ORIGINAL ARTICLE

Method Development and Validation for the Simultaneous Estimation of Rosuvastatin and Amlodipine in Bulk and its Formulation using Reverse-Phase High-Performance Liquid Chromatography

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Abstract

Background: High-performance liquid chromatography (HPLC) is basically a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres which makes it much faster. All chromatographic separations including HPLC is based upon the resolution of the sample constituents as per the difference in their relative affinities towards stationary phase and mobile phase used. **Aim:** A simple, specific, accurate, and precise reverse-phase HPLC method was developed and validated for the estimation of rosuvastatin and amlodipine (Rosudapin) in pharmaceutical dosage form. **Materials and Methods:** An Aquasil column reversed phase C-18, 5 µm column having 4.6 mm × 250 mm i.d. in gradient mode, with mobile phase containing HPLC grade acetonitrile:phosphate buffer (pH 3.8):methanol in proportion 30:60:10 v/v, was used. The flow rate was 1 ml/min and effluents were monitored at 251 nm using PDA detector. Linearity was observed over a range of 5–25 µg/mL of rosuvastatin and 2.5–12.5 µg/mL of amlodipine, respectively. **Results and Discussion:** The method was validated for linearity, accuracy, precision, limit of detection, limit of quantitation, robustness, and ruggedness. The limit of detection and estimation of analytes was found to be 3.1 µg/ml and 2.98 µg/ml, and the limit of quantification of analytes was found to be 102 µg/ml and 9 µg/ml, respectively, for rosuvastatin and amlodipine. **Conclusion:** The proposed method was successfully applied for the quantitative determination of rosuvastatin and amlodipine in pharmaceutical dosage form.

Key words: Amlodipine, precision, reverse-phase high-performance liquid chromatography, rosudapin, rosuvastatin, simultaneous estimation

INTRODUCTION

Rosuvastatin belongs to the class of statins[1] (antilipidemic). It is chemically (3R,5S,6E)-7-[4-(4-fluorophenyl)-2-(N-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxy hept-6-enoic acid [Figure 1] and is available in its calcium form, commonly called as rosuvastatin calcium. Rosuvastatin is a hydroxymethylglutaryl-CoA reductase inhibitor or it reduces plasma concentrations of LDL-cholesterol, apolipoprotein B, and triglycerides and prevents cardiovascular disease. Rosuvastatin is commonly used as a statin with mild, asymptomatic, and

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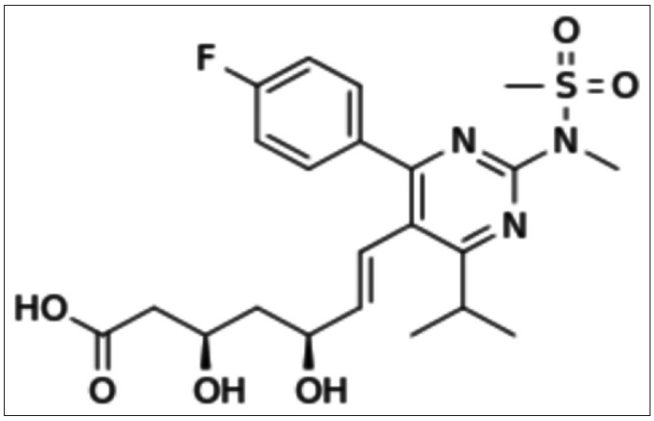

Figure 1: Structure of rosuvastatin

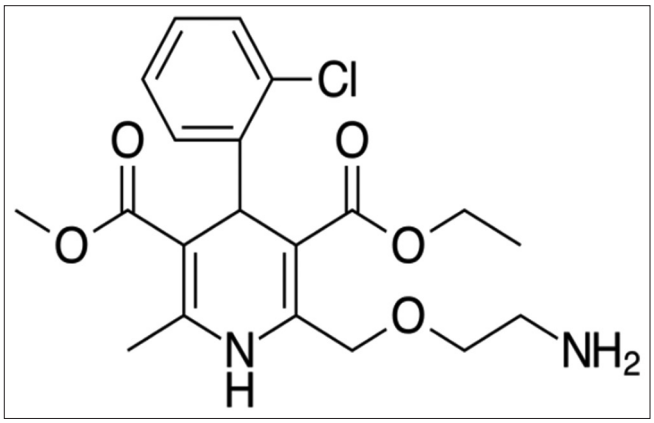

Figure 2: Structure of amlodipine

self-limited serum aminotransferase elevations during therapy.

