Int. J. Chem. Sci.: 8(2), 2010, 1308-1314

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

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ABSTRACT

A simple, rapid, sensitive, reproducible and precise high performance liquid chromatographic method has been developed for the estimation of rosuvastatin in pure form as well as in pharmaceutical dosage form. In this method, RP-C_{18} column (100 mm x 4.6 mm I.D., 3 µm particle size) with mobile phase consisting of 0.02M phosphate buffer pH 6.8 and acetonitrile in the ratio of 60 : 40 v/v in isocratic mode was used. The detection wavelength is 242 nm and the flow rate 0.6 mL/min. The linearity was found in the range of 20-100 µg/mL and shows a correlation coefficient of 0.994. The retention time of the drug was 3.424. The proposed method was validated by determining sensitivity, accuracy, precision and linearity. The proposed method is simple, fast, accurate, precise and reproducible and hence, it can be applied for routine quality control analysis of rosuvastatin in pure and tablet dosage form.

Key words: Rosuvastatin, HPLC, Validation.

INTRODUCTION

Rosuvastatin[1] is a synthetic lipid lowering agent that blocks the production of cholesterol in the body. It is a competitive 3-hydroxy-3-methyl-glutaryl coenzyme A reductase inhibitor effective in lowering LDL cholesterol and triglycerides, developed for the treatment of dyslipidemia. Chemically[2] rosuvastatin calcium is (3R, 5S, 6E)-7-[4-(4-fluorophenyl)-6-(1-methylethyl)-2-[methyl (methylsulphonylamino)]-5-pyrimidinyl]-3, 5-dihydroxy-6-heptenoic acid calcium. Literature survey reveals that various spectrophotometric[3-6], HPLC[7,8], LC-MS[9-13], HPTLC[14] and capillary zone electrophoresis[15] methods have

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been reported for the determination of rosuvastatin in pure and pharmaceutical formulations. In this study, a simple, rapid, sensitive, accurate and precise HPLC method was developed for the estimation of rosuvastatin in tablet dosage form.

EXPERIMENTAL

Instrumentation

The separation was carried out on isocratic HPLC system (Waters) with Waters 1525 Binary HPLC pump, Waters 2487 dual absorbance detector, Waters Empower software and RP-C_{18} column (100 mm x 4.6 mm I.D., 3 μm particle size).

Chemicals and reagents

Rosuvastatin was a gift sample by Dr. Reddy's Laboratories Ltd., Hyderabad. Acetonitrile of HPLC grade were purchased from E.Merck (India) Ltd, Mumbai. Potassium dihydrogen phosphate and orthophosphoric acid of AR grade were obtained from S.D. Fine Chemicals Ltd., Mumbai.

HPLC conditions

The mobile phase consisting of 0.02 M phosphate buffer (pH 6.8 adjusted with orthophosphoric acid) and acetonitrile (HPLC grade) were filtered through 0.45 μ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 60 : 40 v/v was pumped into the column at a flow rate of 0.6 mL/min. The detection was monitored at 242 nm and the run time was 5 min. The volume of injection loop was 20 μL. Prior to injection of the drug solution, the column was equilibrated for at least 30 min. with the mobile phase flowing through the system.

Procedure

Stock solution of rosuvastatin was prepared by dissolving 25 mg of rosuvastatin calcium in 25 mL standard volumetric flask containing 25 mL of acetonitrile and the solution was sonicated for 15 min. 5 mL of the above solution was transferred to 50 mL volumetric flask and the volume was made up to the mark with mobile phase. Subsequent dilutions of this solution were made with mobile phase to get concentration of 20-100 μg/mL. The solutions were injected into the 20 μL loop and the chromatogram was recorded (Fig. 1). The calibration curve was constructed by plotting concentration of the drug against peak area ratio, which was found to be linear in the concentration range of 20-120 μg/mL. The relevant data are given in Table 1. The regression equation of this curve was computed.

This regression equation was later to estimate the amount of rosuvastation in tablet dosage forms.

Table 1: Calibration data of the method

Concentration (μg/mL)	Mean peak area (n=6)
20	1535547
40	3062594
60	4510391
80	7022270
100	8148476
120	9492092

Validation of the proposed method

The various system suitability parameters like specificity, linearity, precision, accuracy, limit of detection and limit of quantitation were studied systematically to validate the proposed HPLC method for the determination of rosuvastatin. Solution containing 100 μg/mL of rosuvastatin was subjected to the proposed HPLC analysis to check intra-day and inter-day variation of the method and the results are funished in Table 2. The accuracy of the HPLC method was assessed by adding known amounts of sample solutions of rosuvastatin at 50%, 100% and 150% of the specification prepared in triplicate to the test solutions and injected into the HPLC system as per the proposed method. The results are furnished in Table 3. The system suitability parameters are given in Table 4.

