INTERNATIONAL JOURNAL OF PHARMACEUTICS & DRUG ANALYSIS
VOL. 3 ISSUE 9, 2015; 275 – 281 ; http://ijpda.com; ISSN: 2348-8948

RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF CHIRAL HPLC METHOD FOR QUANTITATION OF ENANTIOMER IN ROSUVASTATIN CALCIUM

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Date Received: 21st September 2015; Date Accepted: 24th August 2015 Date published: 25th September 2015

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Abstract: A new, simple, precise, rapid and accurate normal phase enantioselective high performance liquid chromatographic method was developed for enantiomeric resolution of Rosuvastatin, which is used for the treatment of hypercholesterolemia. This is a fourth highest selling drug in the United States, accounting approximately $5.2 billion in the year of 2013. The enantiomer of Rosuvastatin and lactone impurity of Rosuvastatin were resolved on a CHIRALPAK IB (250 x 4.6mm, 5µm) column using a simple mobile phase system containing n-heptane, 2-propanol and trifluoroaceticacid (85:15:01v/v). The resolution between Rosuvastatin and enantiomer, Rosuvastatin and lactone impurity was good with resolution factors more than 2.0 and 4.0, respectively. The effect of organic modifier, namely 2-propanol in the mobile phase was optimized in order to obtain the best separation. The Limit of Detection and Limit of Quantitation of enantiomer were found to be 0.07µg/mL and 0.2µg/mL, respectively, for 10µL injection volume. The sample solution and mobile phase were stable for at least 48 hours. The proposed reproducible and accurate method can be useful for quantification of enantiomer of Rosuvastatin in the bulk drug substance.

Key words: Rosuvastatin calcium; Normal phase HPLC; Enantiomer; Impurities; Chiral method.

INTRODUCTION:

Nowadays chiral separations are playing more important role for the analysis of single enantiomers in the field of pharmaceutical industries. It is not uncommon for one enantiomer to be active while other isomer is toxic in biological systems. Enantiomers of racemic drugs often show different behaviors in pharmacological action and metabolic process.[1-4]

Rosuvastatin calcium is a potent inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, is designated as (3R,5S)-7-[4-(4-fluorophenyl)-2-(N-methylmethanesulfonamido)-6-(propan-2-yl) pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid (Figure 1). HMG-CoA reductase inhibitors reduce the production of mevalomic acid from HMG-CoA, resulting in a reduction in hepatic cholesterol synthesis. [5-7] Rosuvastatin calcium is more potent than other statins such as atorvastatin, simvastatin and is 8 fold more potent than the hydrophilic comparator, pravastatin.[8-10]

According to our knowledge, there is no enanioselective HPLC method for the quantification of the enantiomer at the 0.2µg/mL level existing in the literature of Rosuvastatin calcium. The reported analytical methods are three stability-indicating HPLC methods,[11-13] their mass detection methods for the determination of Rosuvastatin calcium in plasma and biological fluids,[14-16] a stability indicating related substance method by UPLC[17] and three UV spectrometric methods.[17-19] But none of the methods either individually or combined are capable of quantifying the enantiomer of Rosuvastatin calcium at the level of 0.2µg/mL.

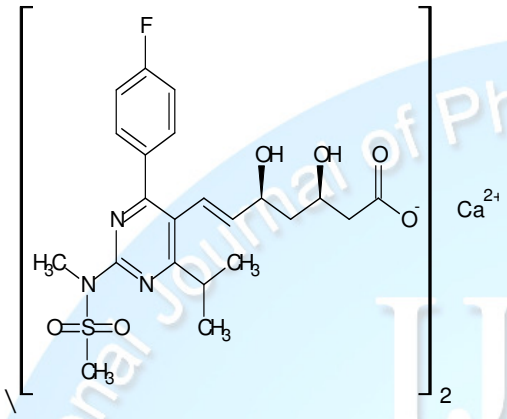
The aim of this work is to optimize the HPLC analysis condition in terms of mobile phase composition in order to separate, identify and quantify the enantiomer of Rosuvasatin calcium. The developed chiral HPLC method is precise and accurate for the quantitative determination. As per ICH (Q3C) guidelines, theist is better to avoid the Class-1 and Class-2 solvents in the manufacturing process to control the environment safety and other safety aspects[20-21]. We have applied the same principle to analytical method development to reduce the usage of Class-1 and Class-2 solvents in HPLC chiral method for Quantitation of enantiomer of Rosuvastatin calcium.

