ISSN 0975-6299

Vol 2/Issue 2/Apr-Jun 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

ANALYTICAL CHEMISTRY

DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR ESTIMATION OF ROSUVASTATIN CALCIUM IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, specific, accurate and precise high performance thin layer chromatography (HPTLC) method has been developed for determination of Rosuvastatin Calcium (RC) in bulk and pharmaceutical dosage forms. The method uses aluminium plates coated with silica gel 60 F_{254} as stationary phase and Ethyl Acetate : Toluene : Methanol (6 : 2 : 2, v/v/v) as mobile phase. Densitometric evaluation of the separated bands was performed at 254 nm using Camag TLC Scanner-3 with win CAT 1.4.4 software. The R_F value of Rosuvastatin Calcium (RC) was 0.32 ± 0.05. The validated calibration range was 500-2500 ng per spot (r^2 = 0.9996). Results of analysis were validated statistically and by recovery studies. The method was validated according to the ICH guidelines with respect to linearity, accuracy, precision and robustness. Thus the proposed method can be used successfully for routine analysis of Rosuvastatin Calcium (RC) from tablet formulations.

KEY WORDS

Rosuvastatin Calcium (RC), HPTLC, Validation, Pharmaceutical formulation.

INTRODUCTION

Rosuvastatin Calcium (RC) [1] is a member of statins, used to treat high cholesterol and related conditions, and to prevent cardiovascular disease. Chemical name of Rosuvastatin Calcium is (3R,5S,6E)-7-[4-(4-fluorophenyl)-2-(N-methylmethanesulfonamido)-6-(propan-2yl)pyrimidin-5-yl]-3,5dihydroxyhept-6-enoicacid. It is having a molecular formula of $C_{22}H_{28}FN_3O_6S$ and its molecular weight is 481.539 g/mol. Its molecular structure;

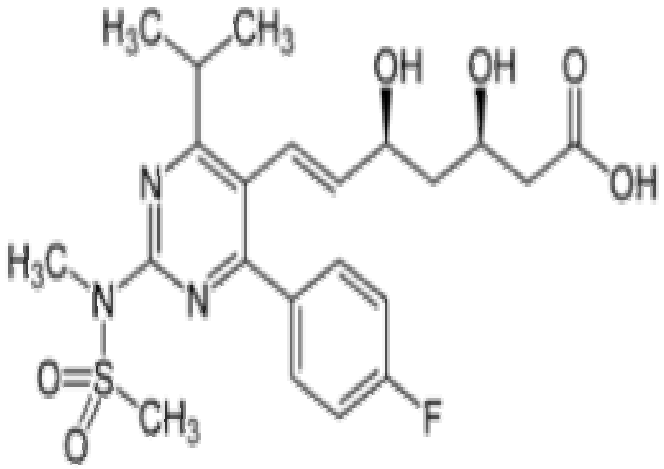

Fig: 1
Structure of Rosuvastatin Calcium

Literature survey reveals that several analytical methods have been reported for the estimation of Rosuvastatin Calcium by HPLC[5] and UV[2,3,4,6,7] methods. No validated HPTLC methods for quantitative determination of Rosuvastatin Calcium in bulk drug samples and formulations were reported till date. The aim of this study was to develop a HPTLC method, which could be employed for the routine analysis of the drug in pharmaceutical dosage forms using simple mobile phase composition.

Experimental:

Reagent and Pharmaceutical preparations:

An analytically pure sample of Rosuvastatin Calcium was procured as gift sample from Cipla Health Care, Ahmadabad (India). All chemicals including Ethyl Acetate, Toluene and Methanol were of A.R.grade from S.D.Fine-chem, Merck, Fischer scientific, and Spectrochem, Mumbai. Tablets of 2 mg were procured from local pharmacy.

4.2 Preparation of Solutions

4.2.1 Preparation of standard stock solution:

The standard Rosuvastatin Calcium 100 mg was weighed accurately and transferred to volumetric flask 100 ml. It was dissolved for sonication for few minutes and diluted up to the mark with methanol to obtain final concentration of 1000 µg/ml and the resulting solution was used as working standard solution.

