JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Rosuvastatin Calcium, Clopidogrel Bisulfate and Aspirin in Solid Dosage Form

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Article History:

Received 15 April 2023
Accepted 31 May 2023
Available online 05 July 2023

Citation:
Patel B. N., Naik S. N. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Rosuvastatin Calcium, Clopidogrel Bisulfate and Aspirin in Solid Dosage Form **J Pharm Sci Bioscientific Res**. 2023. 11(5): 124-128

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(www.jpsbr.org)

ABSTRACT:

The present paper describes the simple, specific, accurate, precise and cost-effective reverse phase high-performance liquid chromatographic method for the simultaneous estimation of rosuvastatin calcium, clopidogrel bisulfate and aspirin in solid dosage form. Chromatographic separation was achieved using ODS C18 RP column, 250 mm × 4.6 mm, 5µm using a mobile phase consisting of a mixture of phosphate buffer: acetonitrile (60:40) and pH adjusted to 3.00 with 0.5% orthophosphoric acid. The detection was made at 235 nm. The retention times were about 3.007, 4.113 and 6.987 minutes for ASP, CLO and ROS. The method was validated according to the ICH cases. The method was linear in the range of 10-30 µg/ml for ASP, CLO and 2.5-7.5 µg/ml for ROS. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation and robustness. The proposed method can be used for the estimation of these drugs in combined dosage form.

KEYWORDS: RP-HPLC, Aspirin, Rosuvastatin calcium, Clopidogrel bisulfate.

1 INTRODUCTION [1-10]

Aspirin (ASP) (fig. 1a) is 2-(acetyloxy) benzoic acid, an analgesic, antipyretic, anti-inflammatory and antithrombic agent is official in IP [1], BP [2] and USP-NF [3]. It is an antiplatelet agent approved by the Food and Drug Administration, USA, for use in secondary prevention of heart attacks and stroke [4, 5].

Rosuvastatin calcium is (ROS) (E)-(3R, 5 S)-7-{4-(4-fluorophenyl)-6-isopropyl-2-{methyl (methyl sulphonyl amino)] pyrimidin-5-yl}-3, 5 dihydroxyhepten-6-oic acid calcium (fig. 1b), a synthetic lipid-lowering agent is official in IP [1]. It is a selective competitive inhibitor of the enzyme HMG-CoA reductase, which catalyses the conversion of HMG-CoA to mevalonate, an important rate limiting step in cholesterol biosynthesis.

Clopidogrel bisulphate is (CLO) (+)-(S)-methyl2-(2-chlorophenyl)-2-(6, 7-dihydrothieno [3, 2-c] pyridin-5(4H)-yl) acetate (fig. 1c), sulfate is a new thienopyridine derivative an antiplatelet agent, which selectively inhibits the binding of adenosine diphosphate (ADP) to its platelet receptor and blocks the subsequent ADP-mediated activation of the glycoprotein GPIIb/III a complex, thereby inhibiting platelet aggregation. It is official in USP-NF [3]. It has been shown to prevent ischemic stroke, myocardial infraction and vascular disease and has demonstrated its clinical efficacy superior to that of aspirin. Thus, clopidogrel is indicated for the patients with atherosclerosis documented by recent stroke, recent myocardial infraction or cardiovascular disease. The ternary combination of ASP, ROS and CLO is used for atherosclerotic patients suffering from various heart diseases.

Many dosage forms of ASP, ROS and CLO as a single or as combination dosage form with others are available in local market for effective therapy. Literature survey revealed many reported analytical methods such as spectroscopy, stability indicating HPTLC, GC, simple HPLC and LC-MS-MS

have been reported for the determination of ASP [6-8], ROS [9-13] AND CLO [14-18] in pharmaceutical dosage forms and biological samples.

To the best of our knowledge there is no analytical method reported for simultaneous determination of ternary mixture containing ASP, ROS and CLO. Therefore, an attempt has been made to develop a simple, accurate, rapid and reproducible reverse phase HPLC method for simultaneous determination of ASP, ROS and CLO in solid dosage form and validate it, in accordance with ICH guidelines [19].