MATERIALS AND METHODS

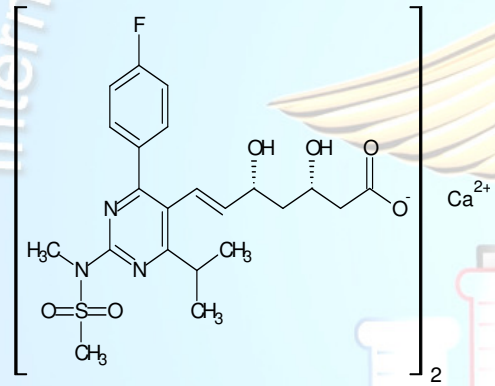
Chemicals and Reagents

Rosuvastatin calcium, enantiomer of Rosuvastatin calcium, lactone impurity were obtained from the Spira lifesciences, Hyderabad, India. HPLC grade, n-heptane, 2-

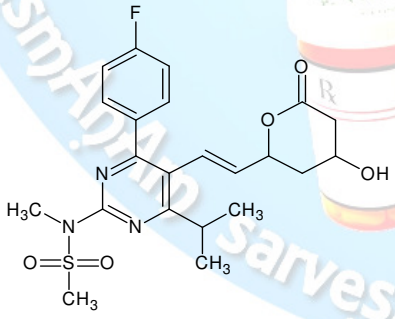
propanol and trifluroacetic acid were purchased from Merck, Mumbai, India. The CHIRALPAK® IB column was procured from Daicel, Japan. The HPLC grade methanol was purchased from Spectrochem, Mumbai, India.



Rosuvastatin Calcium



Enantiomer of Rosuvastatin Calcium



Lactone impurity

Figure 1: Structures of Rosuvastatin calcium, enantiomer of Rosuvastatin calcium and Lactone impurity

Chromatographic condition

Chromatographic method was carried out by using Waters instrument equipped with column oven, photo diode array detector, Alliances 2695 series low pressure quaternary gradient pump equipped with auto sampler has been used for the analysis of samples and the data was processed using a software program, Empower. Chromatographic conditions were optimized using a chiral stationary phase, CHIRALPAK IB column (250 mm x 4.6 mm, 5µm). The isocratic mobile phase composition was a mixture of n-heptane, 2-propanol and trifluoroacetic acid (85:15:0.1 v/v), which was pumped at a flow rate of 1.0 mL/minute. The temperature of the column was maintained at 25°C and the eluent was monitered at a wavelength of 242 nm (Figure 2) which was selected based on the UV spectrum of Rosuvastatin. The injection volume was 10µL.

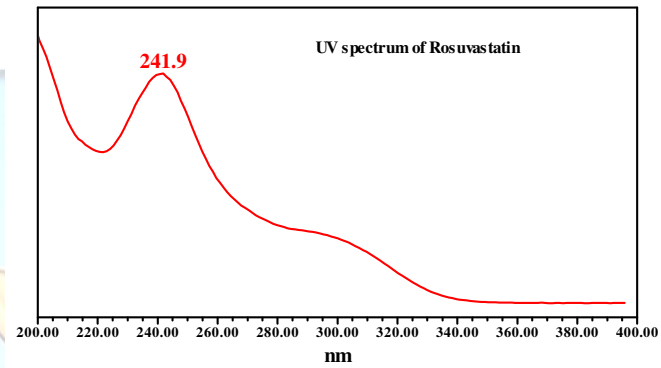


Figure 2: UV spectrum of Rosuvastatin

Sample preparation

Stock solutions of Rosuvastatin calcium (1mg/mL) and enantiomer (1mg/mL) were prepared separately by dissolving appropriate amounts of the substances in methanol. The analyte concentration of Rosuvastatin calcium was fixed as 1000 µg/mL. The 0.15% w/w level of enantiomer of Rosuvastatin solution was spiked with calculated amount of Rosuvastatin calcium stock solution. The system suitability solution containing 1mg/mL of Rosuvastatin calcium and a 0.15% w/w level of the Rosuvastatin enantiomer and 0.15% w/w of lactone impurity (the lactone impurity was included to ensure its separation from Rosuvastatin as it was the close-eluting impurity) was prepared (Figure 3).

