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Research Article

CLEANING VALIDATION OF A SIMPLE AND RAPID REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE SIMULTANEOUS ESTIMATION OF ASPIRIN AND ROSUVASTATIN

CELINA NAZARETH*, GISELLE FIZARDO, CHARMAINE VAZ

Department of Pharmaceutical Chemistry, P.E.S's Rajaram and Tarabai Bandekar College of Pharmacy, Farmagudi, Ponda, Goa, India.
Email: celinanaz@yahoo.com

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ABSTRACT

Objective: This study describes a new, simple, precise, accurate, and reproducible reversed-phase high-performance liquid chromatography (RP-HPLC) cleaning validation method for simultaneous estimation of rosuvastatin and aspirin.

Methods: The proposed RP-HPLC method was carried out on AGILENT-ZORBAX RP-Inertsil column (250 mm × 4.6 mm, 5 µm) in an isocratic mode utilizing potassium dihydrogen phosphate buffer (pH 2.5 with OPA):acetonitrile (50:50,v/v) as mobile phase, at a flow rate of 1.5 ml/min. Detection was carried out at 243 nm using UV detector.

Results: The method was found specific as there was no swab interference. The Beer–Lambert's law was obeyed in the concentration range of 0.5–20 µg/ml for both rosuvastatin and aspirin. The mean percentage recoveries at 100% level were 89.4% for rosuvastatin and 82.1% for aspirin. The limit of detection and limit of quantification for rosuvastatin and aspirin were 0.03 µg/ml and 0.1 µg/ml, respectively. The method was found to be robust and precise with percentage RSD <2.0%.

Conclusion: A simple, novel, and economical RP-HPLC method for cleaning validation has been developed for the simultaneous estimation of rosuvastatin and aspirin. The method was validated as per ICH guidelines for specificity, linearity, accuracy, precision, and robustness. The developed method can be used as a sensitive analytical tool for ensuring the effectiveness of the cleaning procedure adopted.

Keywords: Rosuvastatin, Aspirin, Reversed-phase high-performance liquid chromatography, Cleaning validation.

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INTRODUCTION

Rosuvastatin (Fig. 1) is a hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor. It acts in the liver. Chemically, rosuvastatin 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 is a statin with antilipidemic and potential antineoplastic activities. It selectively and competitively binds to and inhibits hepatic hydroxymethyl-glutaryl coenzyme A (HMG-CoA) reductase, the enzyme which catalyzes the conversion of HMG-CoA to mevalonate, which is a precursor of cholesterol. This leads to a decrease in hepatic cholesterol levels and increase in uptake of LDL cholesterol [1,2].

Aspirin (Fig. 2) is an anticoagulant agent. Chemically, it is 2-acetobenzoic acid. It blocks the production of prostaglandins by inhibiting cyclooxygenase (prostaglandin H synthase), with greater selectivity toward the COX-1 isoform. The antithrombotic effect is due to the inhibition of COX-1 in platelets that block thromboxane production and platelet aggregation. It is chemopreventive against colorectal and other solid tumors [1,2].

Equipment contamination may come from any of the materials that have been in contact with the equipment surfaces. It is critical to avoid carryover of the trace amounts of either active or other materials from one batch to another to avoid cross-contamination of the subsequent product. Hence, equipment used in pharmaceutical manufacturing must be cleaned meticulously and the cleaning procedure used must be validated [3,4].

Literature survey reveals that many HPLC and UV methods have been reported for the determination of rosuvastatin and aspirin, either alone or in combination [5-19]. However, no method has been reported for cleaning validation for rosuvastatin and aspirin in combination.

