ANALYTICAL METHOD DEVELOPMENT FOR QUANTIFICATION OF ROSUVASTATIN CALCIUM THROUGH REVERSE PHASE HPLC

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ABSTRACT

The planned RP-HPLC method is well-suited for analyzing Rosuvastatin Calcium in tablet form on a regular basis. It offers several advantages over other methods, including being simple, precise, accurate, sensitive, and capable of quantifying Rosuvastatin Calcium with just one injection. The separation was achieved by using YMC C8 column with sizes of 150 mm x 4.60 mm ID and 5.0 µm particle size was used for chromatographic analysis. The mobile phase comprised of a mixture of acetonitrile and water which pH is adjusted to 3.5 by phosphoric acid in a proportion of 40:60 (v/v). The flow rate set to 1.5 mL/min, column temperature at 25°C, and the injection volume at 20 µL. Detection was performed at 242 nm of wavelength by means of a photodiode array detector (PDA).

Keywords: Rosuvastatin Calcium, HPLC, Method Validation, Method Optimization, LOD, LOQ

1. INTRODUCTION

Rosuvastatin calcium is a medication used as a lipid-lowering agent that functions as an Anti-HMG- CoA Reductase, which is enzyme that limits speed in production of cholesterol. It is an effective lowering of dyslipidemia and has a longer half-life compared to other statins. Rosuvastatin belongs to the drug class of statins and is classified as BCS class II drug. It is chemically 6-[4-(4-fluorophenyl)]- 7-Heptenoic Acid-6-(1-methylethyl)2. [Methyl (methylsulfonyl) amino]-5-pyrimidinyl]Calcium salt, 3,5-dihydroxy, (3R,5S,6E) - (2:1) which is Water sparingly soluble, methanol barely soluble, readily soluble in acetonitrile, and freely soluble in N,N-Dimethyl formamide.

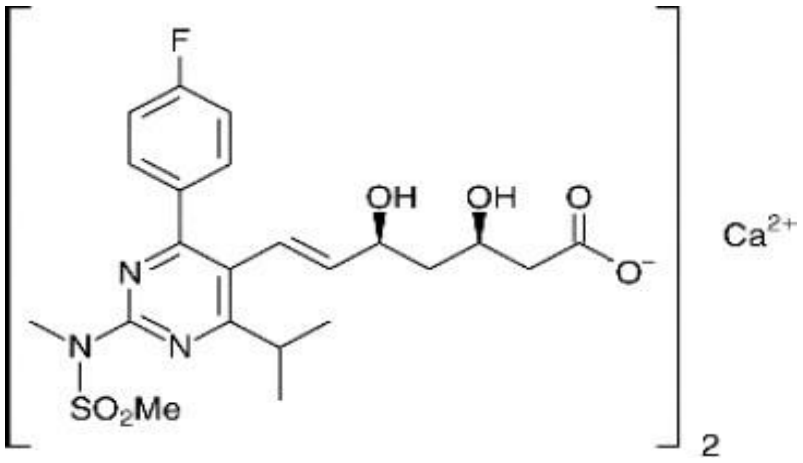


Fig: 1.1 Molecular Structure of Rosuvastatin Calcium

2. Materials and Method

2.1 Materials and Methods

The Rosuvastatin calcium reference standard used in the study was obtained from Glenmark Pharma located in Ankleshwar, India. The Rovastin tablets, which are a pharmaceutical formulation containing rosuvastatin calcium with a 10 mg claim on the label, were manufactured by Quest Pharmaceutical Pvt. Ltd. in Birgunj, Nepal. All the chemicals used in the study, such as Sodium hydroxide pellets, orthophosphoric acid (88%), hydrochloric acid (35%), and a 30% (v/v) hydrogen peroxide solution, remained of high purity and obtained in New Delhi from Ranbaxy Fine Chemicals, India. HPLC-grade acetonitrile and water, necessary for the analysis, were bought in Mumbai, India, from Spectrochem Pvt. Ltd.. To ensure proper filtration, filters for nylon syringes with 0.45 micron pores, specifically Millex-HN manufactured by Millipore in Mumbai, India, were employed in the study.

2.2 Instrumental

Chromatographic system used for assay method development and validation:

- The binary pump LC-10ATvp
- Photodiode array detector SPD-M10Avp
- 20-loop manual injector Rheodyne model 7725i

Multi-instrument data processing and acquisition system:

- 6.13 SP2 Class-VP
- Provided by Shimadzu, Kyoto, Japan.

2.3 Preparation for the Mobile Phase

Making up the mobile phase:

- Acetonitrile
- Water (with orthophosphoric acid added to adjust pH to 3.5)

- Ratio of acetonitrile to water is 40:60 (v/v)Degassing of the prepared mobile phase:
- Mobile phase remained degassed by means of an ultrasonic bath through sonication.

