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A Validated Reversed-Phase HPLC Analytical Method for the Analysis of Rosuvastatin Calcium in Bulk Drug and Tablet Dosage Formulation

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Authors' contributions

This work was carried out in collaboration between both authors. Author RK developed the idea of research and supervised the project and helped in the interpretation of data. Author AP has performed all the experiments and was responsible for data acquisition. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The current research work has desired the validated reversed-phase analytical technique for the assurance of rosuvastatin calcium in bulk and tablet formulation.
Study design: Experimental research work.
Place and duration of study: UDPS, RTM Nagpur University, Nagpur, Maharashtra State, India between June 2019 and March 2020.
Methodology: The segregation was obtained on a reversed-phase Princeton (C18) column with dimensions (250mm × 4.6mm, 5µ). The solvent system employed was a mixture of buffer, and methanol in the proportion (20:80) v/v, flow rate one ml per minute. Detection wavelength at 240 nm. The retention time (RT)beneath the developed chromatographic condition was found to be 2.848 minutes for rosuvastatin calcium.
Results: The technique indicates linearity within the range of 2-16 µg per ml with a correlation

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coefficient (r^2) is 0.9999. The analysis of marketed tablet formulations was erect to be 99.98%. The percentage RSD was <2% and % recovery was found to be 97.94-100.37%.
Conclusion: The advanced reversed-phase HPLC technique was erect to be simple, specific, linear, sensitive, rapid, accurate, precise, economical, and can be utilized for daily quality control of rosuvastatin calcium in tablet and bulk formulations.

Keywords: Rosuvastatin calcium; validation; RP-HPLC Relative Standard Deviation (RSD).

1. INTRODUCTION

Rosuvastatin calcium, chemically (3R,5R)-7-[4-(4-Fluorophenyl)-2-[methyl(methylsulfonyl)amino]-6-propan-2-ylpyrimidin-5-yl]-3,5-dihydroxyhept-6enoic acid (Fig. 1). It is used as an antilipidemic agent in the dealing of abnormal lipid, hypertension [1,2]. The literature review revealed that several high-performance liquid chromatography techniques were notified for the evaluation of rosuvastatin calcium in tablet and bulk formulations. [3-14]. This paper reports an approved Reversed Phase-HPLC technique for the determination of rosuvastatin calcium. The suggested technique is very simple, rapid& suitable for the routine evaluation of rosuvastatin calcium in tablet and bulk formulations. As per ICH guidelines (ICH, Q2A; ICH, Q2B), the method was developed.

2. MATERIALS AND METHODS

2.1 Chemical Requirements

Pharmaceutical analytical grade Rosuvastatin calcium was buying up as an offering sample from Cadila Pharmaceutical Pvt. Ltd., Ahmedabad, Gujrat. Rosmi 10 a marketed formulation, was procured from a pharmacy shop.

All other chemical reagents like methanol and o-phosphoric acid of analytical quality were bought up from Ranchem, India.

2.2 Instrumentation

Reversed-phase HPLC, Shimadzu LC-6AD system supplied along with a PDA detector. Lab Solution software was used for Data processing in RP-HPLC.

2.3 Optimized Chromatographic Conditions

The developed chromatographic partition was attained by reversed-phase Princeton (C18) column along with dimensions at a temperature applying a solvent system subsist of a mixture of buffer, and methanol in the proportion of (20:80) v/v, at a wavelength 240 nm and flow rate 1.0 milliliter per minute. The pH of the mobile phase was maintained at 3.0, Injection volume of about 10.0µl. All developed various chromatographic conditions are presented below (Table 1).

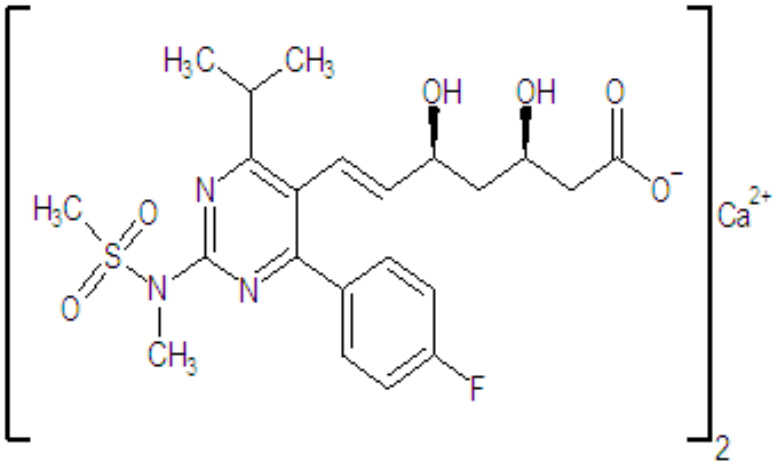

Fig. 1. Chemical structure of rosuvastatin calcium

Table 1. Developed chromatographic condition

Chromatographic condition	
Mobile phase	Water (pH 3 along with ortho-phosphoric acid: Methanol) (20:80) v/v
Flow rate	1ml per min.
Column	Princeton C -18 (Dimensions - 250mm × 4.6mm, 5µ)
Detector wavelength	240 nm
Column temp	30°C
Injection volume	10 µl
Runtime	20 minutes
Diluent	Methanol
Retention time	2.848 minutes

