HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD DEVELOPMENT AND VALIDATION OF ROSUVASTATIN CALCIUM API

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Abstract

A simple and specific liquid chromatographic method was developed and validated to the analysis of Rosuvastatin Calcium API content using the HPLC. All the parameters were determined according to ICH guide lines. The detection wavelength of eluent is 254 nm, MeOH and water (90:10) are used as mobile phases by isocratic elution and a MICROSOLV make Cogent ABX C18 5µ 100Aº (150×4.6mm) Part Number: 85528-15P column is used for the separation with a flow rate 1.0 ml/min at ambient temperature to obtain good chromatographic separation results. The linearity data obtained for the concentration ranges from 200 µg to 10 µg obtained a linear curve. The response of the rosuvastatin calcium is linear at 10% to 200%. The correlation coefficient is greater than 0.99. The developed method is validated as per ICH guidelines for the parameters of linearity, suitability, LOD & LOQ, precision and robustness.

***Keywords**: Rosuvastatin calcium, isocratic elution, ambient temperature.*

INTRODUCTION:

Rosuvastatin calcium is chemically bis((3R,5S,6E)-7-[4-(4-fluorophenyl)-2-(N-methylmethanesulfonamido)-6-(propan-2-yl) pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoate) having a chemical formula $C_{22}H_{27}FN_3O_6S$ and molecular weight 481.538 g/mol. Generally, it is available in Crestor, Ezallor trading names.[1]

Rosuvastatin, a lipid lowering drug belonging to the statin class of medications, was patented in 1991 and approved for medical use in the United States in 2003. It is available as a generic medication and is used to lower the risk of cardiovascular disease and manage elevated lipid levels by inhibiting the endogenous production of cholesterol in the liver. Despite its effectiveness in reducing cholesterol, there have been some adverse effects associated with its use, including abdominal pain, nausea, headaches, and muscle pain.[1]

Several analytical techniques have been described for the estimation of Rosuvastatin calcium in bulk and pharmaceuticals. Madhina Moid, et al.,[2] conducted a method validation study in tablet dosage forms. This study utilized c18 column and mobile phase ACN: phosphate buffer adjusted to pH 4.5.[2] Another simple and consistent method reported for determination of Rosuvastatin calcium Rameshwar Gholve et al., by using mobile phase composed of 10mm phosphate buffer with with 1.1g octane-1-sulfonic acid sodium salt having pH 2.5 and acetonitrile in the ratio of 500:500 v/v. maintained at 25^0C temperature.[3] H.O. Kaila, et al., developed a method for assay of Rosuvastatin calcium for determination of content uniformity. They operated a RP-HPLC instrument by isocratic separation by using c8 5micron particle size column flow rate was regulated at 1.5 ml/min using PDA, mobile phase composition ACN: H_2O (40:60) pH is adjusted to 3.5 with phosphoric acid.[4] Previously used methods were time consuming and

complex with high proportion of organic solvents. Therefore, in the present research a simple and accurate method was developed and validated for determination of Rosuvastatin calcium API.

MATERIALS AND METHODS

Chemicals & reagents: Rosuvastatin calcium API, Acetonitrile HPLC grade, methanol HPLC grade, Formic acid, water RANKEM HPLC grade were used.

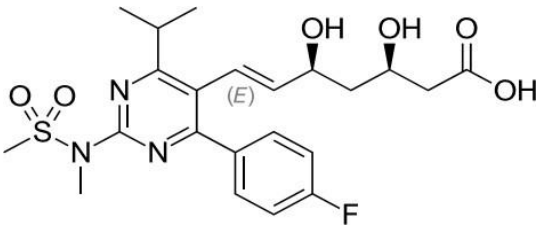


Figure 1: molecular structure of rosuvastatin[1]

Instruments:

A high-performance liquid chromatography- waters alliance was equipped with a compact photo diode array detector with an auto injector. The chromatogram was recorded using empower software. O.45µm filter paper, electronic analytical balance and ultrasonic bath were used.

