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Validated Reverse Phase High Performance Liquid Chromatography Technique for Quality Analysis of Rosuvastatin Calcium Molecule in Tablet Dosage Form

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ABSTRACT

Introduction

A simple, reliable, precise reverse phase high performance liquid chromatography (RP- HPLC) method has been developed and validated for the estimation of Rosuvastatin Calcium in tablet dosage form.

Methods

RP- HPLC separation was achieved on an C18, 5µm particle size (150mm x 4.6mm ID) column using a mobile phase of KH_2PO_4 and methanol (70:30) at a flow rate of 0.75ml/min at temperature of 40^0C, run time 10 min detected at 242nm using UV detector. The method was validated for system suitability, linearity, accuracy, precision, robustness.

Results

The method was found to be linear in the drug concentration range of 38 –72 µg/ml with correlation coefficient of 0.9996. The precision relative standard deviation was 1.07 which shows the method is precise. The accuracy minimum recovery value was 98.5 % and maximum recovery value was 101.97 % respectively.

Conclusion

Therefore, the developed method is basic, fast, precise and reproducible and thus can be used for the routine quality analysis of Rosuvastatin Calcium.

Keywords

Method validation; rosuvastatin calcium; RP- HPLC

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INTRODUCTION

Rosuvastatin Calcium is a widely used medication from the statin group, prescribed as lipid lowering compound which reduces the risk of cardiovascular diseases. Chemically it is a complex calcium salt, closely resembles the natural HMG-CoA to competitively inhibit the enzyme HMG-CoA reductase. Rosuvastatin helps decrease LDL levels (bad cholesterol), total cholesterol, and triglycerides, while slightly increasing HDL (good cholesterol).[1] One of the distinguishing features of rosuvastatin compared to other statins is its relatively hydrophilic nature which offers good efficacy safety profile.[2]

Referring to various literatures, several techniques are documented for the determination of Rosuvastatin calcium - including spectrophotometric methods, stability indicating assays[3], HPTLC, while RP-HPLC remains the gold standard. RP-HPLC is particularly favored due to its superior resolution, sensitivity, and reproducibility in quantifying the drug. Therefore, RP-HPLC is the preferred choice for ensuring stringent quality control.[4, 5]

The objective of the study is to provide a novel, simple RP- HPLC method for determining Rosuvastatin calcium. The method has been validated for accuracy, precision, linearity, specificity, sensitivity, robustness according to established guidelines, set by ICH and USP guidelines. Special attention was given to ensuring clear separation of the rosuvastatin peak from solvents and other components, along with achieving good accuracy, precision, sensitivity, and robustness. Overall, the method is suitable for routine quality control analysis.

METHODS[6, 7]

This is an in vitro analytical experimental study focused on method development and validation of RP-HPLC developed analytical method for Rosuvastatin Calcium. All the experiments were carried out in Department of Pharmacy, Kathmandu University and Mera Biotech Pvt Ltd, Bhaktapur, Nepal. Data processing and statistical analysis were performed using Microsoft Excel (Microsoft Corp., USA). Calibration curves were constructed by linear regression analysis, and results are expressed as percentage and mean ± standard deviation (SD).

Instrumentation

The Shimadzu, LC 2030 plus HPLC system (The Shimadzu, Japan) consisting of binary pump (Shimadzu Prominence I Plus), a system controller, an auto injector and a diode array detector with semi micron flow cell was used with inbuilt data processing system, Shimadzu Lab solutions LCGC version 6.83.

Standards & Chemicals

Rosuvastatin calcium standard and Rosuvasatin calcium tablets were obtained from Meera Biotech Pvt Ltd, Bhaktapur. All AR grade chemicals and HPLC grade solvents were used.

