WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 13, 1439-1446. Research Article ISSN 2277– 7105

RP-HPLC METHOD DEVELOPMENT FOR ESTIMATION OF ROSUVASTATIN IN BULK AND TABLET DOSAGE FORM

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Article Received on
20 June 2023,

Revised on 10 July 2023,
Accepted on 31 July 2023

DOI: 10.20959/wjpr202313-29239

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ABSTRACT

Background: The RP-HPLC method development for rosuvastatin in bulk and tablet dosage form was developed and validated in this research. This was a suitable, clear, accurate and sensitive method for method development of rosuvastatin drug. Acetonitrile: water [75:25 % v/v] was used in concentration sutiable to obtain proper chromatogram. The separation was performed using an HPLC method with a UV detector and Openlab EZchrom program, as well as a Water spherisorb C18 column (100 mm × 4.6; 5m). Acetonitrile was pumped at a flow rate of 0.6 mL/min with a 0.1N NaOH solution balanced to a detected at 252 nm. **Result:** The developed RP-HPLC method yielded a suitable retention time for rosuvastatin of 3.097 min, which was optimized using the Design Expert-12 software. The linearity of the established method was verified with a correlation coefficient (r2) of 0.999 over the concentration range of 5-40µg/ml.percent recovery was 99.6 to 100.3% respectively. The percentage RSD for the method's precision was found to be less than 2.0%. The percentage recoveries were discovered to be between 99.5, 100.4, 99.6% respectively for concentration of 80,100,120 µg/mL. **Conclusion:** The developed and validated RP-HPLC system takes less time and can be used in the industry for routine quality control/analysis of bulk drug and marketed rosuvastatin products.

KEYWORDS: RP-HPLC, QbD, Rosuvastatin, Acetonitrile, Development, Validation, etc.

INTRODUCTION

Rosuvastatin[1,2] chemically is (3R,5S,6E)-7-[4-(4- fluorophenyl)-2-(N-methylmethanesulfonamido)-6- (propan-2-yl) pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid. It belongs to family of statins and used in combination with exercise, diet and weight loss to treat HDL and related conditions which prevent cardiovascular diseases. Rosuvastatin

is a competitive inhibitor of HMG-CoA reductase. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate. This is an early rate-limiting step in cholesterol biosynthesis. Rosuvastatin acts primarily in the liver. Decreased hepatic cholesterol concentrations and stimulate the upregulation of hepatic LDL receptors which increases hepatic uptake of LDL. It also inhibits hepatic synthesis of VLDL. The overall effect is a decrease in plasma LDL and VLDL.

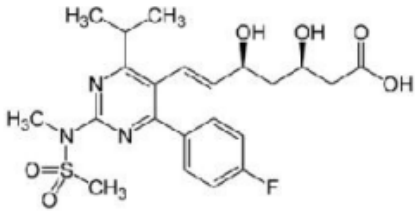

Fig. 1: Rosuvastatin.

MATERIALS AND METHODS

INSTRUMENT

Liquid chromatographic system from Shimadzu (LC-20AT) comprising of manual injector, double reciprocating plunger pump LC-20ATVp for constant flow and constant pressure delivery and Photodiode array detector SPD-M20A connected to software LC solution for controlling the instrumentation as well as processing the data generated was used.

The HPLC system consisted of a binary pump (model Waters 515 HPLC pump), auto sampler (model 717 plus Auto sampler), column heater and PDA detector (Waters 2998). Data collection and analysis were performed using Empower - version 2 software. Separation was achieved on Kromasil C18 column (250 mm × 4.6 mm, 5.0 µ) column maintained at 500C using column oven.

The UV detection was performed at 240 nm, mobile phase was forced at the speed of 2 ml/min and analytical column was retained at 25OC 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 then filtered through 0.2 µm membrane filter before passing through pump 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.
- Run time-8 mins.
- Run mode-isocratic.
- Run mode-isocratic.

