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VALIDATED RP-HPLC METHOD FOR THE ESTIMATION OF ROSUVASTATIN CALCIUM IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A new sensitive, specific, linear, precise and accurate RP-HPLC method was developed and validated for estimation of Rosuvastatin calcium in Bulk and Tablet dosage form. An isocratic, reversed phase HPLC method was developed. Shimadzu shim pack C18 (250mm x 4.5µm, x 5 µ) column. Shimadzu Prominence-I LC-2030C plus equipped with Auto sampler as the instrument model. Mobile phase consists of mixture of 0.02M Phosphate buffer pH 2.5: Methanol: Acetonitrile in the ratio (50:20:30 v/v) at a flow rate of 1.0ml /min with injection volume of 10µL. UV detection was performed at 242nm. The Linearity was established for Rosuvastatin calcium in the range of 5-30µg/ml with correlation coefficient of 0.9998. LOD and LOQ were found to be 0.030µg/ml and 0.093µg/ml respectively. Retention time of Rosuvastatin calcium were found to be 3.42mins. The % Recovery was found to be 99.93-101.28 and %RSD was found with in ± 2. The method has been validated according to ICH guidelines for linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. The developed validated method was successfully applied for reliable quantification of Rosuvastatin calcium in bulk and pharmaceutical dosage form.

KEYWORDS

Rosuvastatin calcium, RP- HPLC, Validation and Pharmaceutical formulations.

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INTRODUCTION

Rosuvastatin is in a class of medications called HMG-COA reductase inhibitors (statins), an enzyme found in the liver that plays a role in producing cholesterol. Rosuvastatin works by slowing the producing of cholesterol in the body to decrease the amount of cholesterol that may build up on the walls of the arteries and block blood flow to the heart, brain, and further parts of the body. It is also used to

prevent the cardiovascular disease in those at high risk and treat abdominal lipids[1].

Literature survey revealed that there were few analytical methods have been reported for the determination of the Rosuvastatin calcium in pure drug and pharmaceutical dosage form by using UV-Spectrophotometric[2-10], RP-HPLC[11-18] and HPTLC[19-22] so far.

The aim of the present work is to develop and validate a novel, rapid, precise and specific Area under curve UV spectrophotometric method for estimation of Rosuvastatin calcium in bulk and tablet dosage form.

MATERIAL AND METHODS

Material and reagents

The Rosuvastatin calcium was obtained as a gift sample from the pharmaceutical industry and Crestor tablet obtained from Pharmacy store. Phosphate buffer Acetonitrile Methanol and distilled water were obtained Bharathi College of pharmacy, Bharathinagara, KM Doddi, Maddur Taluk, Mandya District, India. All chemicals used are of HPLC grade. Distilled water was used throughout the experiment.

Instrumentation

Chromatographic separation was performed on a Shimadzu Prominence-i LC-2030 plus equipped with Auto sampler comprising a variable wavelength programmable UV detector. Shimadzu shim pack C18 (250mm x 4.5µm x 5µ) column is used.

Preparation of solutions Mobile phase preparation

The Mobile phase consisted of a mixture of 0.02M Phosphate buffer pH 2.5(50%) Methanol (20%), Acetonitrile (30%) in the ratio of 50:20:30 v/v, which was filtered through a membrane and degassed before use. pH adjusted with 0.1% of ortho phosphoric acid in Millipore water.

Preparation of sample Standard Solution

The formulation tablets of Rosuvastatin calcium (Crestor 40mg) were crushed to give finely powdered material. From the Powder prepared a 100mg of Rosuvastatin calcium was accurately weighed, transferred in a 100ml volumetric flask, add 30ml of diluents and sonicate to dissolve and dilute to volume with diluent. Transfer 10mL of standard stock solution into 100ml volumetric flask and dilute to volume with diluent. And an appropriate concentration of sample was prepared at the time of analysis. 10µl of these solutions were injected in triplicate into HPLC system and the peak areas were recorded.

Preparation of Standard solution

10mg of Rosuvastatin calcium was dissolved in 10ml of methanol in 10ml volumetric flask (1000µg/ml). Further dilution was made from above in such a way that the final concentration consists of 5, 10, 15, 20, 25, and 30µg/ml.

System suitability requirements from stock and standard solutions

Tailing factor: NMT 2.0

Theoretical Plates: NLT 2000

RESULTS AND DISCUSSION

Validation of the proposed method

The proposed method was validated as per ICH guidelines[23-25]. The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification, and solution stability.