SAMPLE PREPARATION:

Diluent preparation: The diluent of ratio 80:20 v/v was prepared by mixing accurately, measured volumes of 80ml of methanol and 20 ml of water in a 100 ml beaker. Stirred well, sonicated for 5 minutes, and stored well.

Blank preparation: Diluent is taken as blank

Preparation of Rosuvastatin API sample stock solution 1 [2mg]: Accurately weighed 10 mg Rosuvastatin API sample and transferred it into a 5ml volumetric flask and made up to the mark with diluent and kept in the ultrasonic bath for 2 min for complete dissolution of sample.

Preparation of Rosuvastatin API sample stock solution 2 [200µg]: Accurately measured 500µl of sample solution from sample stock solution 1 and transferred it into a 5 ml volumetric flask and made up to the mark with diluent.

Preparation of test sample solution [100µg] Accurately measured 1 ml of sample solution from sample stock solution 2 and transferred it into a glass vial and added 1 ml of diluent into the glass vial and kept on vortex mixture.

METHOD DEVELOPMENT WORKFLOW:

TRIAL-1

Mobile phase A: Accurately measured 0.1 ml of formic acid and transferred into a beaker to this 100ml of HLPC grade water is added and shaken cursorily and transferred into solvent reservoir.

Mobile phase B: Accurately measured 100ml of HPLC grade 100% acetonitrile and transferred into olvent reservoir

Chromatographic conditions:

Column	:	Cogent RP ABX c18 5µ 100^0 (150×4.6mm)
Mobile phase	:	Mobile phase A : mobile phase B (10:90)
Diluent	:	Methanol :water (80:20)
Flow rate	:	0.5ml/min
Column temperature	:	Ambient
Sample temperature	:	Ambient
Injection volume	:	10µl
Run time	:	10 min
Elution	:	Isocratic mode
Absorbance wavelength:		254nm

