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VALIDATED RP-HPLC METHOD FOR DETERMINATION OF ROSUVASTATIN CALCIUM IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT: A rapid, sensitive and specific RP-HPLC method was developed and validated for determination of Rosuvastatin Calcium in bulk and tablet dosage form. Chromatography was carried out on a Enable C18G (250 x 4.6 mm i.d.,5µ) column using filtered and degassed mixture of acetonitrile and water in the ratio of 75:25 % v/v as mobile phase at a flow rate of 0.6 ml/min and effluent was monitored at 252 nm. The method was linear over the concentration range of 5 – 40 µg/ml with a correlation coefficient of 0.999. The retention time of the drug was 3.097 mins. The proposed method was validated by determining sensitivity, accuracy, precision, robustness studies. The developed method was effectively applied to tablets of Rosuvastatin Calcium and the % assay of the drug was found to be 99.98 %. The method is simple, rapid, accurate, precise and reproducible and hence can be applied for routine quality control analysis of Rosuvastatin Calcium in pure and tablet dosage form.

INTRODUCTION: Rosuvastatin calcium, a new member of a class of cholesterol lowering drugs commonly referred to as "statins", was approved for the treatment of dyslipidemia[1-3]. Rosuvastatin calcium (ROS) is chemically bis [(E)-7-[4-(4-fluorophenyl)-6-isopropyl - 2- [methyl - (methyl sulfonyl) amino] pyrimidin-5-yl] (3R, 5S)-3, 5-dihydroxy hept- 6-enoicacid] calcium salt (**Fig. 1**). It is a synthetic lipid lowering agent, selective and competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, the key rate-limiting enzyme of cholesterol biosynthesis in liver. ROS is used to reduce the amounts of LDL cholesterol, total cholesterol, triglycerides and a lipoprotein B in the blood.

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ROS also modestly increases the level of HDL cholesterol in the blood. These actions are important in reducing the risk of atherosclerosis, which in turn can lead to several cardiovascular complications such as heart attack, stroke and peripheral vascular disease.

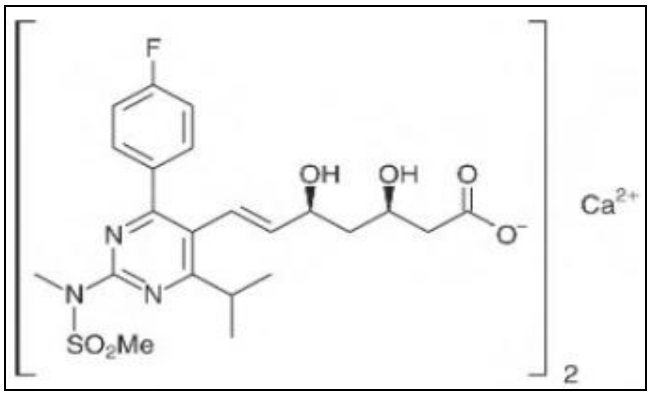

FIG. 1: CHEMICAL STRUCTURE OF ROSUVASTATIN CALCIUM

ROS peak plasma concentrations were reached by 3–5 hrs following oral administration in humans [4].

To the best of knowledge, Rosuvastatin Calcium has been determined by Spectrophotometric methods[5-8], Stability indicating methods[10-12], and RP-HPLC[13-15], LC-MS[16-17]. The present research work describes the rapid, accurate, sensitive and reproducible RP-HPLC method for estimation of Rosuvastatin Calcium from the tablet formulation.

MATERIALS AND METHODS:

Instrumentation:

Shimadzu HPLC comprising of LC-20AD binary gradient pump, a variable wavelength programmable SPD-20A detector and an SCL 20A system controller. A Rheodyne injector fitted with a 20µL loop was used and data were recorded and evaluated by use of LC solutions software.

Chemicals and Reagents:

ROS pure sample was procured as gift sample. The tablets were procured from local pharmacy. Label claim of each tablet for ROS was 10 mg respectively. Acetonitrile HPLC grade was purchased from E.Merck. Milli-Q (Qualigens) water HPLC grade was used throughout the experiment.

Chromatographic Conditions:

Chromatographic analysis was performed on a Enable C18G (250 x 4.6 mm i.d., 5µ) column. The mobile phase consisted of acetonitrile: water (75: 25 v/v) which is degassed and filtered through 0.2 µm membrane filter before pumping into HPLC system.