Specificity

From the chromatograms of blank and standard (Prepared from Formulation). It was found that there is no interference due to excipients in the tablet formulation and also found good correlation between the retention time. The specificity results are shown in Table No.2.

Linearity

The linearity of the response of the drug was verified at six concentration levels, ranging from 5-30µg/ml of Rosuvastatin calcium in each linearity level were prepared. 10µl of each concentration was injected into the HPLC system. The response was read at 242nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were presented in Table No.3.

Precision

Precision of the method was performed as intraday precision, Inter day precision. To study the intraday precision, six replicate standard solutions (10µg/ml) of Rosuvastatin calcium were injected. % RSD was

calculated and it was found to be 0.693 and interday precision done same as intraday, six replicate standard solutions (10µg/ml) of Rosuvastatin calcium were injected. % RSD was calculated and it was found to be 0.641 which are well within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in Table No.4.

Accuracy

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 50, 100 and 150% of the label claim of the tablet (40mg of Rosuvastatin calcium). The recovery values for Rosuvastatin calcium ranged from 98.0 to 102.0%. The average recoveries of three levels of Rosuvastatin calcium were found to be 99.93-101.28%. The results are shown in the Table No.5.

Limit of detection and Limit of quantification

The limit of detection is an analytical method is the smallest amount of analyte in a sample which can be reliable detected by the analytical method. The limit of quantitation is an individual analytical procedure is the smallest amount of the analyte in sample which can be quantitatively determined. LOD and LOQ were calculated using formula LOD = 3.3(SD)/S and LOQ = 10(SD)/S. Results were shown in Table No.6.

Ruggedness

The ruggedness of test method was demonstrated by carrying out precision study in six preparations of sample on a single batch sample by different analysts, the results of the precision study are tabulated as below Table No.7. The % RSD values are less than 2.

Robustness

Robustness is the measure of the capacity of the analytical method to remain unaffected by small but deliberate variation in the procedure. The robustness of the method was evaluated by analysing the system suitability standard and evaluating system suitability parameter data after varying, individually, the HPLC pump flow rate (±0.2ml/min), column temperature (±5C) and detection wavelength (±2nm) shown in Table No.8.

Acceptance criteria

System suitability should pass as per test method at variable conditions.

CHROMATOGRAPHIC CONDITIONS

Table No.1: HPLC method development parameters

S.No	HPLC method development parameters	
1	Column	C18, 250nm X 4.5µm, 5µ
2	Flow rate	1.0ml /min
3	Wavelength	242nm
4	Column temperature	30°C
5	Injection volume	10µL
6	Run time	10minutes
7	Diluents	Mobile phase
8	Elution	Isocratic

Table No.2: Specificity of Rosuvastatin calcium

S.No	Name of the solution	Retention time in min
1	Blank	0
2	Rosuvastatin calcium(Standard)	3.42

Table No.3: Linearity of Rosuvastatin calcium

S.No	Concentration (µg/ml)	Peak area* (mv)
1	5	1321374
2	10	2801237
3	15	4282763
4	20	5679478
5	25	7174721
6	30	8726031

*Average of six determinations.

Table No.4: Results of Precision of Rosuvastatin calcium

S.No	Intraday Names	Studies Peak area	Interday Names	Studies Peak area
1	Injection-1	2821517	Injection-1	2810213
2	Injection-2	2809123	Injection-2	2819571
3	Injection-3	2838276	Injection-3	2855912
4	Injection-4	2812623	Injection-4	2806112
5	Injection-5	2856291	Injection-5	2812283
6	Injection-6	2806283	Injection-6	2822123
7	AVG	2824018.83	AVG	2821035.66
8	STDEV	19582.28	STDEV	18089.451
9	%RSD	0.693	%RSD	0.641

Table No.5: Results of recovery of Rosuvastatin calcium

S.No	Level of addition/ %	Amount added (µg/ml)	Amount found	%Recovery ±Standard deviation*	%RSD
1	50	10	29.72	99.93±0.804	0.804
			30.31		
			29.92		
2	100	20	40.65	101.28±0.543	0.536
			40.69		
			40.21		
3	150	30	50.42	100.006±0.635	0.634
			49.65		
			49.94		

*Average of three determinations.