Fig2: Blank chromatogram of trail1

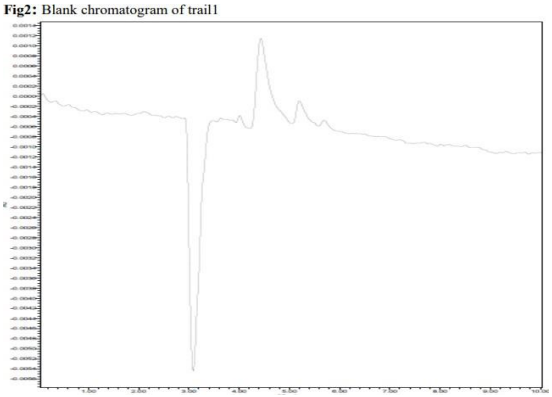


Fig 3: Typical chromatogram of sample trail 1

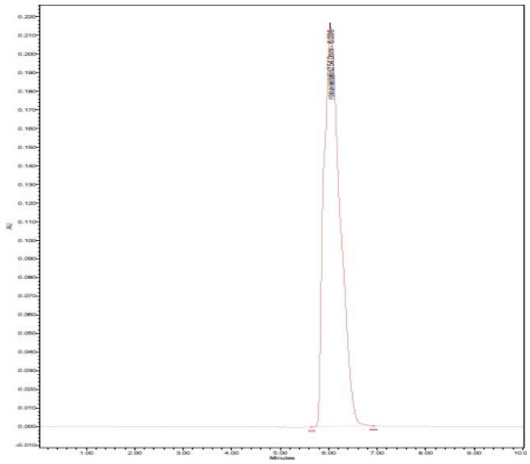


Fig 4: Blank chromatogram of trail 2

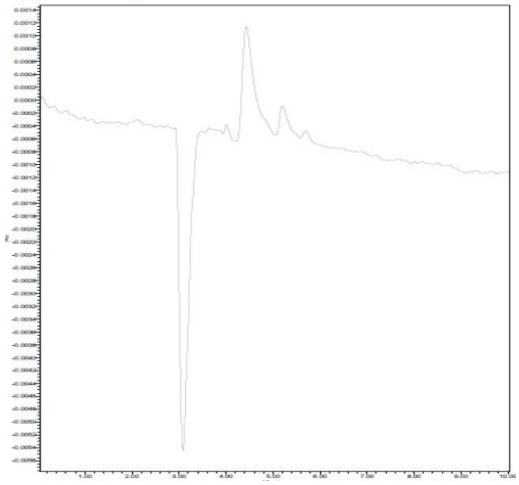


Table 2: Results of trail 1

Parameter	Value
Retention time	6.0 min
Theoretical plate count	1274
Peak area	4952455
Tailing factor	1.48
Peak height	214217

Observation: The peak shape is good but showing higher tailing factor and having a poor amount of plate count which is not acceptable so moved on the next trail.

TRAIL-2

Mobile phase A: Accurately measured 0.1 ml of formic acid and transferred into a beaker to this 100ml of HLPC grade water is added and shaken cursorily and transferred into solvent reservoir

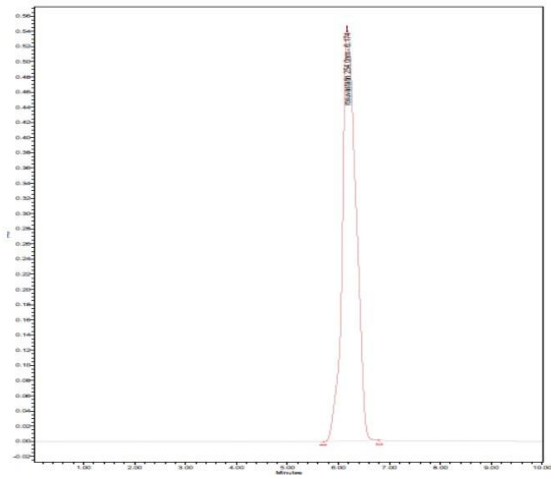
Mobile phase B: Accurately measured 100ml of HPLC grade 100% acetonitrile and transferred into solvent reservoir

Table 3: Results of trail 2

Parameter	Value
Retention time	6.1min
Theoretical plate count	2228
Peak area	10278997
Tailing factor	1.09
Peak height	545380

Observation: The peak shape is good; tailing factor is in acceptance range but having a poor number of plate count which is not acceptable so moved on the next trail.

Fig 5: Typical chromatogram of sample trail 2



TRAIL-3 Optimized method

Mobile phase: accurately measured 180 ml of methanol volumetrically of HPLC grade transferred into the beaker and measured 20 ml of HPLC grade water into the beaker and this methanol water solution is transferred into the solvent reservoir.

Fig 6: Blank chromatogram of trail 3

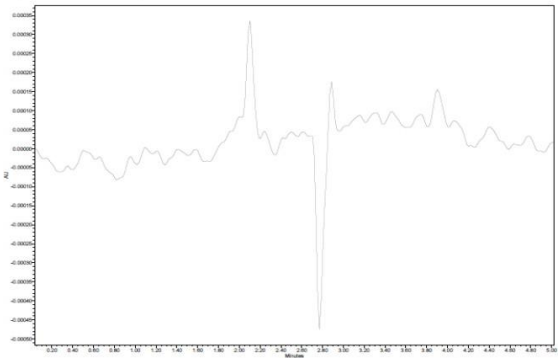


Fig 7: Typical chromatogram of sample trail 3

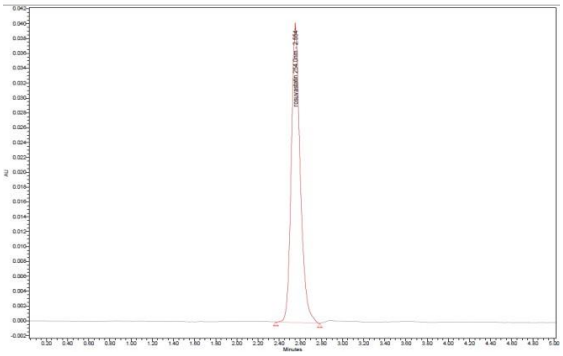


Table 4: Results of trail 3

Parameter	Value
Retention time	2.5min
Theoretical plate count	4345
Peak area	237040
Tailing factor	1.2
Peak height	39883

Observation: The peak shape is good, tailing factor is also in the acceptance range and having an acceptable number of plate count so this method is optimized and validated the method development.