Cleaning validation is required in the pharmaceutical field to avoid potential, clinically significant synergistic interactions between pharmacologically active chemicals. The objective of the determination of the residue of drugs during cleaning validation is to verify the effectiveness of the cleaning procedure for the

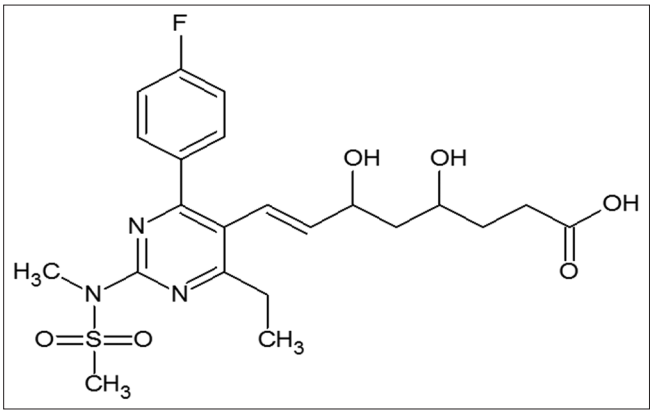

Fig. 1: Rosuvastatin

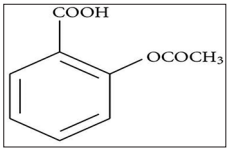

Fig. 2: Aspirin

removal of product residues, degradation products, preservatives, excipients, and/or cleaning agents as well as the control of potential microbial contaminants. In addition, it is needed to ensure that there is no risk associated with cross-contamination of active ingredients [20,21].

Hence, it was thought worthwhile to develop a cleaning validation HPLC method for the simultaneous determination of rosuvastatin calcium and aspirin and validate it as per ICH guidelines.

METHODS

The active pharmaceutical ingredients rosuvastatin and aspirin were supplied as gift samples by Vergo Pharma Research Laboratories, Verna, Goa. Water used for analysis was Milli Q water. Other chemicals used were of analytical/HPLC grade.

The HPLC system used was Agilent-Zorbax RP with a UV detector. Processing was done using Openlab software. The column used was Inertsil (250 mm × 4.6 mm, 5 µm). The mobile phase consisted of pH 2.5 phosphate buffer:acetonitrile (50:50, v/v). The flow rate was 1.5 ml/min. Injection volume was 25 µl and ultraviolet detection wavelength was set at 243 nm.

Preparation of orthophosphoric acid (OPA)

The OPA was prepared by dissolving 1 g of OPA in 1000 ml of water. It was stirred well by adjusting the pH with KOH solution to get pH 2.5 using digital pH meter.

Blank/diluent

The blank/diluent consisted of methanol:OPA adjusted to pH 2.5 with KOH (80:20, v/v).

Preparation of KH_2PO_4 buffer pH 2.5

Preparation of the buffer was carried out by dissolving 1.36 g of KH_2PO_4 in 1000 ml of water. It was stirred well and the pH was adjusted to 2.5 using OPA and filtered.

Preparation of mobile phase

The mobile phase was prepared by adding 500 ml of ACN and 500 ml of previously prepared KH_2PO_4 buffer pH 2.5 in a 1000 ml flask. Further, it was sonicated for 10 min.

Preparation of the standard stock solution of aspirin and rosuvastatin (10 ppm)

About 20 mg of aspirin and rosuvastatin each were weighed accurately and transferred into two separate 200 ml volumetric flask. About 30 ml of diluent was added and sonicated for 5 min and diluted up to the mark with 170 ml diluent and mixed well. Further, 10 ml of each solution was pipetted into two separate 100 ml volumetric flask and 30 ml of diluent was added and sonicated for 5 min and diluted up to the mark with 60 ml diluent and mixed well.

Preparation of mixed standard solution (1 ppm)

About 1 ml from each standard stock solution of rosuvastatin and aspirin were taken and transferred to 10 ml volumetric flask and the volume was made up with diluent.

Method validation

The method validation was performed according to ICH guidelines [22,23].

System suitability

To evaluate system suitability, the mixed drug standard solution was injected 6 times in the HPLC system. The system suitability was then established by calculating the percentage RSD, resolution, tailing factor, and the number of theoretical plates.

Specificity

Swabs used to evaluate the specificity (swab interference) for the proposed method were pretreated by transferring swab sticks in a clean test tube containing 10 ml of diluent and sonicated for 5 min and then drained. The whole procedure was repeated in water and then back in diluent. Two pretreated swabs were then taken and put in two separate test tubes containing 10 ml of diluent and sonicated for about 5 min with intermittent shaking. For the study of specificity, the two prepared swab solutions were injected into the HPLC system.

