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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF ROSUVASTATIN CALCIUM IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A validated simple, sensitive, specific and precise RP-HPLC method was developed for the determination of Rosuvastatin in pure and pharmaceutical formulations. Method was carried on Symmetry C18 column (100 X 4.6 mm i.d., particle size 3.5um,ACE) using acetontrile: phosphate buffer as mobile phase in the ratio of 60:40v/v/. The detection was carried out at 243nm using Waters (2659) UV-Visible detector.The proposed method obeyed linearity in the range of 20-60 µg/mL and met all specifications as per ICH guidelines. Statistical analysis revealed that this method can be used in routine quality control studies of Rosuvastatin in pure and its pharmaceutical formulations.

Keywords: Rosuvastatin; HPLC; Formulation; Validation

INTRODUCTION

Rosuvastatin calcium (ROS) is chemically, bis[(E)-7[4-(4-fluorophenyl)-6-isopropyl- 2-[methyl (methyl-sulphonyl)amino]pyrimidin-5-yl](3R,5S)-3,5-dihydroxyhept-6-enoic acid] calcium salt . It belongs to a class of drugs called statins, which are employed to lower hypercholesterolemia and related conditions and to prevent cardiovascular diseases. It increases the number of hepatic low-density lipoprotein receptors involved in the catabolism of LDL and also inhibits hepatic synthesis of very low-density lipoprotein [1-3].It is indicated for the treatment of hypercholesterolemia and mixed dyslipidemia[4-5].New tablet formulation in combination of Rosuvastatin 10mg commercially available in market (ROSVAS10) for the treatment of mixed Dyslipidemia, Hypercholesterolemia and hypertension. Literature survey revealed that few analytical methods such as UV[6-7],HPLC[8-12] methods have been reported. Hence a new sensitive and efficient HPLC method was developed and validated as per ICH guidelines for the assay of the drug Rosuvastatin calcium in tablet formulations**.** The proposed method was developed,optimized,and validated according to International conference on Hormonization(ICH) guidelines [13].

EXPERIMENTAL

Instrumentation: To develop a high pressure liquid chromatographic method for quantitative estimation of Rosuvastatin using Waters HPLC system on Symmetry C18 column column (150 mm x 4.6 mm, 5µ) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC 7000 UV detector. A 10 µL Rheodyne injector port was used for injecting the samples. Data was analyzed by using Empower 2 software.

Chemicals and solvents: Rosuvastatin was provided as gift sample by Spectrum Labs, Hyderabad, India. All the chemicals potassium dihydrogen phosphate,

orthophosphoric acid and triethylamine were of AR grade and acetonitrile of HPLC grade were purchased from Merck Specialities Pvt. Ltd., Mumbai, India. Commercial tablets of Rosuvastatin were purchased from local market. HPLC grade water obtained from Milli-Q water purification system was used throughout the study.

Preparation of phosphate buffer pH 3.8: 2.72 grams of potassium dihydrogen phosphate was accurately weighed into 1000 mL volumetric flask, added about 900 mL of Milli-Q water and sonicated to dissolve and make up to the final volume with Milli-Q water. 1 mL of triethylamine was added and then pH is adjusted to 3.8±0.5 with orthophosphoric acid solution.

Preparation of the mobile phase and diluent: 350 mL of phosphate buffer was mixed with 650 mL of acetonitrile was used as mobile phase. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µm filter under vacuum. The mobile phase was used as diluent.

Preparation of standard drug solution: 100 mg of Rosuvastatin was accurately weighed, transferred to 100 mL volumetric falsk and is dissolved in 70 mL of the mobile phase. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.25 µm filter and the volume is made up to 100 mL with mobile phase to get a concentration of 1 mg/mL (free base) stock solution. This solution is further diluted with same solvent to obtain required working standard concentrations.

Preparation of sample solution: 20 commercial tablets of Rosuvastatin were finely powdered and the powder equivalent to 100 mg of Rosuvastatin was accurately weighed and transferred to 100 mL volumetric flask and dissolved in 70 mL of mobile phase. The above solution was subjected to sonication for 15 min. After getting clear solution it is filtered through 0.25 µm filter and the solution is made up to 100 mL with mobile phase resulting in preparation of 1 mg/mL solution. This is further diluted so as to obtain required concentration of Rosuvastatin in pharmaceutical dosage form.