METHOD VALIDATION

The validation of a test method through laboratory studies is known as method validation, which ensures that the method's performance characteristics meet the requirements of the intended analytical applications. This process provides documented evidence of the method's reliability during normal use, giving assurance that it performs as intended. The US Food and Drug Administration (FDA) recognizes the specifications listed in the current edition of the United States Pharmacopeia (USP) as legally recognized for determining compliance with the Federal Food, Drug, and Cosmetic Act. The validation of analytical methods is carried out in accordance with the guidelines set by the International Council for Harmonization (ICH).[5]

Validation parameters:

- System suitability
- Linearity
- Limits of detection
- Limits of quantification
- Precision
- Robustness

system suitability:

The assessment of system suitability involves evaluating the elements of an analytical system to demonstrate that the system's performance aligns with the method's specified standards. To verify the system's suitability, 5 duplicate injections (n=5) were administered, in addition to a single bracketing injection, resulting in a total of 6 duplicate injections (n=6). The evaluation of these outcomes encompassed the analysis of tailing factor retention time peak height, peak area, and theoretical plates, considering their RSD% and STD.[6]

Table 5: system suitability of HPLC method for determination of Rosuvastatin calcium.

S.no	RT	Peak area	Peak height	Peak tailing	Plate count
1	2.47	232509	41062	1.1	4385
2	2.54	232339	39899	1.16	4430
3	2.55	237040	39883	1.18	4345
4	2.54	236523	39748	1.2	4708
5	2.55	231578	43496	1.2	4341
Bracketing standard 1	2.55	230030	43547	1.15	4312
Mean	**2.53**	**233336.5**	**41272.5**	**1.16**	**4420.16**
STD	**0.03**	**2813.35**	**1805.70**	**0.03**	**146.83**
%RSD	**1.23**	**1.20**	**4.37**	**3.24**	**3.32**

Acceptance criteria:

- The % RSD for peak areas of rosuvastatin not more than 2.
- The % RSD for retention time of rosuvastatin is not more than 0.5
- The theoretical plate count of rosuvastatin is consisting more than 2000
- The tailing factor of rosuvastatin peaks is not more than 2.

Linearity:

Linearity is the ability of analytical procedure to obtain a linear response that is directly proportional to the concentration of analyte in the sample stock solution 2 (200ug/ml), serial dilutions were prepared in a range of 10, 25, 50, 75, 100, 200 ug/ml (n=6) concentrations. A linearity curve is plotted against prepared concentrations. A linearity curve is plotted against prepared concentrations and peak areas and regression equation computed to obtain value of coefficient of determination (r^2).[5]

Table 6: Linearity data of Rosuvastatin calcium

S.no	Concentration (ug/ml)	Concentration level	Peak area
1	10	10%	12898
2	25	25%	25574
3	50	50%	52723
4	75	75%	78883
5	100	100%	113317
6	200	200%	205380

Fig 8: linearity curve plot between concentration and peak area

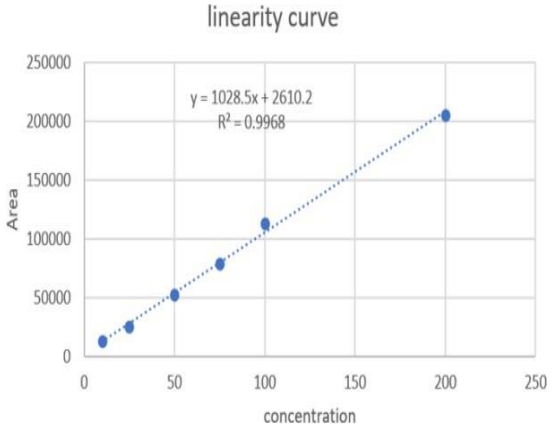


Table 7: Results from linearity data of Rosuvastatin calcium

Correlation coefficient (r)	0.998399
Regression coefficient (R^2)	0.9968
Slope	1028.508
Intercept	2610.196