4.2.2 Preparation of sample solution:

For the estimation of Rosuvastatin Calcium in tablets formulations by this method. 20 branded tablets were weighed and triturated to fine powder. Tablet powder equivalent to 10 mg of Rosuvastatin Calcium was weighed and transferred to 100 ml volumetric flask than dissolved with methanol and further diluted with methanol. It was kept for ultrasonication for few min; the solution was then filtered through Whatman filter paper No. 41 and further dilution was made with methanol to get the final stock solution of 100 mcg/ml.

4.3 Chromatography:

Analysis was performed on 10 cm × 10 cm HPTLC silica gel 60 F_{254} plates (Merck, Darmstadt, Germany) with concentrating zone. The TLC plates were pre-washed with methanol. Activation was done in an oven at 50°C for 5 min. Sample and standard zones were applied to the layer as bands by means of a Camag (Muttenz Switzerland) Linomat V automated spray-on applicator equipped with a 100-µL syringe(Hamilton, Reno, Nevada, USA). The slit dimensions 6 mm × 0.45 mm and scanning speed of 20 mm/sec was employed. Ascending development of the plate, migration distance 60 mm, was performed at 25 ± 2º C with Ethyl Acetate : Toluene : Methanol (6 : 2 : 2, v/v/v) , as mobile phase in a Camag twin trough chamber previously saturated for 30 min. The average development time was 30 min. After development the plate was dried at 50°C in an oven for 5 min. Densitometric scanning at λ = 243 nm was then performed with a Camag TLC Scanner-3 equipped with Win CAT 1.4.4 software, using a deuterium light source.

RESULTS AND DISCUSSION

Literature survey revealed that few HPLC and UV methods have been reported for estimation of RC which are sophisticated, but costly and time consuming. The present study was aimed at development of speedy and cost effective HPTLC technique for determination of RC as bulk and in dosage forms.

Various blends of solvent systems in varying proportions were tried as mobile phase. However, mobile phase consisting of Ethyl Acetate : Toluene : Methanol in the ratio of 6 : 2 : 2 (v/v/v) was found to be more suitable with R_F values of 0.32, with saturation time of 10 minutes. The selection of wave length was based on maximum absorbance for optimum sensitivity. The drug showed good linearity in the range of 500-2500 ng per spot with coefficient of correlation value 0.9996 for peak area. From the recovery studies, the accuracy results were within the range of 98.48 - 99.64 % and were found to be highly accurate. Ruggedness and robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters (% RSD < 2).

Validation of the method:

Linearity:

Appropriate volume of aliquots from standard Rosuvastatin Calcium stock solutions were prepared and applied on the TLC plate in the range of 1.2-2.4 µL to give a series of spots covering the range from 500 to 2500 ng/spot with the help of micro liter syringe using an automatic sample applicator. The plates were developed, dried and scanned densitometrically at 243 nm. The drug peak-area was calculated for each concentration level and a graph was plotted of drug concentration against the peak area and shown in (Fig.2). Calibration parameters are given in table: 1.

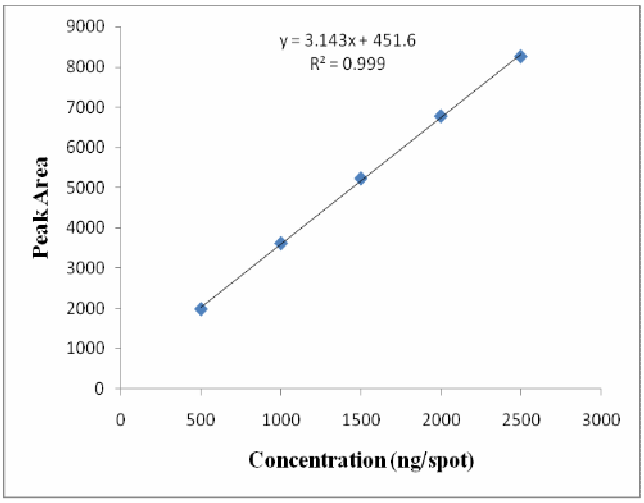

Fig. 2
Calibration curve of RC at 243 nm

Table: 1
Calibration parameters for RC

Parameters	Results
Linearity Range	500-2500 ng/spot
Correlation co-efficient	0.9996
Slope	3.1437
Intercept	451.69

Precision:
The precision expressed as standard deviation or relative standard deviation.