Linearity

To evaluate the linearity of the proposed method, a 10 ppm solution of rosuvastatin and aspirin was prepared by taking 10 ml of aspirin and 10 ml of rosuvastatin (100 ppm stock solution) and diluting to 100 ml using diluent. The concentration range selected was 0.1–20 ppm. The solutions for linearity study were injected in the HPLC system and the linearity range was determined by plotting calibration curves for both the drugs. The linear regression equation and correlation coefficient (r^2) were calculated.

Accuracy

To evaluate the accuracy of the method, recovery study was performed at 100% level concentration of the drugs. A 10 ppm solution of the drugs was prepared by taking 10 ml each of 100 ppm stock solution of rosuvastatin and aspirin and diluting to 100 ml using diluent. For recovery at 100% level, 1 ml of rosuvastatin and aspirin 10 ppm solution was dispersed over 4" × 4" stainless steel plate and dried using an electric fan. After drying, the plate was swabbed and the swabbed samples were collected in a test tube containing 10 ml of diluent, mixed, and sonicated for about 5 min with intermittent shaking to result in approximate 1 ppm solution. The procedure was performed in triplicate to result in three samples which were then injected as per test method and mean percentage recovery for both the drugs was calculated.

Precision

The evaluation of precision was done by injecting six replicates of rosuvastatin and aspirin mixed standard solution at limit of quantification (LOQ) level. The peak areas were recorded and the relative standard deviation for rosuvastatin and aspirin was calculated.

Limit of detection (LOD) and LOQ

The evaluation of the LOD and LOQ of rosuvastatin and aspirin was done based on "signal-to-noise ratio" method. The concentration which gave a signal-to-noise ratio of about 3 for LOD and about 10 for LOQ was derived. The LOD and LOQ tests were carried out using 0.1 ppm solution.

Robustness

The robustness for the developed method was demonstrated by bringing about small deliberate changes in organic phase composition, flow rate, pH of buffer in mobile phase, and column temperature and calculating its impact on system suitability parameters.

RESULTS

Table 1: System suitability parameters

System suitability parameters	Observed value		Acceptance value
	Rosuvastatin	Aspirin	
%RSD (n=6)	1.01	0.84	NMT 2.0%
Average of theoretical plates	8525	8466.16	NLT 2000
Average of tailing factor	1.10	1.13	NMT 2.0
Resolution	10		NLT 1.5

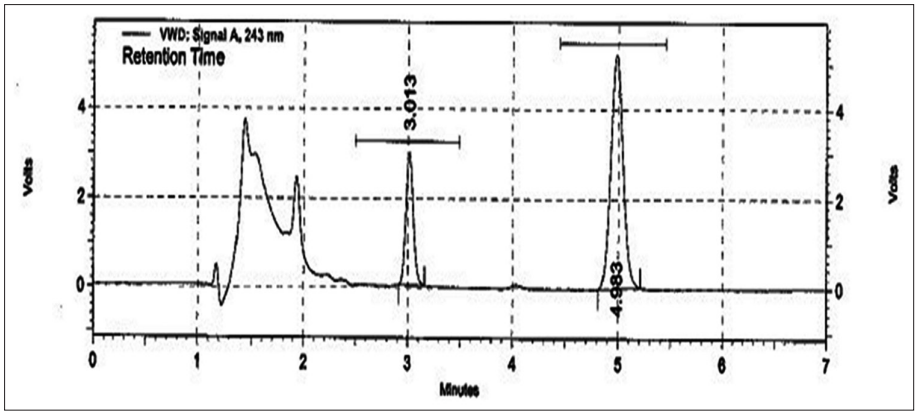

Fig. 3: Representative chromatogram of mixed standard solution for system suitability