Acceptance criteria:

- The correlation coefficient (r) should be not less than 0.99

Conclusion:

Based on the above data, the experimental results meeting the acceptance criteria. Hence the method is linear.

LOD & LOQ

The lowest concentration of an analyte in a sample that can be detected but not quantified is referred to as the limit of detection (LOD), which is determined by the signal to noise ratio (3:1). On the other hand, the limit of quantification (LOQ) is

the lowest concentration of an analyte in a sample that can be quantified with acceptable precision and accuracy by the test method. Typically, the LOQ is estimated from a determination of signal noise ratio (10:1).[7]

LOD can be calculated from the formula

LOD=3.3σ/S[7]

LOD can be calculated from the formula

LOD=10σ/S[7]

Where, σ is the standard deviation of the response at low end of the calibration curve and S is the slope of the calibration curve.

Table 8: LOD & LOQ VALUES of Rosuvastatin calcium

Parameter	RT	Linearity(ug/ml)	Value
LOD	2.53	10-200	2.87ug/ml
LOQ	2.53	10-200	9.47ug/ml

System Precision

The definition of precision for an analytical method is based on the consistency of results obtained from multiple tests on a uniform sample. To assess the precision of the assay method at 100% concentration, six injections were made and peak areas & retention time were measured to determine the %RSD of Rosuvastatin calcium.

Table 9: Results for precision of Rosuvastatin calcium

Injection	Retention time	Peak area
1	2.47	232358
2	2.54	232467
3	2.55	237297
4	2.54	236478
5	2.55	231693
6	2.55	230976
Mean	**2.53**	**233544.8**
STD	**0.031411**	**2656.34**
%RSD	**1.239918**	**1.1374**

Acceptance criteria:

- The %RSD for peak areas of rosuvastatin calcium from six replicate injections of standard not be > 2.

Robustness

To evaluate the robustness of the developed RP- HPLC method, small deliberate variations in the optimized parameters were made in chromatographic conditions like mobile phase composition and wavelengths.

The effect of change in mobile phase composition and wavelength of detection on retention time and tailing factor were examined. The parameters and values obtained are mentioned in the table.

Table 10: Results for Robustness of Rosuvastatin calcium

Parameter	Value	Retention time	Tailing factor
Mobile phase Composition (MeOH:H_2O)	80:20	2.9	1.41
	90:10	2.5	1.12
	100%MeOH	1.8	1.32
wavelength	252nm	2.12	1.25
	254nm	2.13	1.12
	256nm	2.13	1.32

CONCLUSION:

The mobile phase chosen for the HPLC procedure was a mixture of methanol and water (90:10), in which Rosuvastatin was found to be soluble. The run time of the procedure was determined to be 5 minutes. To ensure the reliability of the method, it was validated for system suitability, linearity, precision, robustness, LOD, and LOQ. The system suitability parameters were found to be within the acceptable limits, indicating that the system was suitable for conducting the assay. The method demonstrated linearity across a concentration range of 10-200 ug/ml. Furthermore, the absence of interference from the mobile phase confirmed the method's specificity. Additionally, the method was deemed robust, as evidenced by the insignificant variation in results when the flow rate and wavelength were altered, as well as when different analysts performed the analysis.

The developed method for analyzing pharmaceutical formulation showed a high level of agreement in the assay results. This indicates that the proposed method is a reliable approach for obtaining accurate results and is suitable for routine analysis of Rosuvastatin calcium in pharmaceutical formulations.

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