2.4 Preparation of standard solution of Rosuvastatin Calcium

Precisely weighed 1.0 mg of rosuvastatin calcium was conveyed to a 10.0-milliliter volumetric flask and soften in 5-milliliter of diluent. The quantity was made up to 10.0-milliliter with diluent. 1.0milliliter, the resulting solution was pipetted in a 10.0-milliliter volumetric flask and diluted to 10.0-milliliter with diluent to add a solution of concentration 10.0µg per milliliter of rosuvastatin calcium.

2.5 Preparation of sample solution of Rosuvastatin Calcium

20 tablets were weighed and finely powdered and a precisely weighed quantity of powder equal to 1.0 mg of rosuvastatin calcium was conveyed into a 10.0-milliliter volumetric flask. The powder was added with 5-milliliter of methanol. The solution was diluted up to the mark with diluent and filtered over filter paper (Whatman Grade-I). One milliliter of the filtrate was conveyed to a 10-milliliter volumetric flask and the quantity was made up to the mark with diluent to provide a sample solution consist of 10.0µg per milliliter of rosuvastatin calcium. Six replicate tablet solutions consist of 10.0µg per milliliter of rosuvastatin calcium solutions were prepared similarly.

2.6 Assay Procedure

After equilibration of stationary phase, 3 replicate injections of each of sample solutions were made solely and chromatograms were reported. By using peak area of a quantity of drug present in the average weight of tablet as percent labeled claim was calculated by using formula given below.

$$\%\ Assay = \frac{A_{sam} \times C_{std} \times \mathrm{DF} \times \mathrm{Avg.\,Wt.}}{A_{std} \times \mathrm{Wt.\,taken} \times \mathrm{LC}}$$

Where,

A_{sam} = Area of Sample taken
A_{std} = Area of Standard taken
C_{std} = Concentration of standard, µg/ml
DF = Dilution Factor
Avg. Wt. = Average weight of tablets
Wt. taken = Weight of tablet powder taken
LC = Labeled Claim

2.7 The Method Validation

The developed and validated technique following ICH guidelines for linearity, specificity, accuracy, precision, the limit of quantification (LOQ), the limit of detection (LOD), and robustness [15].

2.8 Accuracy

The analytical accuracy operation suggests the adjacency of covenant midway the value, which is confirmed either as an ideal correct value or a received mention value. It was computed at 3 different levels (80%, 100%, and 120%) of the label claim.

2.9 Precision

The measurement of the precision an area of 6qualified working standards for rosuvastatin calcium calculating the % of relative standard deviation (RSD). The assay technique precision was estimated by operating six independent assays of test samples of rosuvastatin calcium across qualified working standards and considering the %RSD. The intermediate precision of the technique was also proved using different analysts and different days.

2.10 Linearity

Linearity test solutions of rosuvastatin calcium were arranged by diluting the stock solution at concentration levels of 2-16 µg/ml. Linearity was settled by the least-squares linear regression analysis obtained. Peak areas versus linear regression analysis and corresponding concentrations were achieved on the resulting curves. The linear curve of rosuvastatin calcium was shown in Fig. 2.

2.11 Specificity

The developed technique was specified by correlating the chromatograph of the standard and sample solution.

2.12 LOD and LOQ

The limits of detection (LOD) are the lowest analyte concentration that can be noticed and limits of quantification (LOQ) is the lowest analyte concentration that can be evaluated with adequate precision and accuracy. LOQ and LOD were settled, under ICH guidelines, by the purpose of the equations Limit of Detection = 3.3σ/S and Limit of Quantification = 10σ/S, where σ is the standard deviation of the regression line, and S is the slope of the calibration plot.

2.13 Robustness

To specify the robustness study of the validated chromatographic technique, the chromatographic conditions were consciously variation and the resolution for rosuvastatin calcium was estimated. To survey the outcome of wavelength on the assessment, and the wavelength variation by ± 2 nm, i.e., 238 and 242 nm from the original wavelength, 240 nm. To survey the outcome of flow rate on the assessment, the flow rate was varied by ± 0.1 millimeters per minute i.e., 0.9 and 1.1 millimeters per minute from the certain flow rate, 1.0 millimeters per minute.

