DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR ESTIMATION OF ROSUVASTATIN CALCIUM

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Abstract: The present work is aimed at development and validation of HPLC method which is simple, specific, precise, and accurate for estimation of Rosuvastatin Calcium and its process-related impurity in bulk and pharmaceutical dosage forms. Extensive literature survey revealed no method for estimation of the above said. The characterization of synthesized impurities by using FTIR, NMR and MS. The RP-HPLC method was developed according to ICH Q2B guidelines for quantitation of impurity in bulk and formulations. The method was validated as per ICH guidelines. The method was found to be linear, precise, accurate, robust and rugged.
The present study focuses on the various steps, parameters involved in HPLC condition. Various applications of this system also discussed. HPLC process development is important in case of drug discovery, drug development and pharmaceutical products. It can be adopted apparently for routine quality control study of research and formulation tests. The method is carried out on a Symmetry C18 (4.6 mm ID × 150 mm, 5 µm, Make: XTerra) with a mobile phase consisting of acetonitrile and potassium dihydrogen phosphate buffer of pH = 4.0 in the ratio 65:35 volume/volume at a flow rate of 0.7 mL/min. The detection of eluted components is carried out at a wavelength of 216 nm. The retention time of Rosuvastatin Calcium is found to be 2.647 min. The developed method is validated in terms of accuracy, precision, linearity, limit of detection, limit of quantization. The linearity limits, LOD and LOQ of the developed method are found to be 10-50, 0.052 and 0.171 µg mL-1 , respectively. The developed method is found to be simple, fast and economic and hence it can be used as an alternative method in quality control.

Keywords: RP-HPLC, Rosuvastatin Calcium, Impurities, Guidelines, Symmetry.

1: Introduction

The identification and qualification of impurities in Active Pharmaceutical Ingredient's (API's) and pharmaceutical products, is a very intensive activity performed at many levels of the drug discovery and beyond. Impurities related to starting materials, by-products, breakdown products or polymorphs. They can appear at the API production level as well as during or after formulation process. Impurities in APIs are of significant concern as they may carry activity responsible for the eventual undesirable side effects or toxicity and/or may interfere with the drug's activity. Thus monitoring impurities in API and drug product is a prerequisite to insure drug safety and quality. In Pharmaceutical World, an impurity is considered as any other organic materials, besides the drug substances, or ingredients, arises out of synthesis or unwanted chemicals that remains with Active Pharmaceutical Ingredient's (API's). The impurity may be developed either during formulation or upon aging of both API's and formulations. Presence of impurities in trace quantity in drug substance or drug product is inevitable. Therefore, their level should be controlled and monitored. They reinforce or diminish the pharmacological efficacy of the Active Pharmaceutical Ingredient's. [1] ICH defines impurities profile of a drug materials is "A description of the identified and unidentified impurities, present in a new drug substance."For Pharmaceutical products, impurities are defined as "substance in the product that are not the API itself or the excipient used to manufacture it " i.e. impurities are unwanted chemical that remains within the formulation or API in small amounts which can

influence Quality, Safety and Efficacy, thereby causing serious health hazards. [2] Qualification of the impurities is the process of acquiring and evaluating data that establishes biological safety of an individual impurity; thus, revealing the need and scope of impurity profiling of drugs in pharmaceutical research. [3] Identification of impurities is done by a variety of Chromatographic and Spectroscopic techniques, either alone or in combination with other techniques. [3-5]

Methodology and Experimental Work:

The author had developed a liquid chromatographic in bulk samples and pharmaceutical formulations. In this study PEAK 7000 isocratic HPLC with rheodine manual sample injector with switch (77251) was employed and the column used was thermohypersil BDS C18 (250 mmx4.6 mm, particle size 5 µm) column, Waters 2695 alliance with binary HPLC pump and a Waters 2998 PDA detector. Waters Empower 2 software was used for monitoring chromatographic analysis and data acquisition. Spectra lab DGA 20 A3 ultrasonic bath sonicator was used for degassing the mobile phase. Electronic balance ELB 300 was used for weighing the materials. The syringe used for injecting was 20 µL Hamilton syringe. DIGISUN pH meter was used for all pH measurements.