Table 2: Precision of the proposed HPLC method

Cocnentration of rosuvastatin (μg/mL)	Measured concentration of rosuvastatin (μg/mL)			
	Intra-day		Inter-day	
	Mean (n = 5)	% C.V.	Mean (n = 5)	%C.V.
100	100.41	0.215	99.97	0.276

Table 3: Accuracy studies

Concentration	Amount added (mg)	Amount found (mg)	% Recovery	% Mean recovery
50%	50	46.6	99.3	
100 %	100	100.1	100.1	100
150%	150	151.1	100.7	

Table 4: System suitability parameters

Parameter	Result
Linearity (μg/L)	20-100
Correlation coefficient	0.994
Theoretical plates (N)	4909
Tailing factor	1.4
LOD (μg/mL)	0.017
LOQ (μg/mL)	0.052
Percentage recovery	99.64

Assay

Two commercial brands of rosuvastatin calcium tablets were chosen for testing suitability of the proposed method to estimate rosuvastatin in tablet dosage form. Twenty tablets were weighed accurately and powdered. A quantity equivalent to 25 mg of rosuvastatin was weighed accurately and transferred to 25 mL volumetric flask. About 20 mL of acetonitrile was added and kept in ultrasonic bath for 15 min and the volume was made up to the mark with mobile phase, thoroughly mixed and filtered through 0.45 μ membrane filter. From the above solution, 5 mL was transferred to 100 mL volumetric flask and the volume was made up to 100 mL with mobile phase. Sample solution was injected under the chromato-graphic conditions and the chromatogram was recorded. The amount of rosuvastatin present in tablet formulation was determined by comparing the peak area from the standard. All determinations were conducted in triplicate. The results are furnished in Table 5.

Table 5: Assay and recovery studies

Formulation	Label claim (mg)	Amount found (mg)	% Amount found	% Recovery
Brand-1	10	9.85	98.5	98.74
Brand-2	10	9.92	99.2	99.36

RESULTS AND DISCUSSION

By applying the proposed method, the retention time of rosuvastatin was found to be 3.770 min (Fig. 1). Linearity range was observed in concentration range of 20-100 µg/mL.

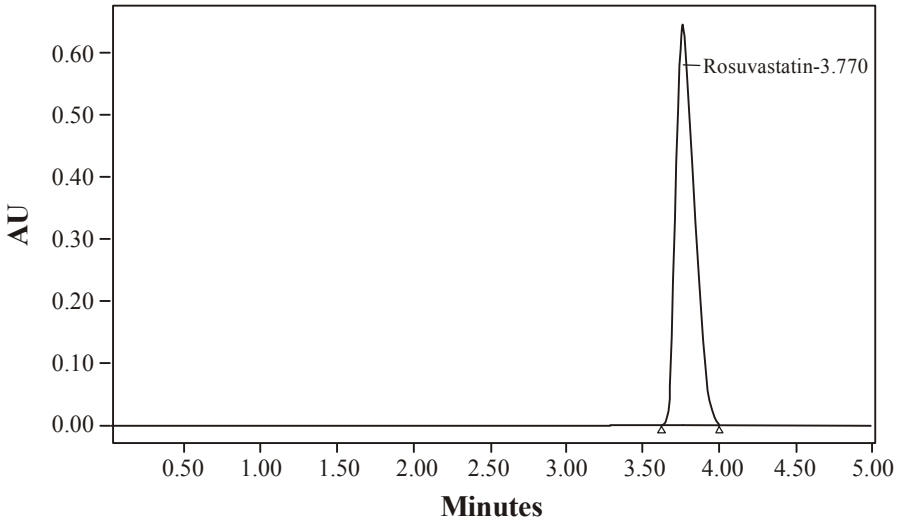

Fig. 1: Typical chromatogram of rosuvastatin

The regression equation of rosuvastatin concentration over its peak area ratio was found to be Y = -126663.333 + 82217.5X (r = 0.994) where Y is the peak area ratio and X is the concentration of rosuvastatin (µg/mL). The number of theoretical plates was found to be 4990, which indicates efficient performance of the column. The tailing factor was found to be 1.4, which indicates good shape of peak. The limit of detection and limit of quantification was found to be 0.017 µg/mL and 0.052 µg/mL, indicating the sensitivity of the method. The high percentage of recovery (99.64) indicates that the proposed method is highly accurate. The use of phosphate buffer and acetonitrile in the ratio of 60 : 40 v/v resulted in peak with good shape and resolution. No interfering peaks were found in the chromatogram within the

run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by proposed HPLC method.

CONCLUSION

The proposed HPLC method was found to be highly accurate, sensitive and precise. Therefore, this method can be applied for the routine quality control analysis of rosuvastatin in pure and its tablet dosage form.

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Accepted : 18.11.2009