2.14 Stability of solution

The stability of the sample was noticed for 24 hours. % relative standard deviation of 0.9 indicates the stability of the technique for 24 hours. Thus, the technique was found to be specific.

3. RESULTS AND DISCUSSION

The work re-presents, the HPLC technique was utilized for the evaluation of degraded products which was a method developed and validated. Initially, the pure drugs sample solution was chromatographed solvent system containing a mixture of buffer and methanol in a proportion of (20:80) v/v, the flow rate of 1.0 millimeters per minute. shows well-resolved peaks of drugs. Detection was done at 240 nm. Beneath the developed chromatographic condition of rosuvastatin calcium, retention time was 2.848 minutes. The entire run time of the chromatogram was around 20.0 minutes. chromatograph of the standard solution and sample solution of rosuvastatin calcium is presented in Fig. 3 and Fig. 4 respectively.

3.1 Method Validation

3.1.1 System suitability

The system suitability of the validated technique was well-settled by injecting 6imitate injections of the standard solution. The tailing factor of rosuvastatin calcium was 1.62, theoretical plates were erect to be 2866, and %RSD of peak area was found to be 0.9 for rosuvastatin calcium (Table 2). All specifications are within the limits, illustrate applicable for operating the analysis.

3.2 Linearity

Linearity was settled by the least-squares linear regression analysis. Calibration plots within the range of 2-16 µg per millimeter for rosuvastatin calcium. To obtain the resulting curve areas across the linear regression analysis and corresponding concentrations. The calibration plot equation of rosuvastatin calcium was Y=47945x+1511.5, the correlation coefficient is0.9999(Table 3).

3.3 Accuracy

The percentage of recoveries was found to be 97.94-100.37% for rosuvastatin calcium. The % relative standard deviation value well within the limit (Table 4).

3.4 Precision

The intraday and interday precision of the proposed technique was determined by analyzing the standard solution of rosuvastatin calcium at concentration three times on the same day and three different days. The outcome is

notified in terms of RSD. The % relative standard deviation values for rosuvastatin calcium w within the limit ± 2.0%, express that the technique was precise. The result of intraday and interday for rosuvastatin calcium was 0.8 and 0.9 respectively.