Sample preparation:

Accurately weighed Quantity of sample powder equivalent to 10 mg of Rosuvastatin Calcium was transferred into 100 mL of volumetric flask added 50mL of water and sonicated for 30 mins and make up the volume with mobile phase and filtered through the 0.45 µm membrane filter paper. 5 mL of the above solution is taken into 25 mL volumetric flask make up the volume with mobile phase. An aliquot of this solution was injected into HPLC system.

. Method Development and Optimization of Chromatographic Conditions

Inorder to develop the method, a study base line was recorded with the optimized chromatographic conditions set for Rosuvastatin Calcium and stabilized for about 30 minutes. A non-polar C18 column was chosen as the stationary phase for this study. The following studies were carried for this purpose.

Mobile Phase:

For getting sharp peak and line separation of the components, successive aliquots of the sample solution were recorded by the author until the reproducibility of the peak areas was adequate.

For ideal separation of the drug isocratic conditions, mixtures of commonly used solvents with or without different buffers in different combinations were tested as mobile phases on C18 stationary phase. A mixture of 0.01 M sodium acetate solution and methanol in the ratio of 600: 400 (v/v) was found to be the most suitable of all the combinations since the chromatographic peaks were better defined, resolved and showed a low tailing factor of 1.622 for Rosuvastatin Calcium. The analysis was carried at a flow rate of 1 mL/min. The injecting volume is 20 µL and the total run time 7 minutes.

Detection Wavelength:

UV-spectrophotometer was used to record the spectra of diluted solution of Rosuvastatin Calcium in methanol. The peaks of maximum absorbance wavelengths were observed. The spectra of the Rosuvastatin Calcium showed that a balanced wavelength was found to be 216 nm.

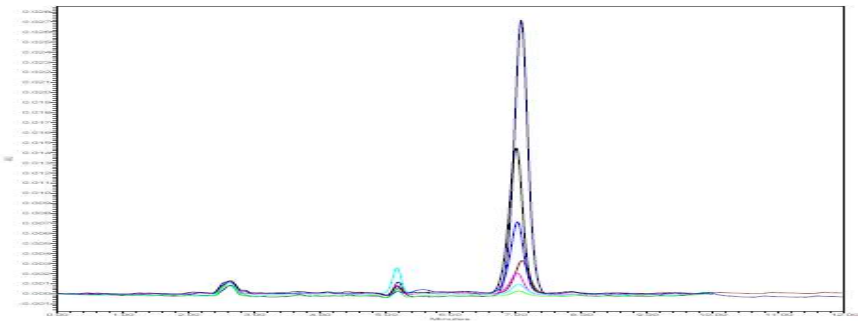

Fig 1: Standard chromatogram for Rosuvastatin Calcium

Table 1: Optimized Chromatographic conditions

S.No	Parameter	Value
1	Column	Inertsil- ODS C18 (250 mmx4.6 mm, particle size 5 µm)
2	Mobile phase	water (pH 5.2 adjusted with sodium acetate) and methanol in the ratio of 600: 400(v/v)
3	Flow rate	1.0 mL/min
4	Diluent	Mobile phase
5	Column temperature	25°C
6	pH	5.2
7	API Concentration	Rosuvastatin Calcium - 20 µg/mL
8	Run time	6 min
9	Retention time	Rosuvastatin Calcium -1.4 min.
10	Volume of injection	10 µL
11	Detection wave length	216 nm

Table 2: Linearity data of Rosuvastatin Calcium

S.No	Concentration (µg/mL)	Peak area	
1	10	1877189	Slope = 37490 C.C = 0.99 (~1.0)
2	15.00	2812563	
3	20.00	3747683	
4	25	4688354	
5	30	5621489	