Amlodipine [Figure 2] is used to treat high blood pressure and coronary artery disease such as chronic stable angina.[2] It is a long-acting 1,4-dihydropyridine calcium channel blocker. It is chemically (RS)-3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate. Amlodipine decreases arterial smooth muscle contractions and inhibits the influx of calcium ions through L-type calcium channels. Inhibition of the initial influx of calcium decreases the contractile activity of arterial smooth muscle results in vasodilation.

A combination of rosuvastatin and amlodipine (Rosudapin)[3] is given in adults for the treatment of increased blood pressure and with high cholesterol level, when changing diet and doing more exercise were not enough to prevent cardiovascular events.

A detailed survey of the analytical literature for rosuvastatin and amlodipine revealed few methods based on a number of techniques such as ultraviolet-spectrometry[4,5] and HPLC methods.[6-10] Since a HPLC method has many advantages, it is often the first choice for developing an analytical. Conformation of the applicability of the developed method was validated according to the ICH guidelines.[11,12]

MATERIALS AND METHODS

Instruments

A water module equipped with autosampler and PDA detector (996 model) 2695 separation module for finding out the λmax values of the drug was used throughout this study. An Aquasil C18 (4.6 mm × 250 mm, 5 µm) (Make: Thermo Scientific) column was employed for the method development. The chromatographic system was monitored by EMPOWER2 software. The digital ultrasonicator and pH meter were from Enertech and Lab India, respectively.

Chemicals

HPLC grade acetonitrile and orthophosphoric acid were obtained from Merck India Ltd., Mumbai, India. Analytical grade methanol was obtained from Lichrosolv (Merck India Ltd.) and 0.45 µm membrane filter was obtained from Pall Life Sciences, Mumbai, India. High purity deionized water was obtained from a Milli-Q (Millipore, Milford, MA, USA) purification system. Anhydrous dihydrogen phosphate and citric acid were from Finar Chemicals.

Preparation of phosphate buffer (ph - 3.8)

About 0.9 g of anhydrous dihydrogen phosphate and 1.298 g of citric acid monohydrate were dissolved in sufficient water to produce 1000 mL with pH adjusted to 3.8 using orthophosphoric acid.[13-15]

Preparation of mobile phase

Nearly 300 ml (30%) of HPLC acetonitrile, 600 ml of phosphate buffer (3.8) (60%), and 100 ml HPLC Methanol (10%) were mixed and degassed in a digital ultrasonicator for 10 min and then filtered through 0.45 µ filter under vacuum filtration.

Diluent preparation

The mobile phase was used as the diluent.

Preparation of standard solution

About 10 mg of rosuvastatin and 10 mg of amlodipine working standard wereee accurately weighed and transferred into 10 mL volumetric flask each. 10 ml of diluents were added, sonicated, and diluted with diluent up to the mark. Further pipette 0.15 ml and 0.075 ml of the above rosuvastatin and amlodipine stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluents to form 15 µg/ml of rosuvastatin and 7.5 µg/ml of amlodipine solutions.[16,17]

Preparation of Sample Solution

10 combination tablets were crushed and powder weight equivalent to 10 mg of Rosuvastatin and Amlodipine was weighed and added to 10 mL clean dry volumetric flask. Make up this with 10 mL of diluent and sonicate to dissolve it completely. Further pipette 0.15 ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents to form 15 µg/ml of sample solution.

Optimized Chromatographic Conditions

Separation was achieved using a mobile phase consist of acetonitrile:phosphate buffer (pH 3.8):methanol in proportion 30:60:10 v/v, respectively, at a flow rate of 1 ml/min. The eluent was monitored using PDA detector at a wavelength of 251 nm. The column was maintained at ambient temperature, and an injection volume of 20 µl was used. The run time was 8 min. The mobile phase was filtered through 0.45 µm filter before use. Solubility of the compounds was enhanced by sonication on an ultrasonicator (Bandelin Sonorex).

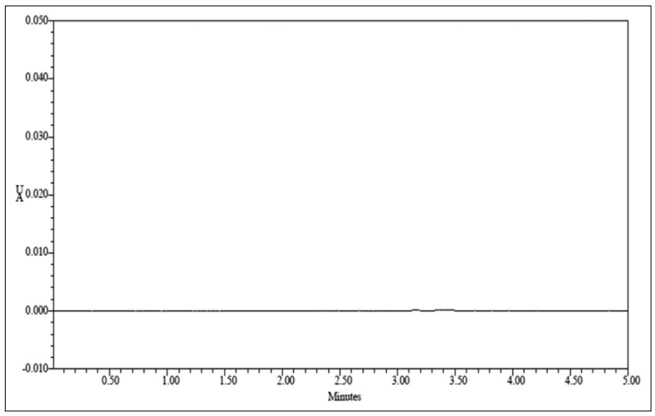

Figure 3: Blank chromatogram

RESULTS AND DISCUSSION

Validation

Before validation studies, blank solution was injected and chromatogram was noted [Figure 3]. Optimized conditions were maintained with good retention time and resolution which were shown in Figure 4.