Table No.6: System suitability parameters

S.No	Parameters	Rosuvastatin calcium
1	Linearity	5-30µg/ml
2	Regression equation	y = 294516.86x – 156444.533
3	Correlation coefficient	R^2 = 0.9998
4	Retention time	3.42min
5	Run time	10min
6	Limit of detection (LOD)	0.030µg/ml
7	Limit of quantification (LOQ)	0.093µg/ml
8	Tailing factor	1.21
9	Theoretical Plate	4866

Table No.7: Results of Ruggedness of Rosuvastatin calcium

By changing the Analysts

S.No	Concentration	T1	T2	Mean	SD	%RSD
1	5	1390213	1362532	1376372.5	19573.42	1.42
2	10	2832074	2798243	2815158.5	23922.12	0.84
3	15	4306783	4292173	4299478	10330.83	0.24
4	20	5701302	5684174	5692738	12111.32	0.21
5	25	7203271	7199263	7201267	2834.08	0.039
6	30	8751743	8792106	8771924.5	28540.95	0.32

*Average of three determinations.

By changing the instrument

S.No	Concentration	T1	T2	Mean	SD	%RSD
1	5	1372921	1391763	1382342	13323.30	0.96
2	10	2850742	2891271	2871006.5	28658.33	0.99
3	15	4279821	4291913	4285867	8550.33	0.19
4	20	5698134	5720187	5709160.5	15593.82	0.27
5	25	7172471	7189272	7180871.5	11880.10	0.165
6	30	8788172	8752172	8770172	25455.84	0.29

*Average of three determinations.

Table No.8: Robustness results for rosuvastatin calcium

S.No	Parameters	Conditions	Tailing Factor	% RSD
1	Column Temperature	Decreased (-5°C)	1.19	0.33
		Increased (+ 5°C)	1.016	
2	Flow rate (ml/min)	Decreased (-2min/min)	1.21	0.58
		Increased (+2 min/min)	1.018	
3	Wavelength	Decreased (-2nm)	1.021	1.02
		Decreased (+2nm)	1.2	