- Mobile phase: Acetonitrile: water (75:25 % v/v).
- Detection wavelength: 252 nm.
- Flow rate: 0.6 ml/min.
- Injection volume: 20 µl.
- Column temperature: ambient.
- Runtime: 8 mins.
- Run mode: isocratic.

Preparation of standard stock solution:

10mg of ROS was accurately weighed and transferred in 100 ml volumetric flask and the content in the flask was dissolved in 25ml of Acetonitrile. After the immediate dissolution, the volume was made up to the mark with same solvent. This standard stock solution was further diluted with mobile phase to get 100µg/ml of ROS.

The chromatogram for standard ROS was shown in **Fig. 2**.

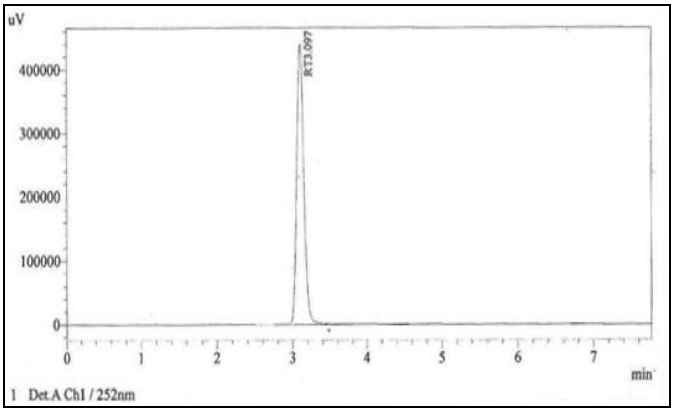

FIG. 2: CHROMATOGRAM OF ROSUVASTATIN CALCIUM

Preparation of sample solution:

Commercial tablets of ROS were taken and their average weight was determined, they were crushed to fine powder. Powder equivalent to 10mg of ROS was taken in 100ml volumetric flask and dissolved in 75ml of acetonitrile with shaking for 5-10 minutes and then sonicated. The supernatant liquid was filtered through 0.2µm membrane filter and then transferred to 100 ml volumetric flask and volume was made up with acetonitrile. After that 10 ml of the above solution was diluted up to 100 ml with mobile phase.

RESULTS:

Method Validation: [18]

System Suitability: System suitability 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, tailing factor, HETP were investigated by injecting standard solutions of the drugs six times and the results are given in **Table 1**. From the results it was observed that all the values are present within the limits indicating good performance of the system.

TABLE 1: SYSTEM SUITABILITY PARAMETERS

Parameter	Result
Retention time	3.097 (mins)
Theoretical plates	4590
Tailing factor	1.1
HETP	32.67

Linearity: Standard solutions for linearity tests were prepared from the primary standard stock solution in the concentration range of 5-40µg/ml.

The prepared solutions were injected and their peak areas were measured and tabulated in **Table 2**. The calibration curve was constructed by plotting concentration on X- axis versus and peak areas on Y- axis and linear regression equation was calculated as shown in (**Fig. 3**).The calibration curve was found to be linear with correlation coefficient of 0.999.

TABLE 2: CALIBRATION CURVE DATA

S.no	Concentration (µg/ml)	Peak area
1	5	282595
2	10	591671
3	15	965607
4	20	1282481
5	30	1936825
6	40	2607083

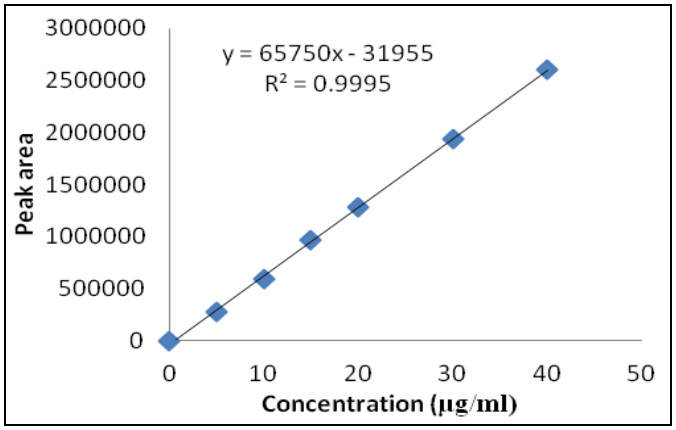

FIG. 3: CALIBRATION CURVE OF ROSUVASTATIN CALCIUM

Specificity:

Specificity was checked for the interference of excipients in the analysis of sample solution and was determined by injecting sample solution with added exipients under optimized chromatographic conditions to demonstrate separation of ROS from excipients. There is no interference of excipient peak on the peak of ROS indicating the high specificity of method.