2.4 Diluent Preparation

Solvent composition:

- Acetonitrile
- Water

Acetonitrile:water in the solvent mixture: 50:50 (v/v).

2.5 Preparation of stock solution (500 µg/ml) and standard solutions (50 µg/ml):

Preparation of Stock Solution:

- Accurately weigh 25 mg of Rosuvastatin calcium reference standard.
- Transfer the weighed standard to volumetric flask, 50 ml.
- Add 20 ml of 50/50 water-acetonitrile to the flask.
- Sonicate the combination for 2 minutes near complete dissolution of Rosuvastatin calcium.
- Dilute the solution to the mark using the same solvent combination. Preparation of Standard Solution:
- Take 5 ml of the stock solution (500 µg/ml).
- Transfer the 5 ml aliquot to volumetric flask, 50 ml.
- Dilute the solution using the same solvent combination to mark..

2.6 Preparation of stock (500 µg/ml) and test solutions (50 µg/ml):

Preparation of Stock Solution:

- Weigh and combine the contents of 20 tablets.
- Accurately weigh an amount of the mixed powder weighing the same as five tablets.
- Transfer the balanced powder to volumetric flask, 100 ml.
- Add 60 ml of water-acetonitrile (50:50, v/v).
- Sonicate the combination for 10 minutes while performing normal hand shaking.
- Allow flask to cool at room temperature.
- Diluted the solution using the same solvent combination and volume.
- Through a 0.45-m nylon syringe filter, filter the solution.. Preparation of Test Solution:
- Take 5 ml of the stock solution prepared above (500 µg/ml).
- Transfer the 5 ml aliquot to volumetric flask, 50 ml.
- Dilute the solution to the (50:50, v/v) volume with water-acetonitrile.

Use the typical average tablet's weight (obtained by weighing 20 tablets) for assay calculation.

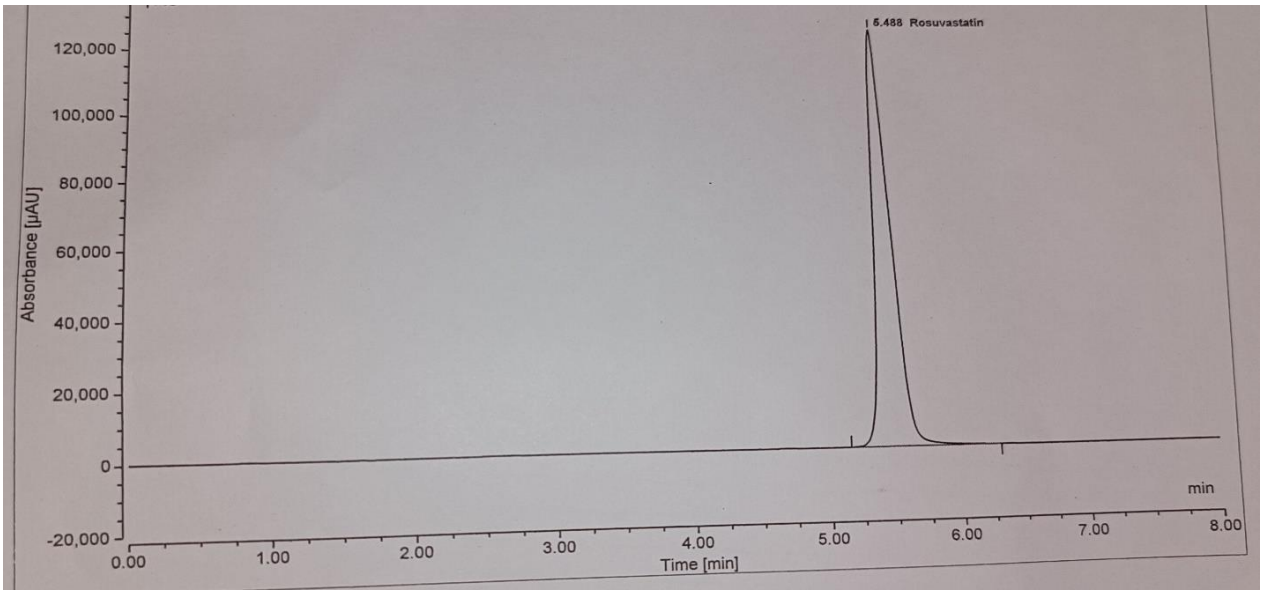
3. Result and Discussion

3.1 Method Introduction and Modernization of the chromatographic conditions

To progress an accurate and appropriate approach for quantifying Rosuvastatin, several factors were evaluated, including diluents, buffers, a buffer's level, organic solvents used for the mobile phase, as well as other chromatographic conditions. Initial experiments utilizing several mobile phases comprising Methanol and orthophosphoric acid in acidic water showed unsatisfactory peak shape. However, substituting methanol with acetonitrile improved peak shape and resulted in shorter retention times. The ratio of components of the mobile phase were tuned to minimize retention times then achieve good separation between Rosuvastatin and its decomposition products. Examining the typical solution across a variety of 190 to 370 nm using a PDA detector, a 242 nm detecting wavelength was chosen due to its excellent response and linearity. Based on multiple considerations, the selected diluent was deemed the most suitable among all the tested options.