Validation of method

The method was validated for the following parameters as per ICH guideline Q2 (R1). The specificity of the method is performed by injecting both isomers, lactone impurity and recemic mixture individually. The specificity determined by using peak purity and resolution. The system suitability of the method was performed by using known concentration of enantiomer of Rosuvastatin calcium, Rosuvastatin calcium isomer and lactone impurity.

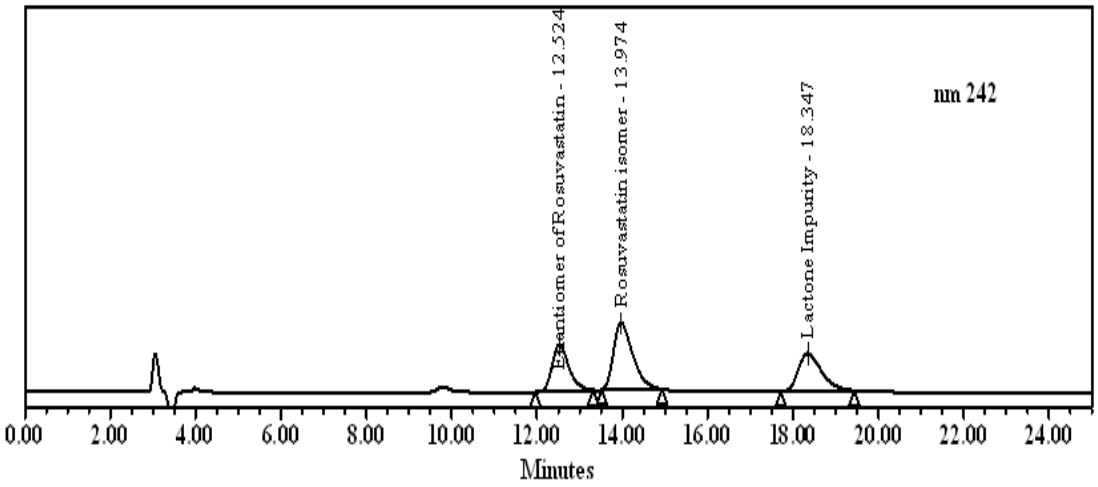


Figure 3: A typical HPLC chromatogram of Rosuvastatin calcium, spiked with enantiomer of Rosuvastatin calcium and Lactone impurity