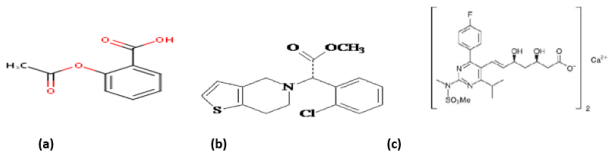

Figure 1 Molecular structure of (a) aspirin (b) Clopidogrel bisulfate and (c) Rosuvastatin calcium.

2 EXPERIMENTAL WORK

2.1 Materials and reagents

ASP, ROS and CLO were provided as a gift sample by S.KANT Pvt Ltd. (Vapi, Gujarat). Capsule dosage form (Rosutor gold; 75 mg ASP, 75 mg CLO and 20 mg ROS per capsule) of Ajanta Pharma Ltd was purchased from local market. Methanol, acetonitrile and water (HPLC grade) were obtained from Lichrosoly-E. Merck Ltd. (Mumbai, India). Orthophosphoric acid (analytical grade) was also obtained from E. Merck Ltd. (Mumbai, India).

2.2 Instrumentation

The chromatographic separation was carried out using a HPLC – [Shimadzu-LC Solution] and a UV detector. The output signal was monitored and processed by LC solution software. A UV – Visible Spectrophotometer – [Labindia (3000+)], Analytical balance and Sonicator were also used.

2.3 Chromatographic conditions

Stationary phase: C18 column (250 mm x 4.6 mm i.d, 5 µm particle size) was used at ambient temperature.
Mobile Phase: Phosphate buffer: acetonitrile (60:40) (pH adjusted to 3.0 using 0.5% Ortho Phosphoric acid)
Flow rate: 1.0 ml/min
Injection volume: 20 µL
Detection: At 235 nm with UV Visible detector.
Before analysis the mobile phase was filtered through a 0.2 µm membrane and degassed by ultra-sonication.

2.4 Preparation of Standard Stock Solutions and calibration graphs for chromatographic measurement

Stock standard solutions were prepared by dissolving separately 20 mg ASP, 20 mg CLO and 5 mg ROS in 100 ml methanol (200 µg/ml, 200µg/ml and 50µg/ml). An aliquot of the stock solution (1ml) was transferred to a 10 ml volumetric flask, and diluted to the mark with mobile phase to obtain a mixed working standard of ASP (20 µg/ml), CLO (20 µg/ml) and ROS (5 µg/ml).

2.5 Preparation of sample solutions

Powder of 20 capsules (rosutor gold), each containing 75 mg ASP, 75 mg CLO, and 20 mg ROS, were weighed and analysed: a quantity of powder equivalent to one capsule was weighed and transferred to a 100 ml volumetric flask containing 50 ml methanol, sonicated for 15 min and dilute to 100 ml with methanol. The original stock solution was further diluted to get sample solution of drug concentration of 20 µg/ml ASP, 20 µg/ml CLO and 5 µg/ml ROS. A 20 µL volume of sample solution was injected into HPLC. The peak area for the drugs was measured at 235 nm and amount of ASP, CLO and ROS were determined using the related linear regression equations.

2.6 Method validation

The developed RP-HPLC method was validated to confirm that the present method was suitable for its intended purpose as described in ICH guidelines Q2 (R1). The described method extensively validated in terms of specificity, linearity, accuracy, precision, limit of detection, limit of quantitation and robustness.

2.6.1 Linearity and Range

The linearity of the method was determined at concentration levels ranging from 10-30 µg/ml of ASP, CLO and 2.5-7.5 µg/ml. The calibration curves were constructed by plotting peak area versus concentration of ASP, ROS and CLO. The slope, Y-intercept and correlation coefficient were calculated.