Methodology: The HPLC system was stabilized for thirty minutes by passing mobile phase, detector was set at 243 nm, flow rate of 0.7 mL/min to get a stable base line. One blank followed by six replicates of a single standard solution was injected to check the system suitability. Six replicates of each standard solutions 20, 30, 40, 50 and 60 µg/mL were injected. Calibration graph was plotted by concentration of Rosuvastatin on X-axis and peak area on Y-axis and linearity curve was shown in Figure 2. The amount of drug present in sample was computed in calibration graph. Chromatographic conditions for estimation of Rosuvastatin were described in Table 1.

Pharmaceutical formulations: Prepared dilution of pharmaceutical formulation is injected and the procedure described under bulk samples was followed. The amount of drug present in sample was computed in calibration graph. The assay results in commercial formulations of Rosuvastatin were described in Table 2.

RESULTS AND DISCUSSION

The objective of the present work is to develop simple, precise and reliable HPLC method for the analysis of Rosuvastatin in bulk and pharmaceutical dosage form. This is achieved by using the most commonly employed column Symmetry C18 column detection at 243nm. The representative chromatogram indicating Rosuvastatin is shown in Figure 3.

Parameter fixation: In developing this method, a systemic study of effects of various parameters was under taken by varying one parameter at a time and controlling all other parameters. The following studies were conducted for this purpose.

Stationary phase characteristics: Based on nature and solubility characteristics of, reverse phase mode of HPLC was selected Rosuvastatin for chromatography. Among different RP-HPLC stationary phases tried Symmetry C18 column was found to be optimum.

Mobile phase characteristics: In order to get sharp peak with base line separation from interfering peaks carried out a number of experiments by varying the composition of solvents and mobile phase flow rate. To have an ideal separation of the drug under isocratic conditions, mixtures of solvents like acetonitrile, methanol with different buffers in different combinations were tested as mobile phase. A mixture of acetonitrile:phosphate buffer in the ratio 60:40 v/v was proved to be the most suitable of all the combinations, since the chromatographic peak obtained was better defined and resolved and almost free from tailing.

Linearity: The linearity range was found in the range of 20-60 µg/mL. The response for the drug was linear and the regression equation was found to be

y=74000x--10164 and correlation coefficient was found to be 0.999 and the results are given in Table 3.
Precision: Precision is the degree of repeatability of an analytical method under normal operational conditions. Precision of the method was performed as intra-day precision and inter-day precision.

Intra-day precision: To study the intra-day precision, six replicate standard solutions (100 ppm) of Rosuvastatin were injected. The percent relative standard deviation (%RSD) was calculated and it was found to be 0.35which are well within the acceptable criteria of not more than 2.0.

Inter-day precision: To study the inter-day precision, six replicate standard solutions (100 ppm) of Rosuvastatin were injected. The percent relative standard deviation (%RSD) was calculated and it was found to be 0.71 which are well within the acceptable criteria of not more than 2.0.

Specificity: The effect of wide range of excipients and other additives usually present in the formulation of Rosuvastatin in the determinations under optimum conditions were investigated. Chromatographic parameters maintained are specific for Rosuvastatin .

Ruggedness: The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC, Agilent HPLC and Water's Breeze HPLC by different operators using different columns of similar type like XDB C_{18}, Hibar C_{18}, Kromasil C_{18} and Symmetry C18 didn't show any significant change.

Limit of detection and limit of quantification: A calibration curve was prepared using concentrations in the range of 20-60 µg/mL (expected detection limit range). The standard deviation of Y-intercepts of regression line was determined. The LOD and LOQ of Rosuvastatin was 2.95 and10 µg/mL, respectively.

Accuracy: The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed standard solution. The standard addition method was performed at 50%, 100% and 150% level of 100 ppm. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and %RSD was calculated and results are presented in Table 4. Satisfactory recoveries ranging from 98% to 102% were obtained by the proposed method. This indicates that the proposed method was accurate.

Robustness: Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

System suitability: A system suitability test was performed to evaluate the chromatographic parameters (number of theoretical plates, tailing of the peak) before the validation runs. The results of system suitability parameters were given in Table 5. The analytical method validation was carried out as per ICH method validation guidelines [17].

CONCLUSION

The proposed HPLC method is rapid, sensitive, precise and accurate for the estimation of Rosuvastatin and can be reliably adopted for routine quality control analysis of Rosuvastatin in its tablet dosage forms.