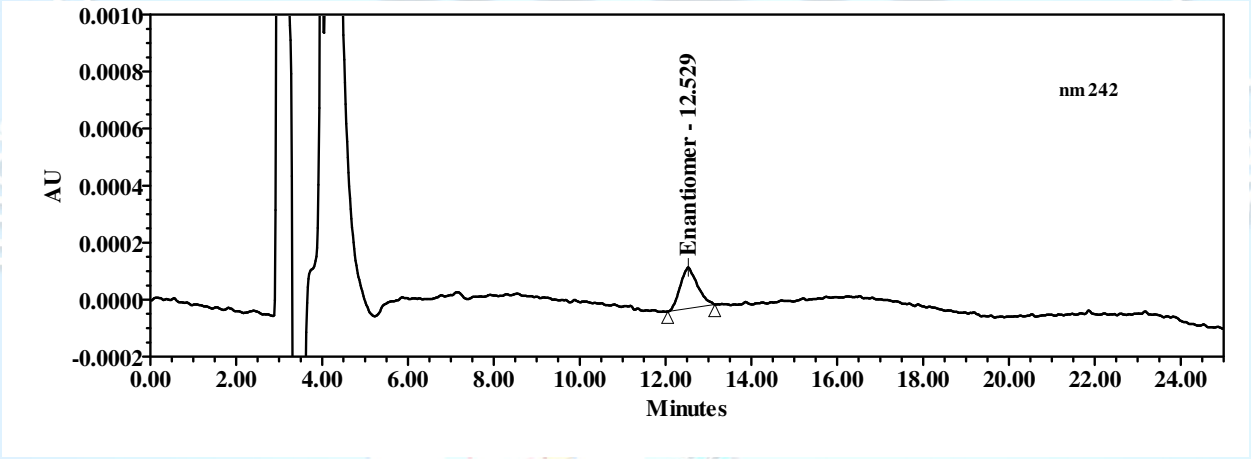


Figure 4: LoQ chromatogram of enantiomer of Rosuvastatin

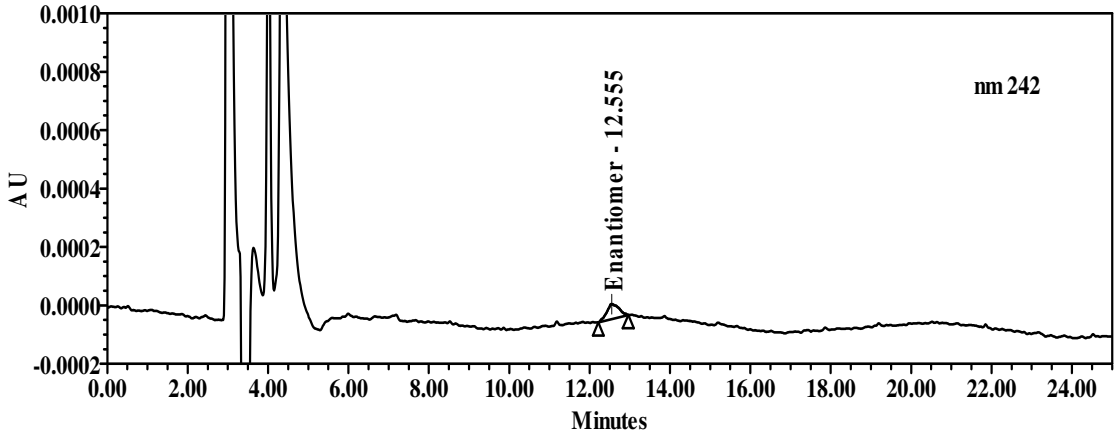


Figure 5: LoD chromatogram of enantiomer of Rosuvastatin calcium

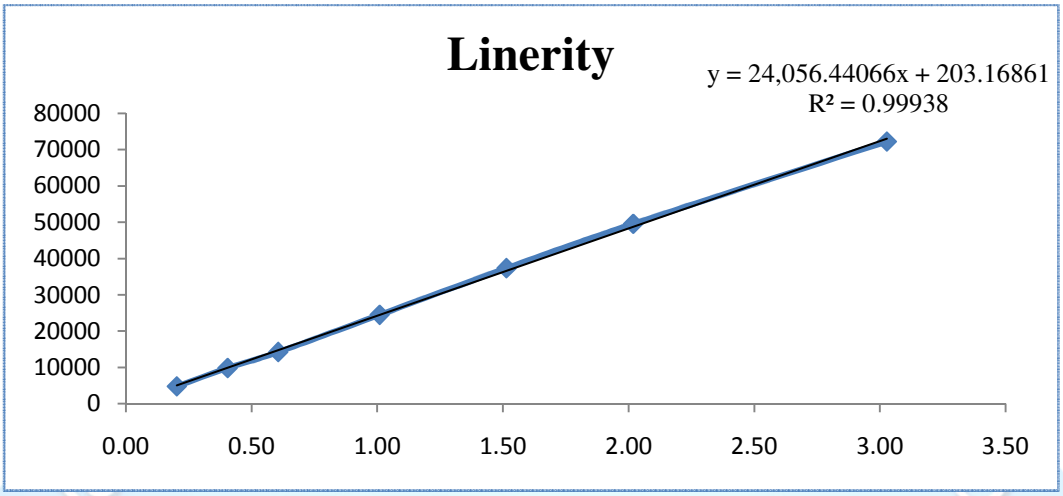


Figure 6: Linearity graph from 0.2µg/mL to 3.03µg/mL vs Average peak area of enantiomer