Chromatographic condition

All Analysis were performed in the LC 2030 plus HPLC system (The Shimadzu, Kyoto, Japan), Code No MQE-49, S.No.L21435782028. Chromatographic separation of Rosuvastatin was achieved at 30^0C temperature using a C-18(150 x 4.6 mm, packed with 5 micron particle size) in an isocratic mode with mobile phase 0.02M potassium dihydrogen phosphate buffer 0.57g in 210 ml water adjusted to pH 6 with ammonia solution: methanol (70:30 % v/v) was used. The flow rate was 0.75 ml/min and the UV detector was set at 242 nm, injection volume 10µl and run time was 10min. The mobile phase was filtered through 0.45 micron membrane filter under vacuum.

Preparation of standard solution

25 mg accurately weighed Rosuvastatin calcium standard was added to 70 ml mobile phase, mixed, sonicated for 10 minutes. The solution was cooled, volume made upto 100 ml with the mobile phase. 5ml of this solution was further diluted to 25ml with the same mobile phase, the final standard solution was filtered through 0.2 micron filter paper and injected in the RP-HPLC system.

Preparation of sample solution

Tablet powder equivalent to 25 mg of Rosuvastatin calcium was weighed and mixed with 70 ml of mobile phase, sonicated for 10 minuates. Cooled, volume made upto 100ml, 5ml of this solution was diluted to 25ml of the same mobile phase, filtered and injected in the RP-HPLC system.

RESULTS

Method Validation[6-10]

System suitability

System suitability test is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The parameters like retention time, number of theoretical plates, peak area, HETP were calculated and the obtained results were shown in Table 1.

Table 1. System suitability parameters

Parameters	Result		Required limit
	Mean	% RSD	
Peak Area	1299120	0.32	NMT 1% RSD
Retention time (min)	8.59	0.01	≤ 1 RSD
Theoretical plates(N)	8061		> 2000
Tailing factor (T)	0.89		≤ 2

Specificity

Specificity was analyzed for the interference of excipients or mobile phase in the analysis. As shown in the chromatogram below the baseline was straight and no interference of excipient peak overlapping or adjoining with Rosuvastatin peak indicating the high specificity of the method.

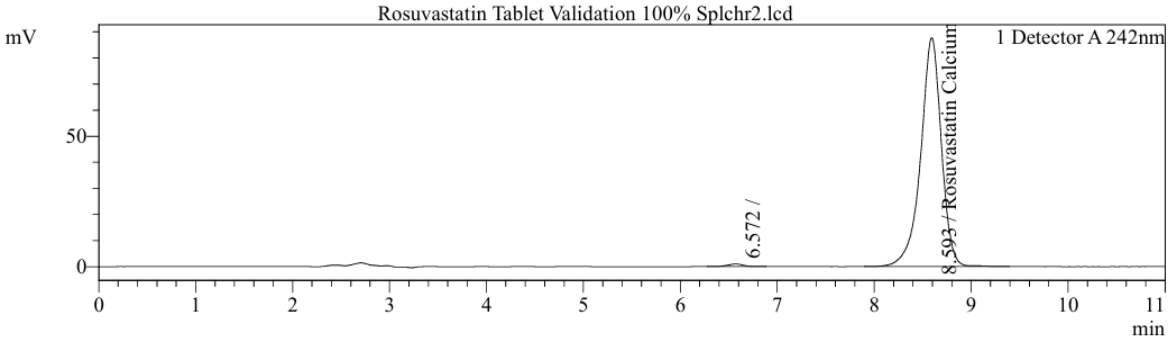


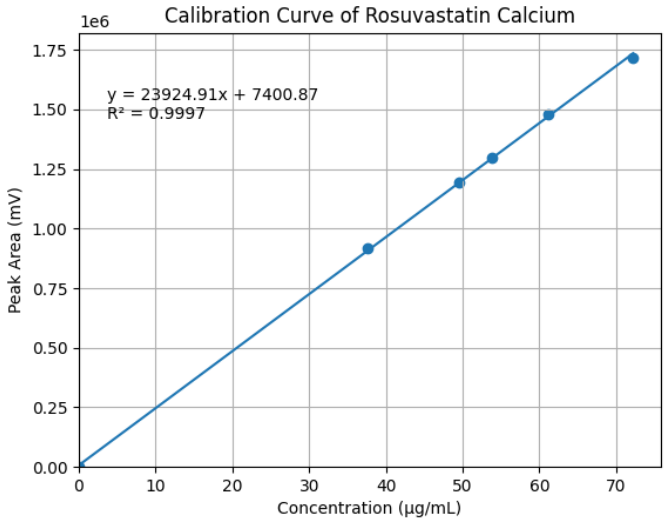
Figure 1. Chromatogram of Rosuvastatin calcium