2.6.2 2.6.2 Accuracy (% Recovery)

The accuracy of the method was evaluated in triplicate at three concentrations levels, 80, 100 and 120% of the target test concentration (20 µg/ml of ASP and CLO, 5 µg/ml of ROS). The percentage recoveries were calculated.

2.6.3 Precision

Precision was investigated using three sample preparation procedure for three samples of commercial capsule (Rosutor Gold).

Intraday: The precision of the method was evaluated by carrying out six independent assay of ASP, CLO and ROS (20µg/ml, 20µg/ml and 5µg/ml) test samples against qualified reference standards.

Intermediate Precision (Inter day): A different analyst on different day in the same laboratory evaluated the intermediate precision %RSD of the method. Six test samples were assayed against reference standard.

2.6.4 Limit of Detection and Limit of Quantitation

The LOD and LOQ were estimated using signal to noise ratio of 3:1 and 10:1 as per ICH guidelines.

2.6.5 Robustness

The robustness of the method was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions: Flow rate (±2), composition of mobile phase (±2) and pH of mobile phase (±2).

2.6.6 System Suitability test (SST)

The system suitability tests represent an integral part of the method and are used to ensure adequate performance of the chromatographic system. The parameters Retention time (RT), theoretical plates (N), Tailing factor (T), Peak asymmetry (As) and Repeatability were calculated using replicate injections of the drug at a concentration of ASP (20µg/ml), CLO (20µg/ml) and ROS (5µg/ml).

3 RESULTS AND DISCUSSION

For successful method validation, preliminary tests were performed with the objective to select adequate and optimum condition. Parameters, such as choice of analytical column, pH of buffer, mobile phase composition and proportion, detection wavelength and other factors were exhaustively studied. Various reversed columns and isocratic mobile phase system were tried. When experiments were performed with methanol instead of acetonitrile as the organic modifier in the mobile phase, late elution of analyte with peak tailing and high column pressure were observed. Hence, the experiments were carried out with acetonitrile as an organic modifier. A satisfactory separation of the three drugs was achieved on a Phenomenex column with a mobile phase of Phosphate buffer (pH: 4) and acetonitrile (60:40, v/v) at a flow rate of 1.0 ml/min. The detection was carried out at 235 nm. Better resolution of the peaks with clear base line separation was found (Figure2).

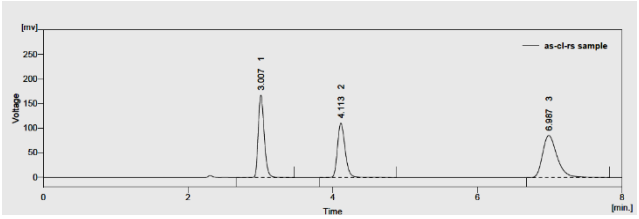

Figure 2 Chromatographic separation of ASP (3.007 min), CLO (4.113 min) and ROS (6.987 min) from their formulation.

3.1.1 Linearity

Linear correlation was obtained between peak area and concentration in the range of 10-30 µg/ml for ASP, CLO and 2.5-7.5 µg/ml for ROS. The linearity of the calibration curves were validated by the value of correlation coefficient of the regression (r). The regression analysis of the calibration curves is shown in Table 1.

Table 1 Results from regression analysis of the calibration curves

Parameters	ASP	CLO	ROS
Slope	48.39	39.47	231.9
Intercept	21.12	7.33	1.91
Correlation coefficient (r)	0.995	0.997	0.995