Table 1. Results of specificity and system suitability

Name	Purity Angle	Purity Threshold	Peak purity	USP Reso-lution	USP Tailing	USP Plate Count
Enantiomer of Ro-suvastatin	0.3569	0.4985	Pass	-	1.32	4710
Rosuvastatin	0.2596	0.6532	Pass	2.12	1.47	4460
Lactone impurity	0.6765	0.9685	Pass	4.64	1.43	5303

Table 2. Precision study results

Study	% RSD
Repeatability (standard injections)	0.96
Method precision (n=6)	1.74
Intermediate precision (n=6)	1.37

Table 3. Linearity results

Concentration in µg/mL	Average Peak Area (n=6)	% RSD
0.20	4824	1.88
0.40	9848	1.01
0.61	14265	1.43
1.01	24521	1.81
1.51	37356	0.96
2.02	49573	1.03
3.03	72211	0.87

Table 4. Accuracy results

S.No.	Spiked amount (µg)	% RSD (n=3)	Recovery (%)
1	0.20	1.85	98.6
2	1.01	1.38	100.9
3	1.51	1.03	100.2
4	3.03	1.28	99.9

The system suitability is confirmed by using resolution between Rosuvastatin and enantiomer, Rosuvastatin and lactone impurity, tailing factor and theoretical plates of Rosuvastatin calcium (Table 1).

The limit of detection (LoD) and limit of quantitation (LoQ) for enantiomer of Rosuvastatin was achieved by

injecting series of dilute solutions and by using signal to noise ratio method ICH Q2 (R1). The precision at LoQ of the developed chiral method for enantiomer of Rosuvastatin was checked by analyzing six injections of enantiomer of Rosuvastatin prepared at LoQ level and calculated the percentage relative standard deviation.

Method reproducibility was determined by measuring repeatability and intermediate precision of retention time and peak area of each enantiomer of Rosuvastatin. The repeatability of the method was determined by analyzing six replicate injections containing Rosuvastatin (1000 µg/mL) spiked with enantiomer of Rosuvastatin (0.15% w/w, 1.5µg/mL). The intermediate precision was determined by analyzing the spiked samples in six replicates (n=6) on a different day, different HPLC system by a different analyst and with a different column (different lot number).

The linearity test was carried out by preparing seven calibration solutions of enantiomer of Rosuvastatin covering from 0.2µg/mL (LoQ) to 3.0µg/mL (0.2, 0.4, 0.6, 1.0, 1.5, 2.0, 3.0µg/mL) in diluent. The regression curve was obtained by plotting peak area versus concentration. The accuracy of the method was determined by analyzing samples with known concentration of enantiomer of Rosuvastatin. The accuracy was calculated in terms of recovery (%).This accuracy test was carried out in triplicate at each concentration covering from 0.2µg/mL (LoQ) to 3.0µg/mL (0.2, 1.0, 1.5, 3.0µg/mL) in diluent. The solution stability of Rosuvastatin calcium and enantiomer of Rosuvastatin was studied by keeping the solution in tightly stoppered volumetric flask at room temperature on a laboratory bench for 48 hours. The content of enantiomer of Rosuvastatin calcium was checked at every 4 hours interval during the storage period.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