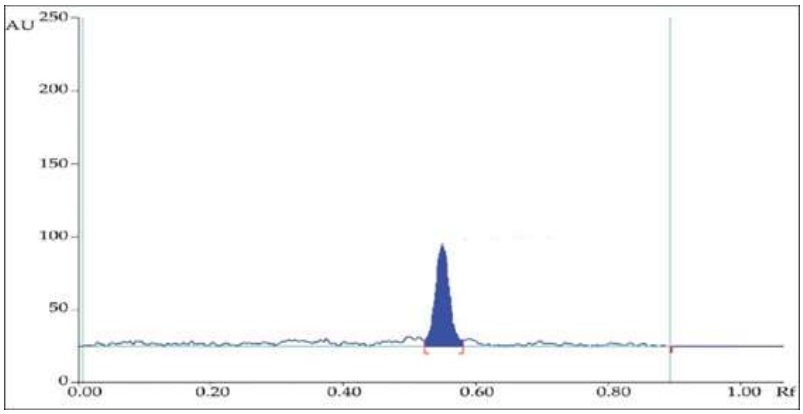

Fig. 2: Chromatograms of Linearity for Rosuvastatin Calcium.

Table 3: Intra – day precision of Rosuvastatin Calcium

S. No	Sample Weight (mg)	Methionine	% Assay
1	482.20	3746397	99
2	482.20	3746266	99
3	482.20	3741869	99
4	482.20	3740761	99
5	482.20	3740569	99
6	482.20	3749990	99
Average Assay:			99
STD			0.10
%RSD			0.10

Table 4: Inter – day precision of Rosuvastatin Calcium

S. No	Sample Weight (mg)	Methionine	% Assay (Methionine)
1	482.20	3746548	99
2	482.20	3745985	99
3	482.20	3746892	99
4	482.20	3748657	99
5	482.20	3746829	99
6	482.20	3748219	99
Average Assay:			99
STD			0.03
%RSD			0.03

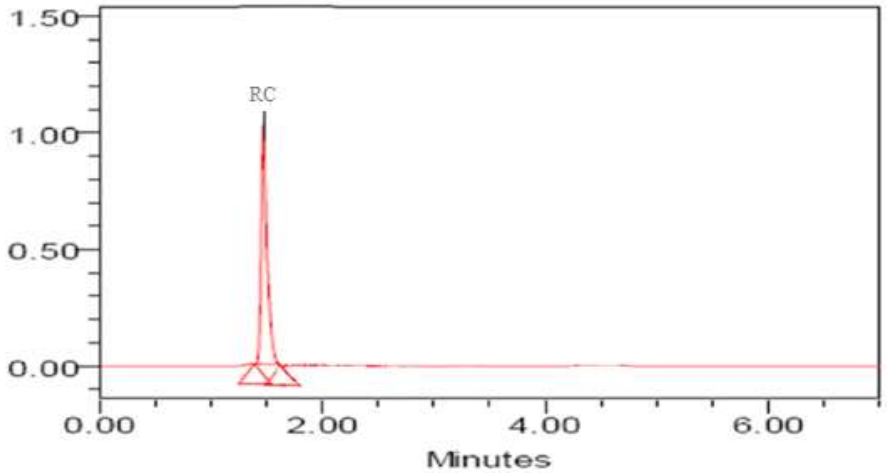

Fig.3: Accuracy Chromatograms-50% of Rosuvastatin Calcium

Table 5: Accuracy data of Rosuvastatin Calcium

Spiked Level	Sample Weight (mg)	Sample Area	µg/mL added	µg/mL found	% recovery	mean
50%	241.50	1875005	9.910	9.92	100.10 (~100)	100.04 (~100)
50%	241.50	1875013	9.910	9.92	100.10 (~100)	
50%	241.50	1879474	9.910	9.94	100.30 (~100)	
50%	241.50	1871012	9.910	9.90	99.89 (~100)	
50%	241.50	1870158	9.910	9.89	99.79 (~100)	
50%	241.50	1876423	9.910	9.92	100.10 (~100)	
100%	483.00	3747599	19.820	19.82	100.00 (~100)	99.99 (~100)
100%	483.00	3745976	19.820	19.81	99.94 (~100)	
100%	483.00	3749990	19.820	19.83	100.05 (~100)	
150%	725.00	5626444	29.751	29.76	100.03 (~100)	99.99 (~100)
150%	725.00	5623774	29.751	29.75	99.99 (~100)	
150%	725.00	5620015	29.751	29.73	99.92 (~100)	
150%	725.00	5624450	29.751	29.75	99.99 (~100)	
150%	725.00	5626008	29.751	29.76	100.03 (~100)	
150%	725.00	5626410	29.751	29.76	100.03 (~100)	