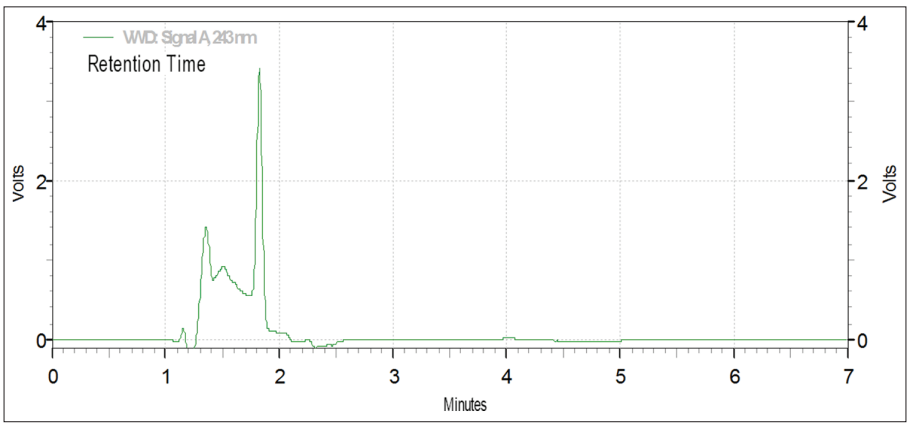

Fig. 4: A representative chromatogram of swab interference

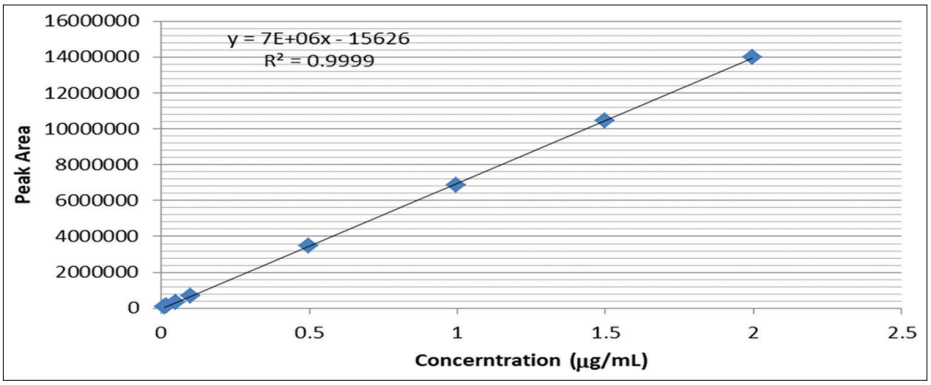

Fig. 5: Calibration curve for rosuvastatin

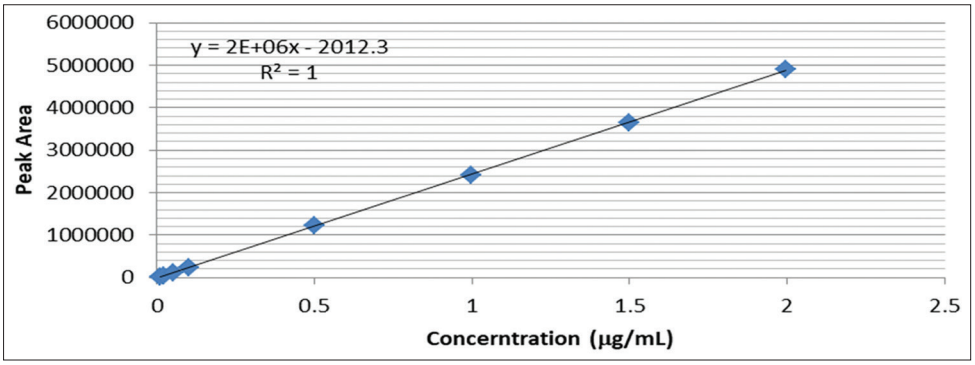

Fig. 6: Calibration curve for aspirin

Table 2: Results for specificity

Swab number	%Interference
1	No peak
2	No peak

Table 3: Statistical data for linearity

Parameters	Rosuvastatin	Aspirin
Linearity range	0.1–20 µg/ml	0.1–20 µg/ml
Slope	6,986,892.213	2,449,625.032
Intercept	−15625.95909	−2012.315733