To develop suitable normal phase HPLC method for the separation of each enantiomer and lactone impurity from Rosuvastatin, different stationary phases and mobile phases were tried. Chiralpak IA, IB and IC and different combinations of mobile phases consisting n-heptane, ethanol, 2-propanol and trifloroacetic acid were used. Noticeable separation could be achieved in CHIRALPAK IC column but the resolution between the enantiomer of Rosuvastatin and Rosuvastatin peak was less than 1.0 (resolution factor). Hence the efforts were continued to select the best stationary phase and mobile phase combinations that would give optimum resolution and selectivity for the enantiomer and lactone impurity from Rosuvastatin. This lead to an excellent separation on CHIRALPAK IB (250 x 4.6mm, 5µm) column using mobile phase consisting of n-heptane, 2-propanol and trifluoroaceticacid (85:15:0.1 v/v). The results of resolution between the enantiomer of Rosuvastatin and Rosuvastatin; and between the lactone impurity and Rosuvastatin are summarized. Based on the data obtained from the method development and optimization activities, CHIRALPAK IB column with mobile phase of n-heptane, 2-propanol and trifluoroaceticacid (85:15:0.1 v/v) was selected from the method development. The flow rate of final method was 1.0 mL/minute with injection volume 10 µL. The column temperature was 25°C and the detection wavelength was 242 nm. Under these conditions, the two enantiomers were separated and also the lactone impurity from Rosuvastatin was separated well and the peak of enantiomer of Rosuvastatin eluted before the peak of Rosuvastatin. In the optimized method, the typical retention time of enantiomer of Rosuvastatin, Rosuvastatin and lactone impurity were 12.5, 13.9 and 18.3 minutes, respectively (Figure 2). Excellent base line separation was obtained with the total run time of 25 minutes.

Validation of the method

The optimized normal phase HPLC method of the final method was evaluated for its specificity, precision, LoD, LoQ, linearity, accuracy, and solution stability. The specificity of the method was determined through photo diode array detector by using peak purity (Table 1).

The LoQ and LoD concentrations were estimated to be 0.02 % w/w (0.2µg/mL) and 0.007% w/w (0.07µg/mL), respectively, for enantiomer of Rosuvastatin. The LoQ and LoD were calculated by using signal to noise ratio method. The method precision for enantiomer of Rosuvastatin at LoQ was less than 5% RSD. Therefore, this method had adequate sensitivity for the detection and estimation of enantiomer of Rosuvastatin calcium. The LoQ and LoD chromatograms are shown in Figure 4 and 5.

Linearity of enantiomer of Rosuvastatin was evaluated over seven levels from 0.2µg/mL to 3.03µg/mL, with the linear regression equation y = mx + c, where x is the concentration in µg/mL and y is the corresponding peak area of undesired enantiomer in mV/s. The correlation coefficient value is more than 0.999 (Table 3) and the linear graph was shown in Figure 6. The accuracy (as percent recovery) was determined by standard addition experiment. The recovery experiments were conducted for enantiomer of Rosuvastatin in triplicate at 0.2, 1.01, 1.51 and 3.03µg/mL. The recovery was calculated by back calculated concentration at each level in each preparation and the recovery was within 98.6 and 100.9 % (Table 4).

The stability of the solution in this method was tested over 48 hours. No significant change in enantiomer content was observed in Rosuvastatin calcium sample during solution stability study. The calculated RSD % value for

replicate analysis was less than 2.0% (0.75%). No unknown peak was observed in the above solution stability study indicating that the solution of enantiomer of Rosuvastatin in the presence of Rosuvastatin calcium solution was stable for at least 48 hours. The repeatability and intermediate precision were expressed as relative standard deviation (RSD). For this study, solution of Rosuvastatin calcium (1000 µg/mL) spiked with enantiomer of Rosuvastatin 0.15 % w/w (1.5µg/mL) was analyzed in six replicates to establish repeatability. The RSD % values were calculated for retention time and peak area of Rosuvasatain and enantiomer of Rosuvastatin. In the intermediate precision, results shown that % RSD values are in the same order of magnitude as those obtained for repeatability studies (Table 2). All the obtained validation results indicated that the method is precise, accurate, specific and linear in the tested range..

CONCLUSION

A simple, specific, linear, accurate and precise normal phase HPLC method was successfully developed by using class 3 solvents (n-heptane and 2-propanol), which was capable of separating the enantiomer of Rosuvastatin calcium and lactone impurity. Amylose based chiral column CHIRALPAK IB was found to be selective for the separation of enantiomer of Rosuvastatin calcium. The developed and validated method can be used for determining chiral purity and quantitative estimation of enantiomer of Rosuvastatin calcium.

Acknowledgment:

The authors would like to thank the management of Advinus Therapeutics Limited, Bengaluru, India, for allowing to carryout the research work.

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