Linearity of Response

Standard solutions for linearity tests were prepared for five different concentration levels 70% to 130% (37.66µg/ml to 72.22 µg/ml). The prepared solutions were injected and their peak areas were shown in table no.2.

Table 1. Calibration curve data

S.N.	Sample Name	Conc. mcg/ml	Avg. Area	% Yield
1	0%	0	0	0
2	70%	37.66	916606	100.86
3	90%	49.50	1194024	99.96
4	100%	53.86	1299670	100.00
5	110%	61.12	1481468	100.44
6	130%	72.22	1716675	98.50



Accuracy

The Accuracy of an analytical method is the closeness of test results obtained by that method compared with the true values. The accuracy method was established using recovery technique. The known amount of standard was added at three different concentration levels, at increasing linearity range, the solutions thus prepared are injected.

Precision

Precision method was performed adding the known amount of standard at three different levels of concentrations 70%, 100%, 130% to sample. Standard solution prepared with 0.025gm in 100ml methanol and placebo 0.287 gm in 100ml methanol. Each determination was performed in triplicate under the same condition and mean peak area and assay% were calculated.

Table 3. Precision Accuracy study data

Std.Wt.(g)	Avg.Area	Assay %			
0.02530	1323356	104.1%			
Injected Item	Active wt. (g)	weight. of placebo(g)	Ave.Area	Observed Concn	Assay %
Spl1(70%)	0.01704	0.26206	821188.5	34.08	99.42
Spl2(70%)	0.01736	0.26284	832756	34.72	98.96
Spl3(70%)	0.01761	0.26232	855938.5	35.22	100.27
Spl1(100%)	0.02585	0.26253	1245536.5	51.70	99.40
Spl2(100%)	0.02577	0.26202	1230961	51.54	98.54
Spl3(100%)	0.02540	0.26201	1213505.5	50.8	98.56
Spl1(130%)	0.03248	0.26275	1559776	64.96	99.07
Spl2(130%)	0.03249	0.26199	1606042.5	64.98	101.97
Spl3(130%)	0.03244	0.26151	1565344	64.88	99.54

Sensitivity (Limit of Detection and Limit of Quantification)

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can be detected by the proposed method. The Limit of Quantification (LOQ) is defined as the lowest concentration of an analyte that can be quantitatively measured with acceptable precision and accuracy. The LOD and LOQ were calculated from the linear calibration curve method shown in table 2 using formulae: LOD= 3.3 * σ / S and LOQ = 10 * σ / S, Where, σ is the standard deviation of the response and S is the slope of calibration curve.

Robustness

Robustness is a measure of a method's capacity to remain unaffected by small, deliberate variations in method parameters. It indicates the method's resilience to typical, expected fluctuations in a laboratory environment like slight change in mobile phase PH or composition of mobile phase, change

Table 4. Robustness Study of RP-HPLC method for Rosuvastatin Calcium

Parameter	Condition	Std avg area	Avg sample assay %
Flow rate	1.0 ml/min	1353358	97.8
	1.2 ml/min	1131580	97.9
	0.8ml/min	1698390	98.04
Column temp	45°C	1360543	97.6
	35°C	1362450	97.5
Column	150 x 4.6mm, 5 µm	1128519	100.9
	25x 4.6mm, 5 µm	1304425	100.4
Buffer Conc	60% buffer	1369715	98.86

in flow rate deviations, variations in column temperature, or column change from the same supplier. The results were shown in Table 4.