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

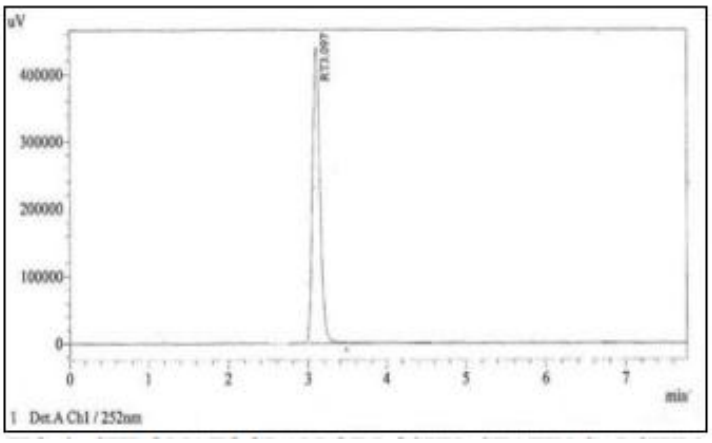

Fig. 2: Chromatogram of Rosuvastatin Calcium.

PREPARATION OF STANDARD STOCK SOLUTIONS

Standard stock solution (1000µg/ml) of Rosuvastatin was prepared by dissolving 100 mg of Rosuvastatin in a 100ml volumetric flask and dissolved it in distilled water to get a concentration of 1000µg/ml.

PREPARATION OF WORKING STANDARD SOLUTIONS

The working standard solutions of Rosuvastatin was prepared by diluting 1 ml of standard stock solution to 10 ml with distilled water in a 10ml volumetric flask to get the concentration of 100µg/ml.

PREPARATION OF CALIBRATION STANDARD

Accurately 1 to 6ml of working standard solution of Rosuvastatin was transferred to a series of 10ml volumetric flasks and the volume was made upto the mark with 0.1N NaOH to produce 1-6µg/ml solutions. The absorbance of the resulted solutions was measured at 240nm. The calibration curve was constructed by plotting absorbance against concentration.

PREPARATION OF SAMPLE SOLUTIONS

Preparation of sample solution 10 tablets of Roseday. Each tablet containing 10mg of Rosuvastatin. Weighed accurately and made into a fine powder. The tablet powder equivalent to 10mg of rosuvastatin was weighed accurately. Transferred it into a 10ml volumetric flask and add 4ml of 0.1N NaOH, mixed it well. Sonicated it for 10 mins using ultrasonicator. The volume was made upto the mark with the same solvent to get 1000µg/ml. Rosuvastatin 10µg/ml sample solution was prepared by diluting 0.1ml of 1000µg/ml of the stock solution with 0.1N NaOH. Accurately 3ml of 10µg/ml solution was taken and added to 10ml volumetric flask and made upto the mark with 0.1N NaOH to get 3µg/ml of Rosuvastatin and the absorbance of the was measured at 240nm.

VALIDATION OF THE DEVELOPED METHOD

The developed method was validated for accuracy, precision, linearity, limit of detection, limit of quantitation and robustness as per ICH guidelines.[3]

RESULTS AND DISCUSSION

Method Validation[4]

System Suitability[4]

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**. The number of theoretical plates should be greater than 2500. The tailing factor should be less than 2.0. 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 Parameter.

Parameters	Results
Retention time	3.097 (mins)
Therotical plates	4590
Tailing factor	1.1
HETP	32.67

Linearity[4]

Standard solutions for linearity were prepared from the primary standard stock solution. The concentration range of solution is 5-40µg/ml. The prepared solutions were injected and their

peak areas were measured as shown in in **Table 2.** The calibration curve was ploted by concentration versus peak 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.

