Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF ROSUVASTATIN CALCIUM IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

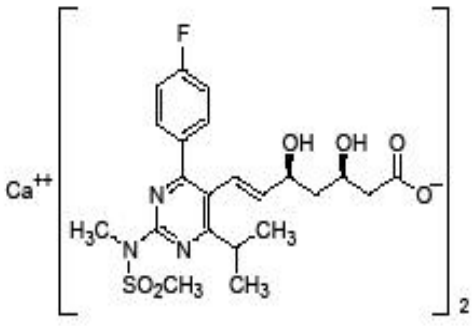
A simple, specific, accurate, and precise reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the estimation of Rosuvastatin Calcium (RC) in pharmaceutical dosage forms. A Thermo hypersil reversed phase C-18, 5 µm column having 100 x 4.6 mm i.d. in gradient mode, with mobile phase containing HPLC grade Acetonitrile : Potassium dihydrogen orthophosphate (50 : 50 v / v, pH 3) was used. The flow rate was 0.5 ml / min and effluents were monitored at 243 nm. Chromatogram showed a main peak of RC at retention time was 3.333 ± 0.004 min. The method was validated for linearity, accuracy, precision, limit of detection, limit of quantitation, robustness and ruggedness. The limit of detection and limit of quantitation for estimation of RC was found to be 0.14 µg / ml and 0.46 µg / ml, respectively. Recovery of RC was found to be in the range of 98.50-100.17 %. Proposed method was successfully applied for the quantitative determination of RC in pharmaceutical dosage forms.

Keywords: RP-HPLC, Rosuvastatin Calcium, Validation, Pharmaceutical dosage form.

INTRODUCTION

Rosuvastatin Calcium[1] is official in indian pharmacopoeia. It is chemically (E)-(3R,5S)-7-{4 - (4-fluorophenyl) - 6 - isopropyl - 2 - { methyl (methylsulphonyl amino)] pyrimidin -5-yl}-3,5-dihydroxyhepten-6-oic acid calcium (Figure 1).

Figure 1: Structure of Rosuvastatin Calcium



It is used as a lipid lowering agent act by inhibition of 3-hydroxy-3-methylglutaryl-coenzymeA (HMG-CoA) reductase. Rosuvastatin is orally administered as calcium salt. Various analytical methods have been reported for determination of RC including Spectrophotometric methods[2-4], Stability indicating method[5], HPTLC[5] and RP-HPLC[6-8]. The present paper describes a new quantitative reversed-phase high-performance liquid chromatographic method, coupled with UV detector, as an alternative technique for quality control of RC products. The purpose of this investigation was to develop and validate a method using a simple, rapid, sensitive, precise, accurate and specific reversed phase HPLC assay. The method uses a simple mobile phase composition and the rapid run time of 5 min. Hence, this method can be used for the analysis of large number of samples. This work describes the validation parameters stated by the ICH guidelines[9,10] to achieve an analytical method with acceptable characteristics of suitability, reliability and feasibility. Ensuring, in this way that the findings achieved, when this method is applied, are correct, and so the drug fulfils are the required specifications showing its quality is the right one.

MATERIALS AND METHODS

Chemicals and Reagents

An analytically pure sample of RC was procured as gift sample from Zydus Pharmaceuticals Ltd. (Ahmedabad, India). HPLC grade acetone and methanol was procured from E. Merck (Ahmedabad). Liquid chromatographic grade water was obtained by double distillation and purification through Milli-Q water purification system. Potassium dihydrogen orthophosphate (AR grade, purity 99.5%) was procured from Qualigens. Tablet formulations ROZAVEL (Sun Pharma) and ROSUVAS (Ranbaxy) were procured from a local pharmacy with labeled amount 20 mg per tablet.

Instrumentation

The HPLC system consisted of a Waters Alliance (Waters Corporation, MA, USA) equipped with a Waters 2695 solvent delivery module in a quaternary gradient mode and a Waters 2487 PDA detector. Data acquisition was performed by the EM-power 2 software.

Chromatographic Condition

Chromatographic analysis was performed on a Thermo Hypersil reversed phase C-18 column with 100 x 4.6 mm i.d. and 5 µm particle size. The mobile phase consisted of phosphate buffer: acetonitrile (50 : 50 v/v) and pH adjusted to 3.0 with ortho-phosphoric acid that was set at a flow rate of 0.5 ml/min. The mobile phase was degassed and filtered through 0.2 µm membrane filter before pumping into HPLC system. The eluent was monitored by UV detection at 243 nm.