Linearity

The linearity of the method was established by determining the absorbance of different concentrations over a range of 5-25 µg/mL of Rosuvastatin and 2.5-12.5 µg/mL of Amlodipine respectively. The calibration curve of Rosuvastatin and Amlodipine were given in Figures 5 and 6 respectively. The linearity data was given in Table 1.

Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out and percentage recovery and standard deviation are calculated and represented in Tables 2 and 3. Each sample was injected thrice each.

Table 1: Results of method linearity for rosuvastatin and amlodipine

Concentration of rosuvastatin (µg/mL)	Peak area	Concentration of amlodipine (µg/mL)	Peak area
5	1,692,344	2.5	927,035
10	3,214,138	5	1,706,996
15	479,1958	7.5	2,582,231
20	6,385,532	10	347,0152
25	7,730,420	12.5	4,180,508

Table 2: Results of accuracy data for rosuvastatin

Accuracy level (%)	Peak area	Average peak area	Average % recovery	Standard deviation	% RSD
50	2,629,787	2,630,409	98.0%	10905.83	0.415
50	2,641,613				
50	261,9828				
100	5,283,037	5,277,055	99%	8566.092	0.162
100	5,267,242				
100	5,280,886				
150	7,524,348	7,514,836	100.6%	8276.242	0.110
150	7,509,284				
150	7,510,875				

RSD: Relative standard deviation

Table 3: Results of accuracy data for amlodipine

Accuracy level	Peak area	Average peak area	Average % recovery	Standard deviation	% RSD
50%	1,365,757	1,366,666	98%	6960.626	0.51
50%	1,374,036				
50%	1,360,204				
100%	2,782,810	2,777,487	100%	19844.78	0.714
100%	2,794,128				
100%	2,755,524				
150%	4,156,891	4,151,220	99%	7650.882	0.184
150%	4,142,518				
150%	4,154,251				

RSD: Relative standard deviation

Table 4: Results of precision data

Analyte	Parameters	System precision	Method precision
Rosuvastatin	Mean peak area	5,680,917	5,257,650
	Standard deviation	22699.72	45206.32
	% RSD	0.39	0.85
Amlodipine	Mean peak area	2,626,428	2,774,987
	Standard deviation	5215.789	22806.64
	% RSD	0.198	0.82

RSD: Relative standard deviation

Table 5: Results of LOD and LOQ data

Analytes	LOD	LOQ
Rosuvastatin	3.1	10.2
Amlodipine	2.98	9.8

LOQ: Limit of quantification, LOD: Limit of detection

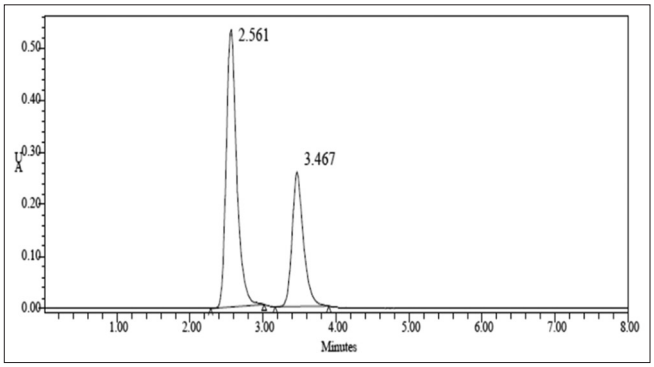

Figure 4: Optimized chromatogram of rosuvastatin and amlodipine

Precision

The precision of the method was demonstrated by method precision and system precision. Five replicate injections of