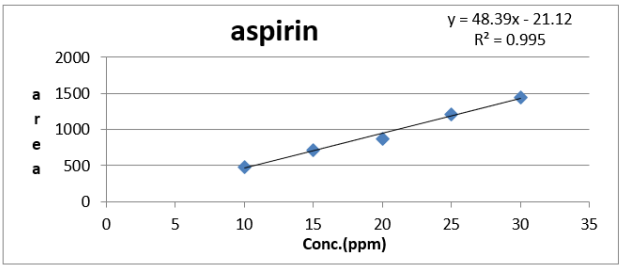

Figure 3. Calibration curve for ASP

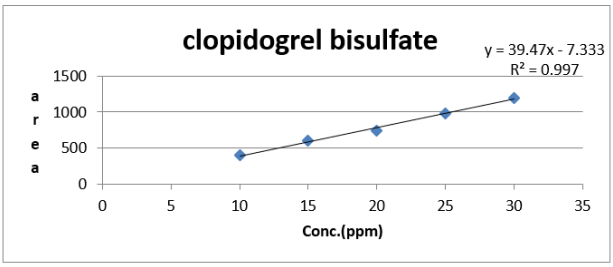

Figure 4. Calibration curve for CLO

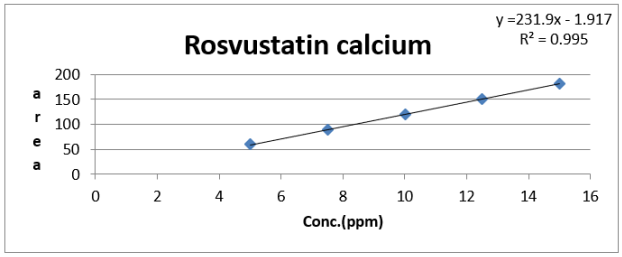

Figure 5. Calibration curve for ROS

3.1.2 Accuracy

The recovery experiments were carried out by the standard addition method. The percentages of the recoveries obtained were 99.468-99.828% for ASP, 99.409-99.925% for CLO and 98.768-99.932% for ROS, respectively (Table 2). The recovery of the method was good.

3.1.3 Precision

The percentage RSD values for the precision study were 0.205-0.462% for ASP, 0.175-1.625% for CLOP and 0.816-1.064% for ROS (inter-day precision) and 0.153-0.377% for ASP, 0.468-0.830% for CLOP and 0.693-1.418% for ROS (intra-day precision) confirming a good precision (Table 2).

3.1.4 Limit of detection and limit of quantification

The LODs for ASP, CLO and ROS were found to be 1.44, 0.220 and 0.221µg/ml, while the LOQs were 4.364, 0.667 and 0.636 µg/ml respectively (Table 2).

3.1.4 Robustness

The method was found to be robust, although small deliberate changes in method conditions did have a negligible effect on the chromatographic behaviour of the solute. The results indicate that changing the mobile phase composition and flow rate had no large effect on the chromatographic behaviour of ASP, CLO and ROS. Even a small change of mobile phase composition did not cause a notable change in the retention time of the used drugs for this method. A minor increase or decrease of the flow rate did also not cause any change in the tailing of peak of each drug. Alteration of the pH (±2) caused no variation of peak areas.

Table 2. Summary of validation parameters for the proposed method

Parameters	ASP	CLO	ROS
LOD	1.440	0.220	0.221
LOQ	4.364	0.667	0.636
Accuracy(%) 80%	99.131-	98.693-	99.0251-
100%	100.585	100.923	101.248
120%	98.383-	98.687-	99.231-
	100.709	100.302	100.634
	99.328-	98.992-	99.271-
	100.312	99.892	100.262
Precision(%RSD)	0.153-0.377	0.468-0.830	0.816-1.064
Interday	0.205-0.462	0.468-0.830	0.693-1.418
Intraday			
Repeatability (%RSD)	0.383	0.716	1.189

4 CONCLUSIONS

The validated RP-HPLC method employed here proved to be simple, rapid, specific, accurate, precise, sensitive and robust. It can be successfully used for routine analysis of ASP, CLO and ROS in combined dosage form without any interference from common excipients and impurity. With the developed method, only this mobile phase is sufficient for quantification of ASP, CLO and ROS either in combination or in single dosage form as per availability of formulation for many pharmaceutical industries. Hence, the proposed method can save labour cost and analysis time for changing mobile phase. This makes the method suitable for routine analysis in quality control laboratories.

5 ACKNOWLEDGMENT

The authors are grateful to the S.Kant (Vapi, Gujarat) for providing gift samples of reference standards.

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