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

Received: 17 October 2018, Revised and Accepted: 28 December 2018

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.

INTRODUCTION

Rosuvastatin (Fig. 1) is a hydroxymethylglutaryl-coenzymeA (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](image1)

![Fig. 2: Aspirin](image2)
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 KH₂PO₄ buffer pH 2.5
Preparation of the buffer was carried out by dissolving 1.36 g of KH₂PO₄ 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 KH₂PO₄ 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.

RESULTS

Table 1: System suitability parameters

| System suitability parameters | Observed value | Acceptance value |
|------------------------------|----------------|------------------|
| %RSD (n=6)                   | 1.01           | 0.84             | NMT 2.0%         |
| Average of theoretical plates| 8525           | 8466.16          | NIT 2.00         |
| Average of tailing factor    | 1.10           | 1.13             | NMT 2.0          |
| Resolution                   | 10             |                  | NIT 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
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 (r²) 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.
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.

REFERENCES
1. Indian Pharmacopoeia. The Indian Pharmacopoeia Commission. 6th ed., Vol. 2. Ghaziabad: Government of India, Ministry of Health and Family Welfare; 2010. p. 1199-2000, 2043-5.
2. Tripathi KD. Essentials of Medical Pharmacology. 7th ed. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd.; 2013.
3. Yang P, Burson K, Feder D, MacDonald F. Method development of swab sampling for cleaning validation. J Pharm Technol 2005;29:84-94.
4. Ghosh A, Dey S. Overview of cleaning validation in pharmaceutical industry. Int J Pharm Qual Assur 2010;2:27-8.
5. Godavariya BD, Prajapati PB, Marolia B, Shah SA. Development and validation of RP-HPLC method for simultaneous estimation of rosuvastatin and aspirin in marketed formulation. Int J Pharm 2012;3:1735-45.
6. Solanki C, Patel N. Development and validation of RP-HPLC method for simultaneous estimation of rosuvastatin calcium and aspirin in capsule dosage form. Int J Pharm Biol Sci 2012;3:577-85.
7. Nazareth C, Shivakumar B, Reddy P. A novel RP-HPLC method for the simultaneous estimation of cardiovascular drugs in a polycap formulation. World J Pharm Res 2018;7:677-87.
8. Nazareth C, Bodke A. Development and validation of a novel cleaning validation and assay method for simultaneous estimation of rosuvastatin and fenofibrate by RP-HPLC. World J Pharm Res 2018;7:1454-65.
9. Nazareth C, Shivakumar B, Reddy P, Acharya S, Verekar S. A simple RP HPLC method for simultaneous estimation of six cardiovascular drugs in bulk and dosage form. IOSR J Pharm Biol Sci 2015;10:32-7.
10. Dumasiya M, Bhattacharya K, Patel B, Joshi N. Development and validation of stability indicating RP-HPLC method for estimation of rosuvastatin calcium and aspirin in combined dosage form. Int J Pharm Sci 2012;3:2421-39.
11. Pandya CB, Channabasavaraj KP. Development and validation of RP-HPLC method for determination of rosuvastatin calcium in bulk and pharmaceutical dosage form. Int J Pharm Sci Res 2010;5:82-6.
12. Turabi ZM, Khatafreh OA. Stability-indicating RP-HPLC method development and validation for the determination of rosuvastatin (calcium) in pharmaceutical dosage form. Int J Pharm Sci Drug Res 2014;6:154-5.
13. Reddy GV, Reddy BV. Development and validation of a stability indicating UPLC method for rosuvastatin and its related impurities in pharmaceutical dosage forms. Int Year Chem 2011;34:250-5.
14. Trivedi HK, Patel MC. Development and validation of a stability-indicating RP-UPLC method for determination of rosuvastatin and related substances in pharmaceutical dosage form. Sci Pharm 2012;80:393-406.
15. Pisal P, Nigade G, Kale A, Pawar S. Development and validation of stability indicating RP-HPLC method for simultaneous determination of aspirin, rosuvastatin, clopidogrel in bulk and pharmaceutical dosage form. Int J Pharm Sci 2018;10:51-6.
16. Yulianita R, Sopyan I, Muchtaridi M. Forced degradation study of statins: A review. Int J Pharm Pharm Sci 2018;10:38-42.
17. Ramadan AZ, Mandil H, Ali RS. Spectrophotometric determination of rosuvastatin in pure form and pharmaceutical formulations through ion-pair complex formation using bromocresol green. Int J Pharm Sci 2015;7:191-8.
18. Ramadan AZ, Mandil H, Shelwahi N. Spectrophotometric determination of rosuvastatin calcium in pure form and pharmaceutical formulations by the oxidation using iodine and formation triiodide complex in acetonitrile. Int J Pharm Sci 2014;6:579-89.
19. Begum S, Bashar SA, Shazia F. Formulation and in-vitro evaluation of mouth dissolving tablets ofamlodipine and rosuvastatin. Int J Curr Pharm Res 2015;7:88-91.
20. Prabu SL, Suriyaprakash TN. Cleaning validation and its importance in pharmaceutical industry. Pharm Times 2010;42:21-4.
21. Fourman GL, Mullen MV, Elilly E. Cleaning validation acceptance limits for pharmaceutical manufacturing operations. Pharm Times 1993;17:54-60.
22. ICH: Q2B. Harmonized Tripartite Guidelines, Validation of analytical procedures: Methodology. IFPMA. Geneva: Proceedings of the International Conference on Harmonization; 1996.
23. ICH: Q2 [R1]. Validation of Analytical Procedures: Text and Methodology; 2005.