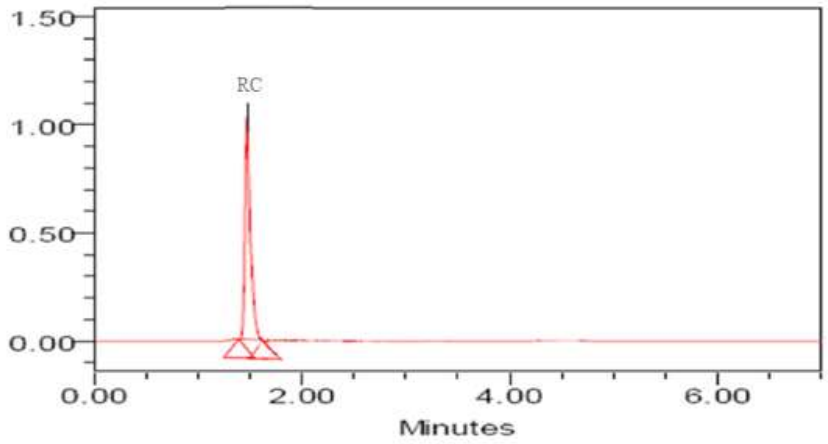

Fig4: Accuracy Chromatograms-100% of Rosuvastatin Calcium

Table 6: Ruggedness of Rosuvastatin Calcium

Injection number	Peak area
1	3742362
2	3740123
3	3739472
4	3720912
5	3701634
6	3692345
7	3681204
Mean	3716864.5714
SD	23370.9432
% RSD	0.62878

Table 7: Robustness data of Rosuvastatin Calcium

S No	Sample name	Change	Name	RT	Area	Tailing	Plate count
1	Flow1	0.2 mL/min (1.0-0.2)	Rosuvastatin Calcium	1.827	4646829	1.524	2701
2	Flow2	0.2 mL/min (1.0+0.2)	Rosuvastatin Calcium	1.294	2973403	1.541	2932
3	Temp1	5oC (25-5)	Rosuvastatin Calcium	1.444	3674010	1.586	2788
4	Temp2	5oC (25+5)	Rosuvastatin Calcium	1.439	3689369	1.580	2652

Table 8: Estimation of Rosuvastatin Calcium from its formulation

Formulation	Dosage	Sample conc. µg/mL	Sample Area	Amount Found µg/mL	% assay
Crestor	Rosuvastatin Calcium	20	3747649.1	19.98	99.9

Conclusion

The development and validation of a High-Performance Liquid Chromatography (HPLC) method for the estimation of Rosuvastatin Calcium was successfully accomplished. The method demonstrated robust and reliable performance, with high precision, accuracy, and reproducibility. Key validation parameters, including linearity, limit of detection (LOD), limit of quantification (LOQ), specificity, and robustness, were thoroughly evaluated and met

the stringent criteria set forth by regulatory guidelines. The optimized HPLC method employed a suitable mobile phase composition, flow rate, and detection wavelength, which ensured efficient separation and detection of Rosuvastatin Calcium with minimal interference from excipients or potential degradation products. The calibration curve exhibited excellent linearity over the concentration range studied, with a correlation coefficient (R^2) consistently greater than 0.999, underscoring the method's sensitivity and appropriateness for quantitative analysis. In summary, the developed HPLC method is highly effective for the routine analysis of Rosuvastatin Calcium in bulk and dosage forms, offering a reliable, efficient, and precise analytical tool that meets regulatory requirements. This validated method can be confidently employed for quality control and routine analysis, ensuring the efficacy and safety of Rosuvastatin Calcium-containing pharmaceutical products.

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