Chromatographic Condition

- Column: YMC C8
- Dimensions: 150 mm x 4.60 mm ID
- Particle Size: 5.0 µm
- Mobile Phase composition: Acetonitrile and water (Phosphoric acid was used to alter the pHto 3.5.)
- Ratio: 40:60 (v/v)
- Flow rate: 1.5 ml/min
- Column Temperature: 25°C
- Injection Capacity: 20 µl
- Detection: Photodiode Array Detector (PDA)
- Wavelength: 242 nm



Chromatogram of Rosuvastatin Calcium

3.2 Method ValidationSpecificity

To determine the method specificity, chromatograms of the API solution compared to the blank solution. The blank solution contained the same ingredients as the drug solution, except for the actualdrug itself. Before injection, they were filtered via a 0.45 m membrane filter. An experiment was performed and it was observed that there were no additional peaks near the drug peak, showing that the method proposed was specific.

Linearity

A curve comprising seven data points was created by graphing the peak area vs the strength of Rosuvastatin calcium. The strength range of 20-80 µg/mL was used, covering a broad range of concentrations found in the test solution. By graphing the peak area vs the concentration, a regression formula was derived as y = 511779x + 371608 in which x represents the strength in µg/ml as well as y represents the peak area in units of absorbance. The high regression coefficient of 0.999 indicates a peak area and concentration are strongly correlated linearly, ensuring accurate quantification of Rosuvastatin calcium in the sample.

Fig: 3.2.1 Linearity curve of Rosuvastatin Calcium for Linearity

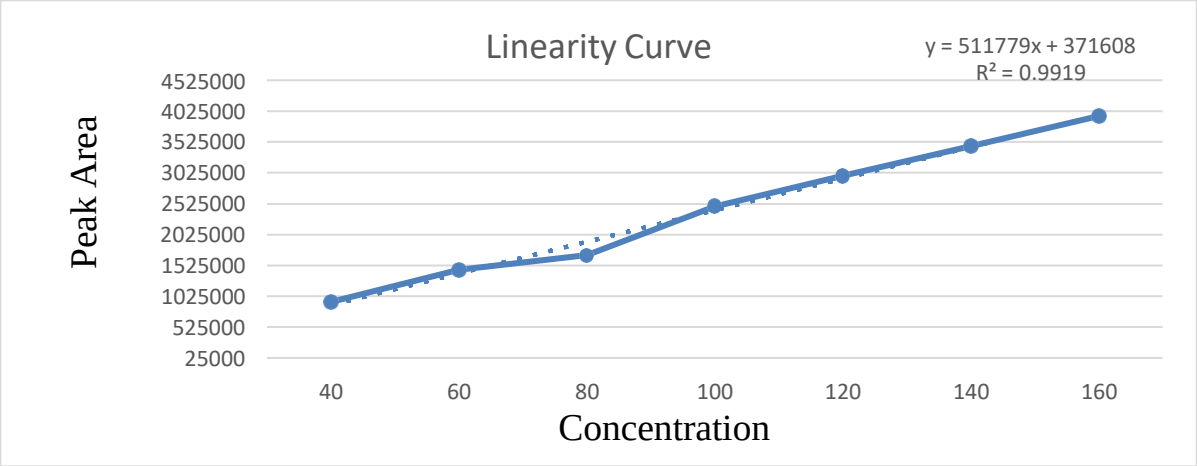


Table 3.2.1 Linearity Data

Linearity Level	Area
(40%)	932659.5
(60%)	1451015
(80%)	1925740
(100%)	2483360
(120%)	2976714
(140%)	3455646
(160%)	3943398

Limit of detection (LOD) and Limit of quantitation (LOQ) study

For determination of the detection limit (LOD) and quantitation limitation (LOQ), the ratio of signal- to-noise (S/N) was calculated for the LOD and LOQ preparations. The concentration used for the LOQ and LOD level were 0.5 μg/ml and 0.1 μg/ml. To assess the reproducibility at the LOQ level, six replicate sample preparations were examined. The relative standard deviation (% RSD) and the standard deviation were designed to evaluate consistency of the outcomes.

Precision Study

The analytical method's precision was assessed through two studies: method precision and intermediate precision.

For method precision, the standard solution was analyzed five times to determine system precision. Six sets of sample preparations were analyzed, and the assay of each set was determined to calculate method precision. Based on the data, the mean% assay value, standard deviation, and% relative standard deviation were determined.