Table 4: Percentage recovery results of rosuvastatin and aspirin

Sample number (Swab number)	% recovery 100% level		Acceptance criteria (%)
	Rosuvastatin	Aspirin	
1	91.3	77.0	70.0–110.0
2	87.4	79.0	
3	89.6	90.5	
Mean	89.4	82.1	

Table 5: Results of precision

Sr. No	Peak area of rosuvastatin	Peak area of aspirin	Acceptance criteria
1	713,778	187,511	NMT 2.0%
2	721,334	189,254	
3	712,494	187,423	
4	720,096	183,902	
5	706,232	181,889	
6	714,138	184,875	
Avg	714,679	185,809	
Σ	5492.97	2729.294	
% RSD	0.76859	1.46886857	

Table 6: Results of LOD and LOQ

Components	LOQ	LOD
	Concentration, in µg/ml	Concentration, in µg/ml
Rosuvastatin	0.1	0.03
Aspirin	0.1	0.03

LOD: Limit of detection, LOQ: Limit of quantitation

DISCUSSION

Preliminary experiments were carried out to achieve the best chromatographic conditions for the simultaneous determination of both the drugs. With the optimized chromatographic conditions, the HPLC instrument was subjected to system suitability. A representative chromatogram is depicted in Fig. 3. The system suitability parameters as summarized in Table 1, complied with the acceptance criteria. Hence, the system was found suitable for the analysis.

A representative chromatogram for swab interference is shown in Fig. 4. The chromatogram showed no interference at the retention time of the drugs. From the data tabulated in Table 2, none of the swabs showed interference. Hence, the method was found to be specific and the swabs were suitable for use.

The calibration curves (Figs. 5 and 6) showed a good correlation between peak areas and concentration of the drugs within the concentration range specified. The correlation coefficient (r^2) values for both the drugs were >0.999. The linearity data are summarized in Table 3. The linearity range was thus established as 0.1–20 µg/ml for both the drugs.

The mean % recovery at 100% level as shown in Table 4 was in the range of 70.0–110.0%. Hence, the method was found to be accurate for both the drugs.

The results of precision study as depicted in Table 5 showed % RSD value for peak areas of both drugs <2.0%. Hence, the method was found to be precise at the LOQ level.

The calculated LOD and LOQ values are shown in Table 6, which proved that the method was sensitive for both the drugs.

For robustness study, it was observed that there were no marked changes in any of the tested method parameters, which demonstrated that the developed method was robust. The results of robustness study are summarized in Table 7.

CONCLUSION

A simple, novel, and economical reversed-phase high-performance liquid chromatography method for cleaning validation has been developed for the simultaneous estimation of rosuvastatin and aspirin. The method was validated as per ICH guidelines for specificity, linearity, accuracy, precision, and robustness. The developed method is thus a sensitive analytical tool for ensuring the effectiveness of the cleaning procedure adopted.

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Table 7: Results of robustness

Parameters	Level	Rosuvastatin			Aspirin		
		R.T. (min)	%RSD	Tailing factor	R.T. (min)	%RSD	Tailing factor
Change in mobile phase composition	45% of acetonitrile variation	6.0	1.1	1.20	3.2	1.5	1.16
	50% of acetonitrile variation	4.99	1.01	1.09	2.99	0.84	1.11
	55% of acetonitrile variation	4.82	0.1	1.87	2.83	1.2	0.83
Change in pH	pH=2.3	2.92	0.1	1.7	4.82	0.7	1
	pH=2.5	4.99	1.01	1.09	2.99	0.84	1.11
	pH=2.7	2.98	0.5	1.15	4.95	1.7	1.08
Change in column oven temperature	25°C	4.99	1.01	1.09	2.99	0.84	1.11
	30°C	4.89	0.7	1.17	2.94	1.1	0.95
Change in flow rate	1.3 ml/min	5.47	0.8	1.16	3.45	2.5	1.07
	1.5 ml/min	2.99	1.01	1.09	4.99	0.84	1.11
	1.7 ml/min	4.42	1.6	1.13	2.65	1.9	1.10

AUTHORS' CONTRIBUTIONS

All authors have equally contributed toward the preparation of manuscript.

CONFLICTS OF INTEREST

All authors declare that there are no conflicts of interest.

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