Precision:

Method Precision: Method precision was performed by preparing six different samples from the same sample pool. Each solution was injected in triplicate under the same conditions and mean value of peak area response for each solution was taken. The relative standard deviation of ROS in six sample solutions was calculated. Relative standard deviations obtained for ROS was 0.21%. The results are tabulated in **Table 3.**

TABLE 3: METHOD PRECISION DATA

S.no	Concentration (µg/ml)	Peak Area
1	20	1282481
2	20	1287925
3	20	1286725
4	20	1283221
5	20	1282913
6	20	1280535
Mean		1283967
S.D		2790.218
%RSD		0.21

Inter-Day Precision:

The Inter-day precision of the sample was measured on three concentrations of the drug on three different days .The measurement of the peak areas were expressed in terms of % RSD and were found to be <1%. The results are shown in **Table 4**.

TABLE 4: INTER-DAY PRECISION

Con. taken (µg/ml)	Mean Peak Area*	%RSD
10	592571	0.16
15	975617	0.24
20	1283731	0.14

*average of six determinations

Accuracy:

The accuracy of the method was established using recovery technique i.e. external standard addition method. The known amount of standard was added at three different levels to pre analysed sample. Each determination was performed in triplicate. The results of recovery study were presented in **Table 5**.

TABLE 5: RECOVERY STUDIES

Drug	Spiked level (%)	Amount taken (µg/ml)	Amount found (µg/ml)	Percent Recovery (% w/w)±RSD
ROS	80	8	7.97	99.6±0.56
	100	10	10.03	100.3±0.74
	120	12	11.96	99.6±0.63

Robustness:

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as changes in wave length, composition of mobile phase and flow rate. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust. The results were shown in **Table 6**

TABLE 6: ROBUSTNESS STUDIES

Condition	Modification	Peak area	Mean % RSD
Mobile phase composition (v/v)	80: 20	591523	0.11
Flow rate (ml/min)	0.5	589162	0.21
Wavelength (nm)	257	592123	0.15

Sensitivity:

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision and variability. The LOD and LOQ were calculated from linear curve using formulae

$LOD = 3.3 * \sigma / S$

$LOQ = 10 * \sigma / S$

(Where σ = the standard deviation of the response and S = Slope of calibration curve).

The results were shown in **Table 7**.

TABLE 7: LOD AND LOQ DATA

Drug	LOD (µg/ml)	LOQ (µg/ml)
ROS	0.017	0.052

Application of Proposed method:

The assay of marketed sample (Tablet formulation) for Rosuvastatin calcium is summarized in **Table 8**.

TABLE 8: ASSAY RESULTS OF TABLET FORMULATION

Tablet	Label claim (mg)	Assay (%label claim)	%RSD
ROZAVEL	10	99.98	0.26
ROSUVAS	10	99.87	0.15

DISCUSSION: By applying the proposed method, the retention time of Rosuvastatin calcium was found to be 3.097mins. Linearity range was observed in concentration range of 5-40µg/ml. The regression equation of concentration over peak area was found to be y = 65750x - 31955 (r = 0.999) where y is the peak area and x is the concentration of Rosuvastatin Calcium (µg/ml). The number of theoretical plates was found to be 4590, which indicates efficient performance of the column. The tailing factor was found to be 1.1, which indicates good shape of peak.

The limit of detection and limit of quantification was found to be 0.017µg/ml and 0.052µg/ml, indicating the sensitivity of the method. The percentage of recovery in the range of 99.6-100.3% indicates that the proposed method is highly accurate. The % RSD value <1% for both method and interday precision indicate the high precision of method. The use of acetonitrile and water in the ratio of 75: 25 % v/v resulted in peak with good shape and resolution. No interfering peaks were found in the chromatogram within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by proposed HPLC method.

CONCLUSION: The proposed method has advantage of simplicity and convenience for the separation and quantitation of Rosuvastatin Calcium and can be used for the assay in its dosage form. Also, the low solvent consumption and short analytical run time lead to environmentally friendly chromatographic procedure. The method is accurate, precise, rapid and selective for estimation of Rosuvastatin Calcium in tablet dosage form. Hence it can be applied routine analysis of Rosuvastatin Calcium in formulation.

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