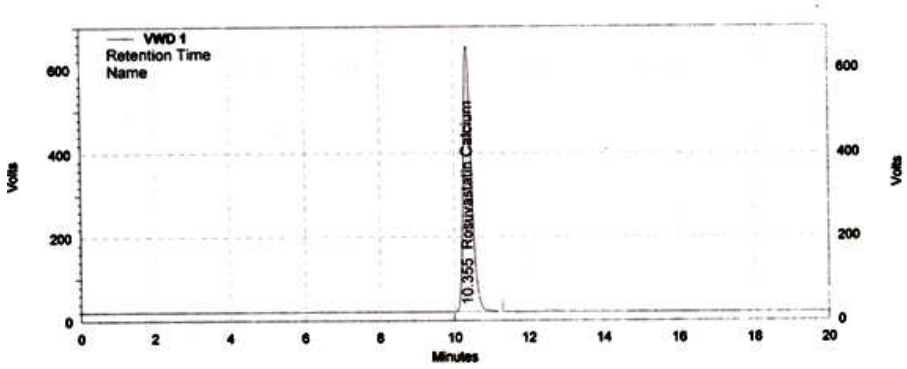
Buffer molarity of 10, 20 and 50 mM was tested. There were no significant changes in the chromatographic response and peak shape with change in buffer molarity. A buffer molarity of 20 mM was selected for further analysis.

After several trials, the method was optimized as a mixture of 20mM potassium dihydrogen phosphate buffer (pH 4.5) and Acetonitrile, methanol (45:25:35 v/v), at a flow rate of 1mL/min, at 248nm for run time of 20min. These chromatographic conditions achieved satisfactory resolution, retention time and tailing factor for rosuvastatin. The **[Fig-2]** shows that chromatogram of rosuvastatin.

Table – I : System suitability parameters of Rosuvastatin

PARAMETERS	RESULTS		Required limits
	Mean±SD	%RSD	
Retention time in minutes (R_t)	9.97±0.04	0.419	RSD≤1
Theoretical plates (N)	11194±94.9	0.85	N>2000
Tailing Factor (T)	1.312±0.01	0.50	T≤2

Fig -2: Standard chromatogram of rosuvastatin



Method validation:

Method was validated accordance to ICH guidelines (7-9), for system suitability, linearity, precision, accuracy, limit of detection, limit of quantification, robustness, specificity and solution stability.

System suitability:

For system suitability, six replicates of standard sample were injected and studied the parameters like plate number (N), tailing factor (T), resolution (R_s) and retention time (R_t), HETP, capacity factor (k^I) and peak symmetry of samples. The results are presented in **Table – I, [Fig-2].**

Linearity:

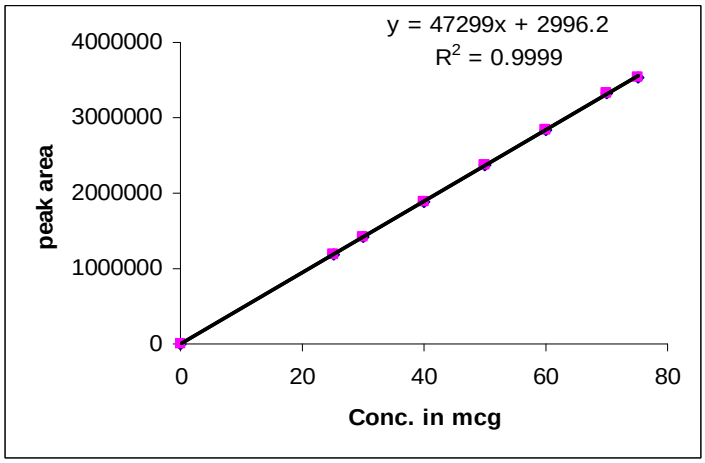
The linearity of this method was evaluated by Linear Regression Analysis, which was calculated by Least Square method and the drug was linear in the concentration range of 0.5-20 µg/ml for both the drugs. Calibration standards were prepared by spiking required volume of working standard (100µg/mL) solution into different 10 ml volumetric flasks and volume made with methanol to yield concentrations of 25, 30, 40, 50, 60, 70 and 75µg/ml. A 20µl aliquot was injected in to the analytical column. The resultant peak areas of the drugs were measured. Calibration curve was plotted between peak areas of drug against concentration of the drug. These results show there was an excellent correlation between peak area and analyte concentration. The linearity results are presented in **Table – II, [Fig-3].**