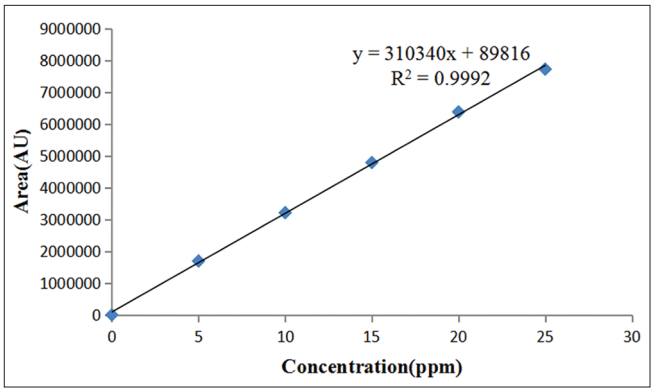

Figure 5: Calibration curve of rosuvastatin

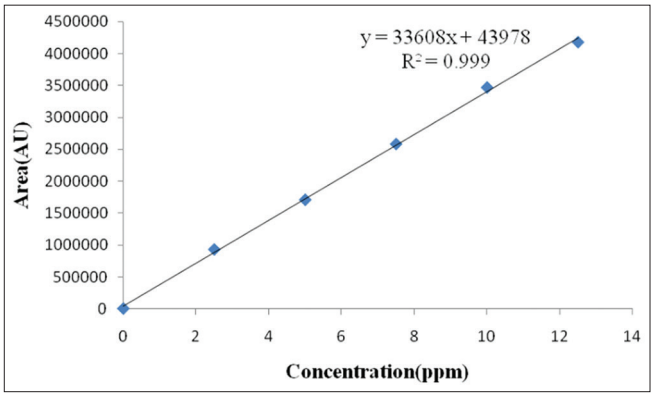

Figure 6: Calibration curve of amlodipine

the sample solutions were made, and the percentage relative standard deviation was calculated and is represented in Table 4.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The values for LOD and LOQ are given in Table 5.

Robustness

The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical

parameters like flow rate, mobile phase composition which may differ but the responses were still within the limits of the assay. The results of Robustness data are given in Table 6.

ASSAY

The commercial combination tablets (Rosudapin – 10 mg Amlodipine and 5 mg of Rosuvastatin) were analyzed by the proposed method. The assay procedure was performed and the assay percentage was calculated as per the given formula.[18] The value was found to be in good agreement with the labeled amounts, which confirms the suitability of the method for the analysis of the analytes, Rosuvastatin and Amlodipine, in pharmaceutical dosage forms.

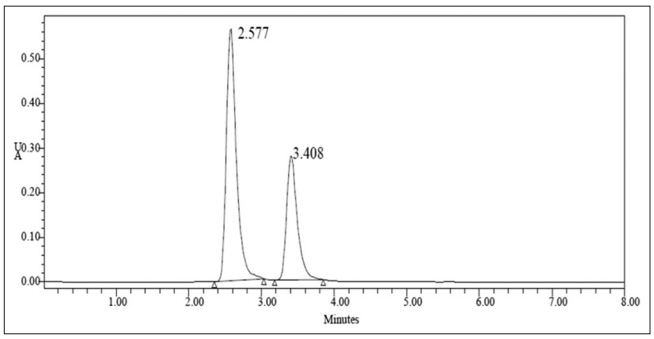

Figure 7: Chromatogram of assay sample

Table 6: Results of robustness data

Analytes	Proposed variation	Peak areas	Theoretical plates
	Flow rate		
Rosuvastatin	0.9 mL/min	5,386,971	4479
	1.0 mL/min	5,489,452	4759
	1.1 mL/min	5,478,984	3072
Amlodipine	0.9 mL/min	2,685,942	4508
	1.0 mL/min	2,623,598	3695
	1.1 mL/min	2,888,145	3072
	Mobile phase composition		
Rosuvastatin	Less (50%)	5,369,821	3700
	Actual	5,689,452	4759
	More (70%)	5,478,529	3794
Amlodipine	Less (50%)	2,698,715	3312
	Actual	2,623,259	3211
	More (70%)	2,888,304	3247

Formulae:

$$\text{Assay}\,\% = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AV}{LC} \times 100$$

Where
AT = Average area counts of sample preparation.
AS = Average area counts of standard preparation.
WS = Weight of working standard taken in mg.
DS = Weight of sample taken in mg.
DF = Dilution factor.
WT= Average weight.
P = Percentage purity of working standard.

The assay results were given in Table 7 and the chromatogram of assay sample is given in Figure 7.

CONCLUSION

The proposed reverse-phase HPLC method is sensitive and accurate and can be used for routine quality control analysis for the determination of rosuvastatin and amlodipine in its tablet dosage form. It can be seen from the results presented that the proposed procedure has good precision and accuracy. Results of the analysis of pharmaceutical formulations revealed that proposed methods are suitable for their analysis with virtually no interference of the usual additives present in the pharmaceutical formulations.

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Table 7: Summary of assay results

Analytes	Peak areas of standard	Mean peak area of standard	Peak areas of sample	Mean peak area of sample	Label claim (mg)	Amount found (mg)	% purity/ % assay
Rosuvastatin	5,401,466	5,388,158	5,386,083	5,363,315	10	9.9	99%
	5,446,288		5,382,363				
	5,316,721		5,321,498				
Amlodipine	2,843,567	2,852,191	2,830,177	2,808,339	5	4.9	98%
	2,866,874		2,840,718				
	2,846,132		2,754,122				

CONFLICTS OF INTEREST

Authors declare that there are no conflicts of interest to disclose.

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