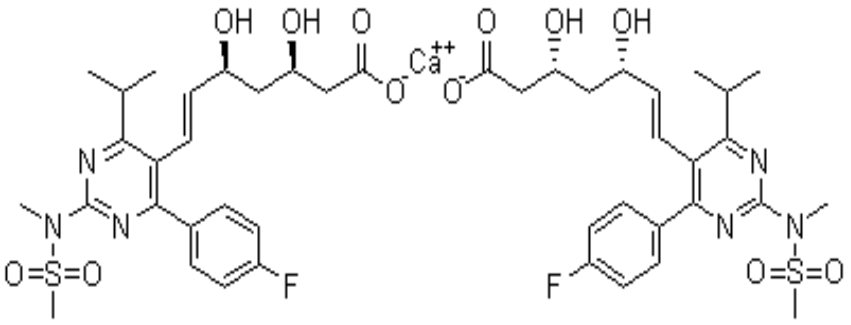

Figure 1: Chemical structure of Rosuvastatin Calcium.

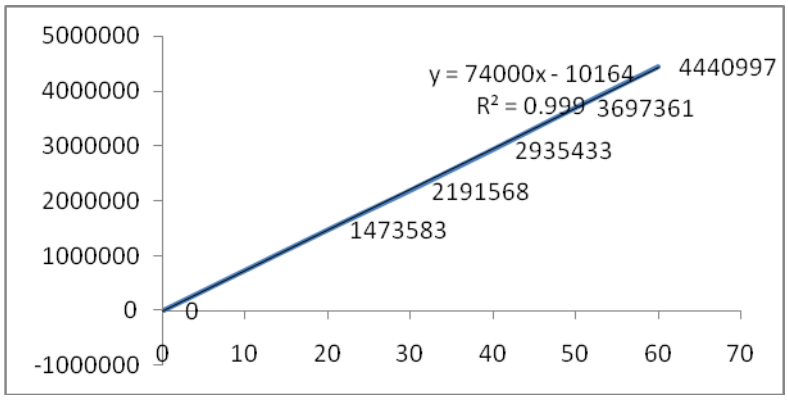

Figure 2: Linearity curve of Rosuvastatin Calcium

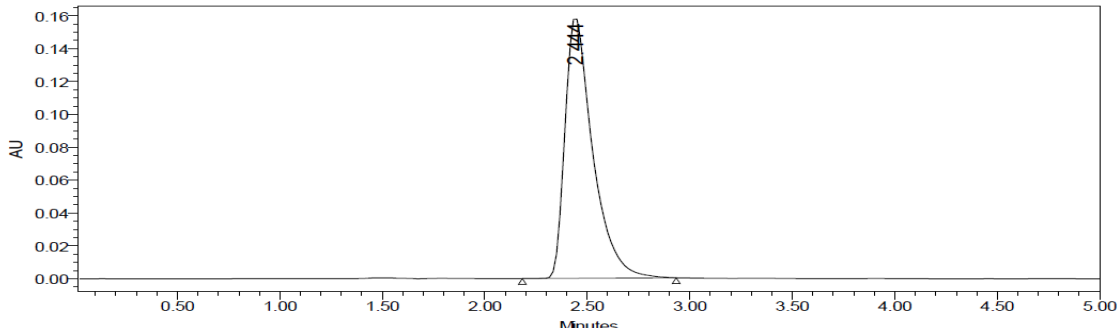

Figure 3: Typical chromatogram of Rosuvastatin calcium

Table 1: Optimized chromatographic conditions of Rosuvastatin calcium

Parameter	Condition
Mobile phase	Acetonitrile : Phosphate buffer (60:40 v/v)
pH	3.8
Diluent	Acetonitrile
Column	Symmetry C_{18} column (150 mm x4.6 mm, 3.5µm)
Column temperature	30°C
Wave length	243 nm
Injection volume	10 µL
Flow rate	0.7 mL/min
Run time	5 min
Retention time	2.444 min

Table 2: Assay results of Rosuvastatin calcium

Formulation	Label claim	Amount found	%Assay
ROSVAS	10 mg	9.85 mg	98.5 %

Table 3: Linearity results of Rosuvastatin calcium

Concentration (µg/mL)	Area
20	1473583
30	2191568
40	2935461
50	3697361
60	4440997

Table 4: Recovery results of Rosuvastatin calcium

Level	Concentration (µg/mL)	Concentration added (µg/mL)	Concentration recovered (µg/mL)	% Recovery
50%	10	5	4.96	99.3
100%	10	10	9.89	98.9
150%	10	15	14.89	99.2

Table 5: Validation parameters of Rosuvastatin calcium

System suitability	Results
Linearity range (µg/mL)	20-60
Correlation coefficient	0.999
Theoretical plates (N)	2250.6
Tailing factor	1.3
LOD (µg/mL)	2.95
LOQ (µg/mL)	10

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