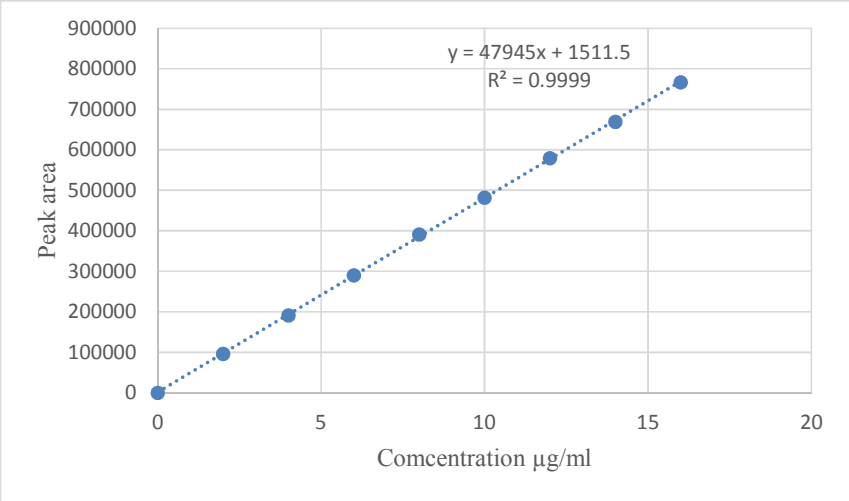

Fig. 2. Linear curve of rosuvastatin calcium

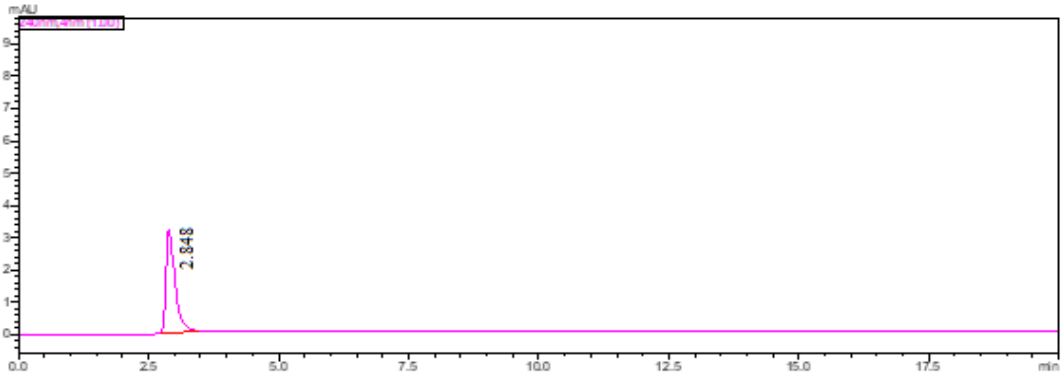

Fig. 3. A typical chromatograph of standard rosuvastatin calcium

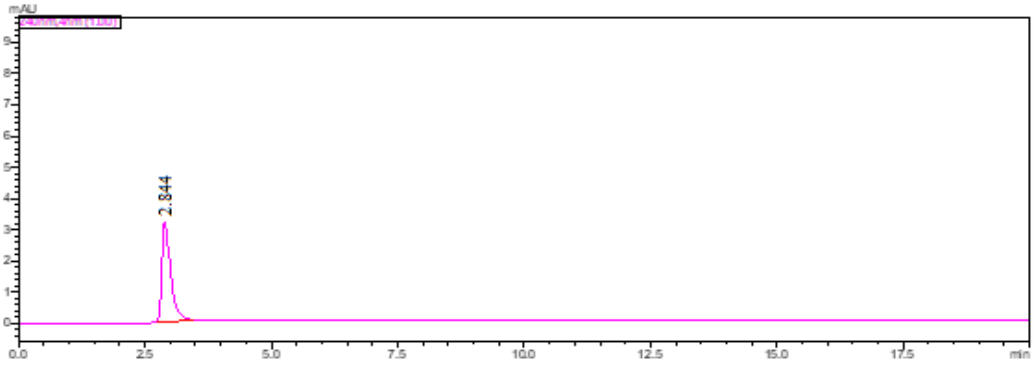

Fig. 4. A typical chromatograph of sample rosuvastatin calcium

Table 2. System suitability results

Parameter	Rosuvastatin Calcium
Theoretical Plate	2866
Retention Time (Rt)	2.848
Tailing Factor	1.62
% RSD	0.9

Rt: Retention time, %RSD: % relative standard deviation

Table 3. Linearity results

Parameter	Rosuvastatin Calcium
Concentration Range (µg/ml)	2-16
Slope (m)	47945
Intercept	1511.5
Coefficient correlation (r^2)	0.9999

Table 4. Recovery results

Level (Percentage)	Quantity taken (µg/millimeter)	Quantity found* (µg/millimeter)	%Recovery*
80	8	7.83	97.94
100	10	9.86	98.68
120	12	12.04	100.37

**Average of 3 determinations*

Table 5. Robustness results

Condition		Rosuvastatin Calcium	
		Amount Estimated* [%]	RSD [%]
Alter in wavelength (240±2 nm)	238 nm	98.21	0.5736
	242 nm	97.28	0.5692
Alter in flow rate (1±0.1 ml/min)	0.9 ml/min	99.25	0.5079
	1.1 ml/min	99.54	0.6383

**%RSD: % relative standard deviation, Average of three determinations*

Table 6. Validation parameter summary

Parameter	Rosuvastatin Calcium
Calibration range	2-16µg/ml
Wavelength	240 nm
Retention Time	2.848
Regression equation	Y=47945x + 1511.5
Intercept	1511.5
Slope	47945
Coefficient correlation (r^2)	0.9999
Precision (% RSD)	
Intraday	0.8
Interday	0.9
% Assay*	99.98
LOD (µg/ml)	0.82
LOQ (µg/ml)	1.90

**LOQ: Limit of Quantification, LOD: Limit of Detection, Average of five determinations*

3.5 LOQ and LOD

The LOQ rosuvastatin calcium was 0.82 µg/ml and LOD was1.90 µg/ml.

3.6 Robustness

The robustness of the technique was accessed by altering the optimized chromatographic condition appropriately. To assess the robustness study of the validated technique, the developed chromatographic conditions were consciously changed and the resolution for rosuvastatin calcium was measured. On the measurement of the result, it can be analyzed that the variation in the altering wavelength, the flow rate do not affect the technique significantly. % relative standard deviation <2% point out that the developed technique was robust (Table 5).

3.7 Analysis of Rosuvastatin Calcium from Marketed Tablets

Percentage analysis of the marketed formulation was erect to be 99.98 for rosuvastatin calcium.

4. CONCLUSION

The developed analytical method enables simple, specific, accurate, economical and, sensitive analysis of the rosuvastatin calcium in tablet and bulk dosage form. The present technique was validated under ICH guidelines. Therefore, the technique utilized for the daily quality control of the rosuvastatin in the tablet dosage form.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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