Table – II : Linearity results of Rosuvastatin

Nominal Concentration (µg/mL)	AVG Peak area	Practical concentration (µg/mL)	Accuracy (%)
25	1186360	25.01	100.08
30	1421245	29.98	99.96
40	1895067	40.01	100.03
50	2369727	50.05	100.10
60	2847064	60.14	100.25
70	3324742	70.25	100.36
75	3530504	74.60	99.47

Values are expressed in Mean ±SD

Fig-3: Linearity graph of rosuvastatin



Intra-day and Inter-day Precision and Accuracy:

Precision and accuracy was studied by quality control samples of standard solutions covering low, medium and high concentrations of linearity range were prepared and injected. Peak areas of three replicated injections of each concentration were measured.

Intra-day precision was studied by six replicate measurements at three concentration levels in the same day. Inter-day precision was conducted during routine operation of the system over a period of 3 consecutive days. Accuracy of the method was determined by calculating recovery

studies. Statistical evaluation revealed that relative standard deviation of the drug at different concentration levels for six injections was less than 2. The results are presented in **Table – III.**

Table – III: Accuracy and precision data of Rosuvastatin (n=6)

Spike level	µg/ml added	µg/ml found	% Recovery	%RSD
80%	40.3	40.23	102.3±0.17	0.169
100%	50.4	50.13	99.46±0.11	0.116
120%	60.5	61.2	101.16±0.15	0.150

Values are expressed in Mean ±SD

limits of Detection and Quantification:

Limit of detection was found to be 3.5 µg/ml (signal to noise ratio 3) and limit of quantification was found to be 10.5µg/ml of rosuvastatin (signal to noise ratio 10). The LOD and LOQ were calculated based on the standard deviation of the response and the slope.

Robustness:

Robustness of the method was done by changing slight variation in the parameters like mobile phase composition, flow rate and wavelength. Present method didn't show any significant change when the critical parameters were modified. The tailing factor for both the drugs was always less than 2.0 and the components were well separated under all the changes carried out. Considering the modifications in the system suitability parameters and the specificity of the method, as well as carrying the experiment at room temperature may conclude that the method conditions were robust.

The developed method shown from the results good correlation between the peak area and concentration of the drugs under prescribed conditions and also the recoveries were found to be 99.46% -102.32% for rosuvastatin. This indicates that commonly used excipients in pharmaceutical formulation were not interfering in the proposed method. The differences of less than 2.0 % for both intra- and inter-day data reflect the precision of the method. The observation of % RSD less than 2.0 for both intra- and inter-day measurements also indicates high degree of precision. In the present method, a Luna C18 column has been used and the buffer pH in the mobile is 4.5, which is within the limits (pH 2-8) specified by the manufacturers. a linearity range of 0.5-20 µg /ml; this linearity range covers all the strengths of rosuvastatin . Hence this method can be applied for quantifying the low levels of rosuvastatin in bulk and pharmaceutical dosage forms.

CONCLUSION

It can be seen from the results and discussion presented above; the proposed method has good sensitivity, and is Specific, Precise and Robust. Results of the analysis of pharmaceutical formulations reveal that the proposed method is suitable for their simultaneous determination with virtually no interference of usual additive present in pharmaceutical formulations. The proposed method is simple, sensitive, reliable, and can be used for the determination of rosuvastatin in pharmaceutical formulation and in pure drug.

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