System precision: Standard solution of RC was prepared as per testing procedure and injected into the HPTLC system in six replicates. The values of % relative standard deviation (RSD) for peak area obtained in six replicate injections were reported in Table: 2.

Table: 2
System Precision results for RC by HPTLC.

Sr. No.	Concentration (ng/spot)	Intraday precision (Area)	Interday precision (Area)
1	1500	5513.1	5423.5
2	1500	5498.4	5476.7
3	1500	5444.3	5521
4	1500	5398	5371
5	1500	5381.8	5457.2
6	1500	5455.8	5475.32
Mean		5448.58	5454.12
Std. Dev.		56.48	57.91
%RSD		1.036	1.061

Method precision: The six different determinations of RC sample (from tablets) were performed as per testing procedure. % RSD was calculated and which are shown in Table: 3.

Table:3
Method Precision results for RC by HPTLC.

Sample	Label claim (mg)	Intra-day			Inter day		
		Amount found*	% Label claim*	% RSD	Amount found*	% Label claim*	% RSD
Tab 1	20	19.76	98.80	0.901	19.85	99.28	0.707

Accuracy:
To check the accuracy of the method, recovery studies were conducted after addition of standard drug solution at three different levels i.e. 50 %, 100 %, and 150 % to pre-analyzed sample solution. The results are given in table: 4

Table: 4
Accuracy results of RC by HPTLC.

Sample	Label claim (mg)	Initial amount (ng/spot)	Amount added (ng/spot)	Amount recovered * (ng/spot)	Recovery ± SD* (%)	% RSD
Tab 1	20	1000	0	991.18	99.11 ± 1.31	1.331
		1000	800	1793.62	99.64 ± 1.33	1.335
		1000	1000	1969.91	98.49 ± 1.06	1.082
		1000	1200	2182.14	99.18 ± 0.28	0.282

Specificity:
To confirm the specificity of the proposed method, the solution of the formulation was spotted on the TLC plate, developed and scanned. It was observed that the excipients present in the formulation did not interfere with the peak of standard Rosuvastatin Calcium as shown in (Fig.3). The R_F value of drug was 0.32.

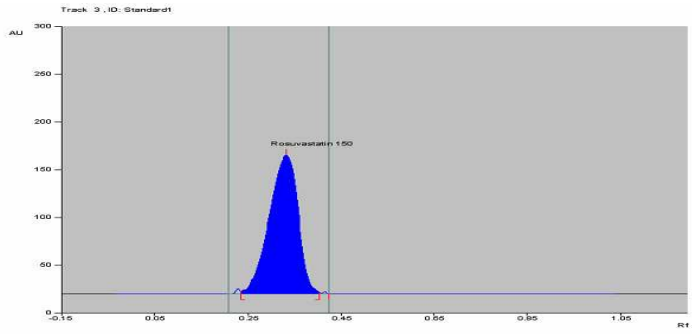

Fig. 3
Typical HPTLC chromatogram of standard solution of RC

Ruggedness and Robustness:

Ruggedness was carried out under the different condition like different days and different analyst. The ruggedness results were shown in Table: 5.

Table:5

Ruggedness studies of RC by HPTLC method.

Sample	Label claim (mg)	Analyst I		Analyst II	
		Amount found* (mg)	Recovery ± SD* (%)	Amount found* (mg)	Recovery ± SD* (%)
Tab 1	20	19.76	98.80 ± 0.89	20.05	100.24 ± 0.59

Table:6

Robustness studies of RC by HPTLC method.

Development distance (cm)	RC assay (%)
	Tab 1
6.0	98.80
6.5	98.35
7.0	101.46

CONCLUSION

The validated HPTLC method proved to be simple, less expensive, fast, accurate, precise and ruggedness and thus can be used for routine analysis of Rosuvastatin Calcium in bulk and pharmaceutical dosage forms.

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