Preparation of Solutions

Preparation of Standard Solutions

The stock solution of RC was prepared by dissolving accurately weighed quantity of 10 mg of the drug in 10 ml of methanol. From this stock solution, standard solution containing 100µg/ml RC was prepared by suitably diluting the appropriate volume of stock solution with mobile phase. Different calibration standards ranging from 5, 10, 15, 20, 25 and 30 µg/ml were prepared by appropriate dilution of standard solution (100µg/ml) with mobile phase.

Preparation of Sample Solution

Twenty tablets were accurately weighed, ground, homogenized and portion of the powder equivalent to 10 mg of the drug was weighed accurately, transferred into a 100 ml volumetric flask and diluted up to mark with methanol. This solution was sonicated for 15 min and filtered through Whatman filter paper No. 41. Further dilution was done with mobile phase to get concentration of 20 µg/ml.

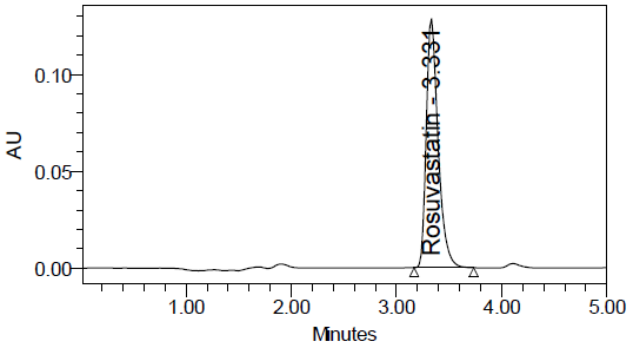
RESULTS AND DISCUSSION

HPLC has gained valuable position in the field of analysis due to ease of performance, specificity, sensitivity and the analysis of samples having complex nature. This technique was employed in the present investigation for estimation of RC in tablet dosage form.

System Suitability

System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standards solutions. The number of theoretical plates should not be less than 2500 and the tailing factor should not be more than 2.0. For all sample analyses, the tailing factor, efficiency, and %RSD were ≤ 1.33, ≥ 3950 and ≤ 0.217 % respectively. A typical chromatogram of RC is presented in Fig 2.

Fig : 2 Typical chromatogram of RC by RP - HPLC



Linearity (Calibration Curve)

To carry out this study, six levels of concentration in the range 5–30 µg/ml were prepared. Each of the levels of concentration was prepared in triplicate. Calibration curve was constructed by plotting average peak area against concentration and regression equation was computed. The results were shown in Table 1. The results show that an excellent correlation exists between peak area and concentration of drugs within the concentration range, regression graph is presented in Figure: 3.

Table 1: Linearity of RC by RP- HPLC method.

Sr. No.	Concentration (µg/ml)	RT	Area (mV)	Height (µV)
1	5	3.332	536259	73073
2	10	3.331	1000212	128237
3	15	3.337	1536487	201312
4	20	3.336	2026566	268989
5	25	3.334	2547691	338040
6	30	3.333	3078485	411458

Table 2: Sensitivity of RP-HPLC method for RC

Baseline noise* (mV)	LOD			LOQ		
	Conc. of solution (noise × 3) (µg/ml)	Signal (height) (µV)	S/N**	Conc. of solution (noise × 10) (µg/ml)	Signal (height) (µV)	S/N**
0.046	0.14	0.136	2.96	0.46	0.438	9.52

*Average of six determinations.

** S/N shall be 3 (2.8 – 3.2) for LOD and 10 (9.5 – 10.5) for LOQ.

Table 3: Recovery studies of RC by RP-HPLC method

Amount of pure RC added to placebo in µg/ml	Amount of RC found in µg/ml	% Recovery* of RC (Mean ± SD)	% RSD
10 (50%)	9.83	98.50 ± 0.01	0.106
20 (100%)	20.09	100.26 ± 0.07	0.357
30 (150%)	30.07	100.17 ± 0.06	0.209

Table 4: Intraday and interday precision studies of RC by RP-HPLC.