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

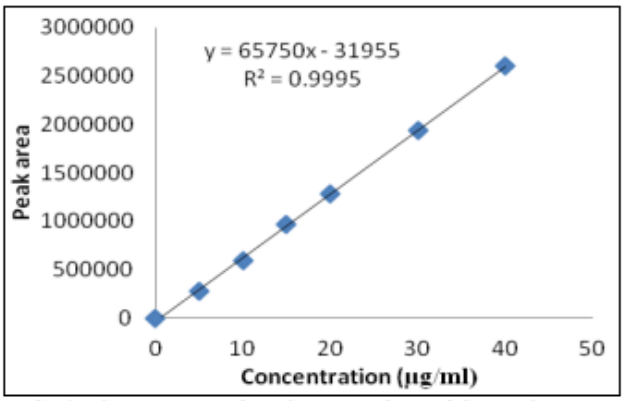

Fig. 3: Calibration Curve Of Rosuvastatin.

Specificity[4]

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

PRECISION

Method for Precision

Method precision was performed by preparing 6 different samples from the same sample pool. Each solution was injected in triplicate under the same conditions. Mean value of peak area response for each solution was taken. The relative standard deviation of Rosuvastatin in 6 sample solutions was calculated. Relative standard deviations obtained for Rosuvastatin was 0.21%. The results are tabulated in **Table: 3**[4]

Table 3: Method Precision Data.

Sr 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[4]

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.(µg/ml)	Mean Peak Area (average of six determinations)	%RSD
10	592569	0.16
15	975615	0.24
20	1283735	0.14

Accuracy[4,5,6]

Accuracy is also called as Recovery study.[5,6] Accuracy of proposed method was established using recovery study which is performed by standard addition method.[5,6] i.e. external standard addition method.[4] A known amount of standard drug solutions were added to pre analyzed sample solutions.[4] The concentrations in the range of 80%, 100% and 120% and re-analyzed it by the proposed method.[5,6] Each determination was performed in triplicate.[4] The absorbance recorded and the % recoveries were calculated using formula.[5,6]

Formula

$$\% \text{ Recovery} = [a - b/ c] \text{ X } 100$$

Where,

a =Total amount of drug estimated

b =Amount of drug found on pre analyzed basis

c= Amount of Pure drug added.

The results are reported in the **Table No. 5**

Table No 5: Recovery Studies.[4]

Spiked level	Amount taken (µg/ml)	(%)Amount found (µg/ml) P	Percent Recovery (% w/w)±RSD
80	8	7.96	99.5±0.56
100	10	10.04	100.4±0.74
120	12	11.95	99.6±0.63

Robustness[4,7,8]

Robustness of the method was done by slight changes slight in the chromatographic parameters like mobile phase composition, flow rate and wavelength.[4,7,8] It was observed that there were no marked changes in the chromatograms, which demonstrated that the RPHPLC method developed is robust.

Table 6: Robustness Studies.

Condition	Modification	Peak area	Mean %RSD
Mobile phase composition(v/v)	80:20	591525	0.11
Flow rate (ml/min)	0.5	589159	0.21
Wavelength (nm)	257	592121	0.155

Limit of detection (LOD) and Limit of quantification LOQ)[4]

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

S = Slope of calibration curve.

The results were found to be LOD 0.017 µg/ml LOQ 0.052 µg/ml.

DISCUSSION

By applying the proposed method the retention time of Rosuvastatin was found to be 3.097mins. Linearity range was found 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 (µg/ml). The number of theoretical plates was found to be 4590. That indicates efficient performance of the column. The tailing factor was found to be 1.1. Which indicates good shape of the 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 to 100.3% indicates that the proposed method is highly accurate. The % RSD value

<1% for the inter-day precision. The use of acetonitrile and water in the ratio of 75:25 % v/v resulted in the peak with good shape and resolution.

CONCLUSION

The proposed method has advantage of simplicity and it is convenience for the separation and quantitation of Rosuvastatin 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 in tablet dosage form. Hence it can be applied routine analysis of Rosuvastatin in formulation.

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