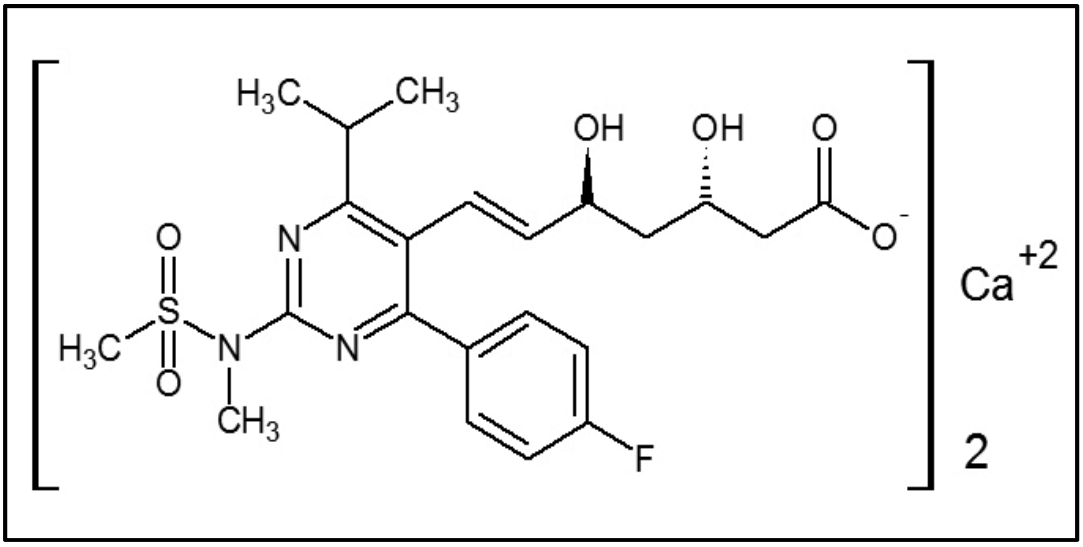

Figure No.1: Chemical structure of Rosuvastatin calcium

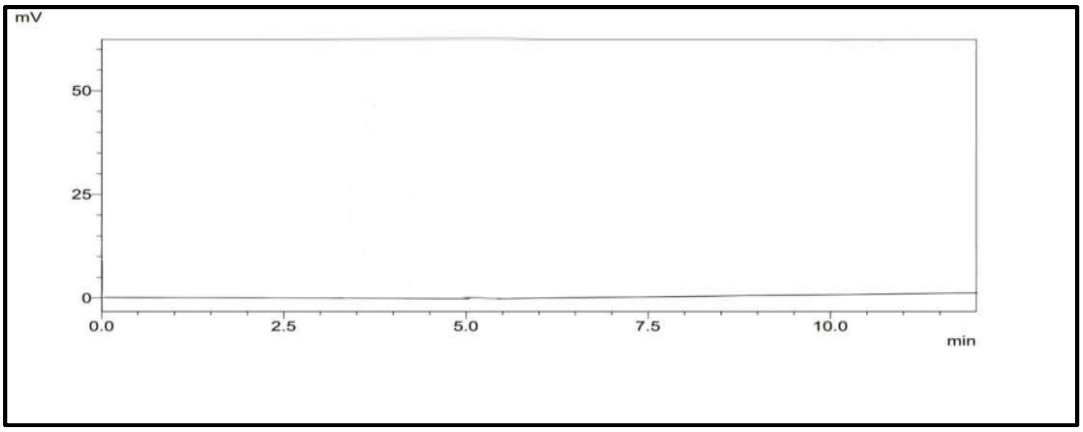

Figure No.2: Chromatogram of blank solution of Rosuvastatin calcium

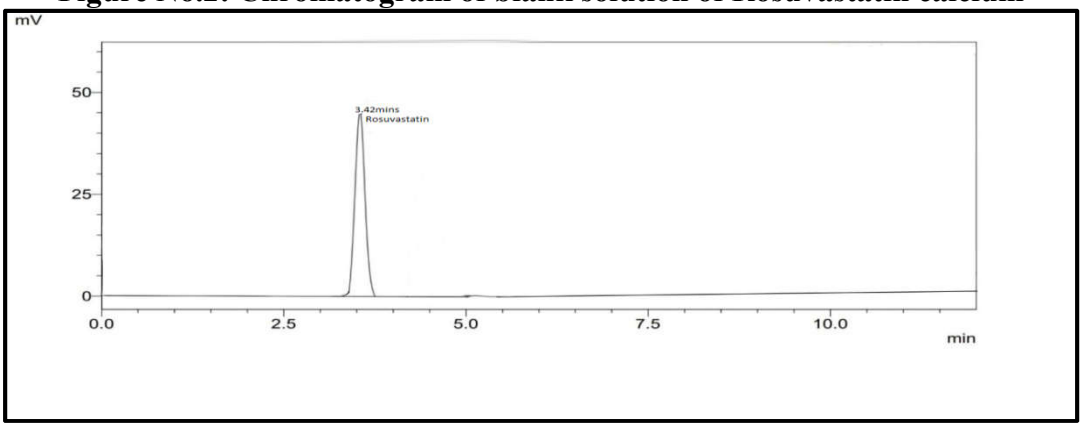

Figure No.3: Chromatogram of standard solution of Rosuvastatin calcium

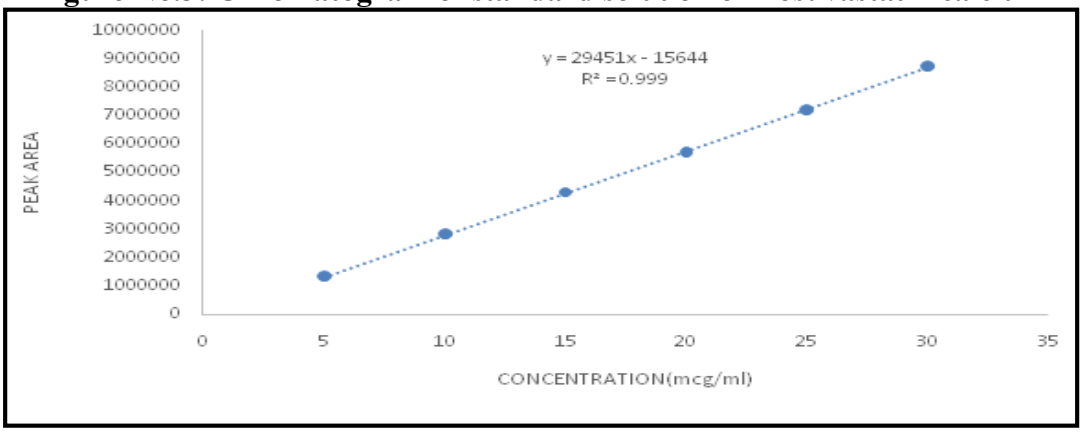

Figure No.4: Linearity of rosuvastatin calcium

CONCLUSION

The present analytical method was validated as per ICH guidelines and met the acceptance criteria. It was concluded that the developed analytical method was simple, accurate, economical and sensitive, and can be used for routine analysis of Rosuvastatin calcium in bulk drug and pharmaceutical dosage forms.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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