Sr. No.	Concentration (µg / mL)	Intraday precision (Area)	Interday precision (Area)
1	20	2037925	2067930
2	20	2036725	2067706
3	20	2033221	2070468
4	20	2029630	2073589
5	20	2029133	2068614
6	20	2030535	2066838
Mean		2032862	2069191
Std. Dev.		3753.713	2475.4
%RSD		0.184	0.119

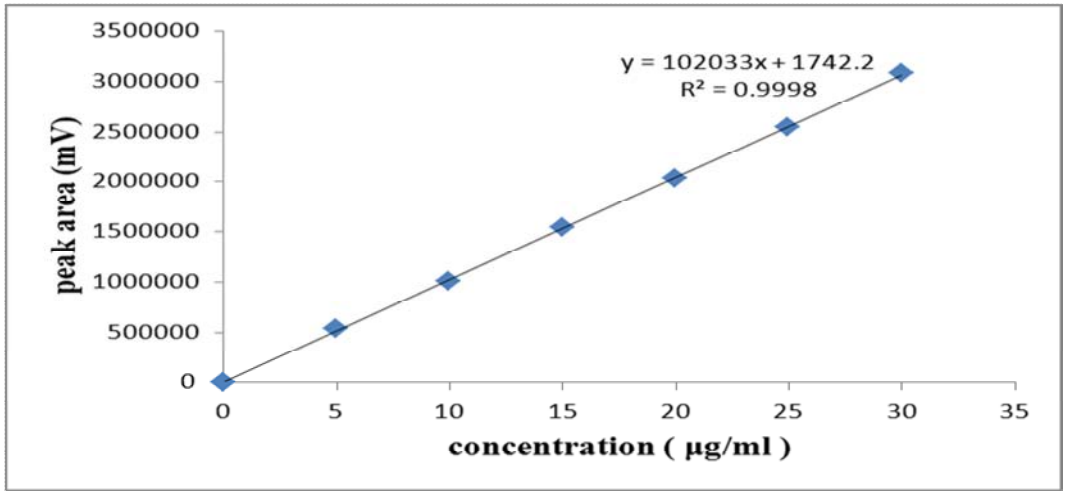
Table 5: Ruggedness studies of RC by RP-HPLC method

Sample	Label claim (mg)	Analyst I		Analyst II	
		Amount found (mg)	Recovery* (%)	Amount found (mg)	Recovery* (%)
Brand I (Rozavel)	20	19.84	99.20 ± 0.22	19.93	99.65 ± 0.34
Brand II (Rosuvas)	20	20.07	100.35 ± 0.47	19.89	99.45 ± 0.27

Table 6: Robustness studies of RC by RP-HPLC method

Condition	Modification	Mean area ± SD	RSD (%)	Mean t_R ± SD (min)
Mobile phase composition Acetonitrile : buffer (v / v)	45 : 55	2427742 ± 4298.473	0.656	4.657 ± 0.012
	50 : 50	2026566 ± 2256.892	0.200	3.353 ± 0.007
	55 : 45	1898274 ± 3567.289	0.472	3.992 ± 0.036
Mobile phase flow rate (ml / min)	0.4	2565357 ± 4207.045	0.510	4.120 ± 0.004
	0.5	2025638 ± 3804.307	0.462	4.026 ± 0.010
	0.6	1837740 ± 5524.929	0.668	3.980 ± 0.067

Figure 3: Calibration curve of RC by RP-HPLC method



Sensitivity

In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ) values, the blank sample was injected six times and the peak area of this blank was calculated as noise level. The LOD was calculated as three times the noise level while ten times the noise value gave the LOQ. Table 2 represents the sensitivity of the proposed method.

Accuracy

Accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out six times by spiked placebo recovery method and the percentage recoveries with standard deviations were calculated. From the data obtained which given in Table 3, the method was found to be sufficiently accurate.

Precision

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. The three components of precision, i.e., repeatability (Intraday Variation), intermediate precision and reproducibility (Ruggedness), in accordance with ICH recommendations, were determined as follows:

Repeatability (Intraday Variation)

Six injections of 20 µg/mL solution of RC were analyzed on the same day at different time intervals and % RSD calculated for injection repeatability.

Intermediate precision (Interday Variation)

Six injections of 20 µg/mL solution of RC were analyzed on the consecutive days and determined the intermediate precision.

Both intraday and interday precision studies are described in Table : 4

Reproducibility (Ruggedness)

The reproducibility of the method was checked by determining precision on the same instrument, but by a different analyst. Results of reproducibility are shown in Table 5.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as change in composition of mobile phase and flow rate. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust. The results are shown in Table 6.

CONCLUSION

A convenient, rapid, accurate, precise and economical RP-HPLC method has been developed for estimation of Rosuvastatin Calcium in tablet dosage form. The assay provides a linear response across a wide range of concentrations and it utilizes a mobile phase which can be easily prepared. The proposed method is simple, fast, accurate and precise for the simultaneous quantification of Rosuvastatin Calcium in dosage form, bulk drugs as well as for routine analysis in quality control. The proposed method can be used for the routine analysis of Rosuvastatin Calcium in bulk preparations of the drug and in pharmaceutical dosage forms without interference of excipient.

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