Table 3.2.2 METHOD PRECISION DATA

Sr. No.	Duplicate	Area	Average	% Assay
1	1	2480436	2480706	100.41
	2	2480976		
2	1	2473167	2474198	100.15
	2	2475229		
3	1	2489347	2488718	100.74
	2	2488088		
4	1	2469725	2466927	99.86
	2	2464129		
5	1	2486584	2484278	100.56
	2	2481972		
6	1	2462743	2463422	99.71
	2	2464100		

For evaluation of intermediate precision, method precision study was recurrent on a distinct day by a distinct analyst under the same experimental conditions. The preparation of six duplicate samples were analyzed, the average, standard deviation, assay value, and relative standard deviation were calculated.

The developed analytical method demonstrated good precision, as indicated by the %RSD for system precision on the same day (intraday) of 0.45% and 0.37%, and on different days (interday) of 0.41% and 0.55%.

Table 3.2.3 INTERMEDIATE PRECISION DATA

Sr. No.	Duplicate	Area	Average	% Assay
1	1	2482122	2482552	100.48
	2	2482981		
2	1	2475893	2475909	100.22
	2	2475925		
3	1	2469567	2470172	99.99
	2	2470777		
4	1	2486027	2485193	100.60
	2	2484359		
5	1	2486451	2489260	100.76
	2	2492068		
6	1	2475133	2473981	100.14
	2	2472828		

Accuracy

The accuracy of the developed analytical method was assessed through a recovery study conducted at three different concentrations: 50%, 100%, and 150% of the test solution concentration. Known amounts of Rosuvastatin calcium (25, 50, and 75 µg/mL) were added to a placebo preparation, and the recovered amount of Rosuvastatin in the presence of placebo interference was calculated. Three sets of samples were prepared at each concentration level, and each set was injected twice for analysis. Thepercentage recovery of Rosuvastatin calcium was calculated at each concentration level and recorded in Table 5.2.6.1. The mean recovery of Rosuvastatin calcium was found to be within the range of 98.00% to 102.00%.

Table 3.2.4 Accuracy Study Results

Evaluation Data of Method Accuracy						
Conc^{n}(%)	No	Conc^{n}. of Drug Added (ug/ml)	Conc^{n}. of Drug Found(ug/ml)	Recovery (%)	Average Recovery (%)	RSD (%)
50	1	25.02	25.11	100.35	101.20	0.78
	2	25.15	25.69	102.14		
	3	25.25	25.53	101.11		
100	1	50.10	50.09	99.88	100.16	0.24
	2	50.40	50.55	100.30		
	3	50.35	50.50	100.30		
150	1	74.77	75.99	101.63	100.74	0.76
	2	76.30	76.53	100.30		
	3	76.59	76.82	100.30		

Robustness Study

The method's robustness was evaluated by analyzing solutions under test with slightly modifiedanalytical conditions. The following factors were selected for evaluation:

- Flow rate: The flow rate was adjusted by ± 0.1 ml/min from the optimized value.
- Mobile phase composition: The mobile phase composition was modified by changing the ratio of acetonitrile and water. Two compositions, acetonitrile:water (38:62 and 42:58, v/v), were tested.
- HPLC column: Different lots of HPLC columns were used for the analysis.

Table 3.2.5 Robustness Study Results

Parameters	**ssay(%)**	**etention Time (min)**	**System Suitability Data**	
			eoreticalplates	**Asymmetry**
Flow rate Variation				
1.4ml/min	100.32	3.84	8562	1.15
1.6ml/min	100.32	5.49	9110	1.11
Mobile Phase Composition Variation				
Acetonitrile:water (38:62,v/v)	101.17	5.49	8647	1.11
etonitrile:water(42:58,v/v)	101.72	3.84	8758	1.08
Column change	100.76	3.84	7870	1.12

System Suitability Study

Before each validation parameter, the chromatographic system was subjected to a system appropriateness test. Asymmetry, theoretical plate, and %RSD of peak area were determined for five replicate injections of standard preparation.

System Suitability Summery (SSS)

System suitability data	RSD (%)	eoreticalplates	Asymmetry
In-House limit	NMT[a]2.0	NLT[b]8000	NMT[c]2.0
Specificity	0.62	8441	1.05
Linearity	0.28	8544	1.02
Precision			

For Assay	0.45	8640	1.06
Intermediate Precision			
For Assay	0.45	8667	1.01
Accuracy	0.34	8591	1.00
Robustness	0.46	8212	1.02

[a]Relative standard deviation, [b]not less than, [c]not more than

4. CONCLUSION

The proposed HPLC method is well-suited for analyzing Rosuvastatin Calcium in tablet form on a regular basis. It offers several advantages over other methods, including being simple, precise, accurate, sensitive, and capable of quantifying Rosuvastatin Calcium with just one injection.

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