DISCUSSION

For the quantitative analysis of Rosuvastatin calcium, an RP-HPLC method was developed and validated, to achieve dependable, repeatable, and high-resolution separation of rosuvastatin calcium, for a moderately lipophilic HMG-CoA reductase inhibitor.[1] The proposed RP-HPLC method utilized reverse-phase chromatography with C18 5 µm particle size stationary phases, since is suitable for moderately polar compound like Rosuvastatin calcium. It demonstrated good efficiency, resolution, and operational back pressure, characteristics which are frequently reported for statin analysis. Similar column characteristics have been shown to produce consistent and symmetrical peaks with minimal tailing in previously developed methods as well.[4, 11-14]

The mobile phase, consists of methanol and potassium dihydrogen phosphate buffer (70:30), was utilized throughout to provide satisfactory resolution and peak symmetry. Buffer systems are crucial for regulating rosuvastatin molecule ionization, which enhances chromatographic performance and reproducibility.[4,13] Using methanol instead of aecetonitrile provides a more cost-effective option without sacrificing method performance and methanol served as an effective organic modifier, providing suitable elution strength without compromising peak shape. The optimized chromatographic conditions, including a flow rate (0.75 mL/min), injection volume (10 µL), and run time (10 min), are consistent with earlier studies where retention times for rosuvastatin typically lies between 3–10 mins.[11-15] The observed retention time of 8.59 mins in this study satisfies the adequacy of the selected conditions. Prior to analysis, the mobile phase, standard, and sample solutions all were filtered using a 0.45 µm membrane filter to ensure removal of particulate matters for enhanced system performance. The best absorbance and linear detector response were obtained by detection at 248 nm, which is consistent with previously published analytical techniques.[3, 14]

The reliability of the devised approach was performed as per Indian Pharmacopoeia 2007 (IP)6, United States Pharmacopoeia 2024 (USP)7, and ICH guidelines9, 10.With a retention time of 8.59 minutes, tailing factor of 0.893, and theoretical plate count of 8061, the system suitability parameters showed good chromatographic performance, indicating effective separation and sharp peak. Column efficiency is further supported by the low HETP value. The method showed excellent linearity with a correlation coefficient (r^2) of 0.999, demonstrated a strong link between concentration and peak area. Recovery studies yielded accuracy values between 98.54% - 101.97%, which fall within the acceptable pharmacopoeial limits of 95–105%, confirming the absence of interference from excipients. Precision was demonstrated by an RSD value of 1.07%.

The sensitivity of the method, expressed as LOD and LOQ, was determined to be 42.60 µg/mL and 129.1 µg/mL, respectively, suggesting it's suitability. Robustness analysis, carried

out by introducing small deliberate changes in analytical conditions, complied assay findings, within the acceptable range of 90–110%.[6] Previous studies have demonstrated similar robustness.[12-14] Overall, the developed RP-HPLC method is simple, economic, precise, accurate, and robust, and is consistent with previously reported methods, thus suitable for routine quality control analysis.

CONCLUSION

The RP-HPLC analytical method was developed as per IP and it was validated in accordance with ICH guidelines and USP. The method employs a simple, economic and easily prepared mobile phase, along with a short run time of 8.5 minutes, enabling rapid and efficient analysis. Validation results confirmed that the proposed method was accurate, with recovery values within 98.54–101.97%, precise with %RSD below 2%, and linear over the studied concentration range with a correlation coefficient of 0.999. System suitability parameters, including a tailing factor below 1 and a high number of theoretical plates, indicate good chromatographic efficiency and peak symmetry.

The method also exhibited adequate sensitivity, and demonstrated robustness under deliberate variations in analytical conditions. Importantly, no interference from excipients was observed, owing to its simplicity, precision, accuracy, and reproducibility. The proposed RP-HPLC method is well-suited for routine quantitative analysis of rosuvastatin calcium in bulk drug substances and pharmaceutical dosage forms, to ensure that every tablet released to the market is safe, effective, and of high quality, protecting general public health.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare. All authors and co-authors have seen and agreed with the contents of the manuscript and certify that the submission is an original work and is not under review at any other publication

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