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Research Article

RP-HPLC Method Development and Validation for Simultaneous Estimation of Aspirin and Rivaroxaban in Bulk and Formulation

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Abstract

A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Aspirin and Rivaroxaban in pharmaceutical dosage form. Chromatographic separation of Aspirin and Rivaroxaban was achieved on Waters Alliance-e2695by using Waters Zorbax SB C18 (150x 4.6mm, 3.5µ) column and the mobile phase containing Acetonitrile: Ammonium Formate pH-3.0/OPA in the ratio of 50:50% v/v. The flow rate was 1.0 ml/min; detection was carried out by absorption at 269nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Aspirin and Rivaroxaban were NLT 2000 and should not more than 2 respectively. % Relative standard deviation of peak areas of all measurements always less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate and robust method for quantitative analysis of Aspirin and Rivaroxaban study of its stability.

Keywords: HPLC; Validation; Aspirin and Rivaroxaban

Introduction

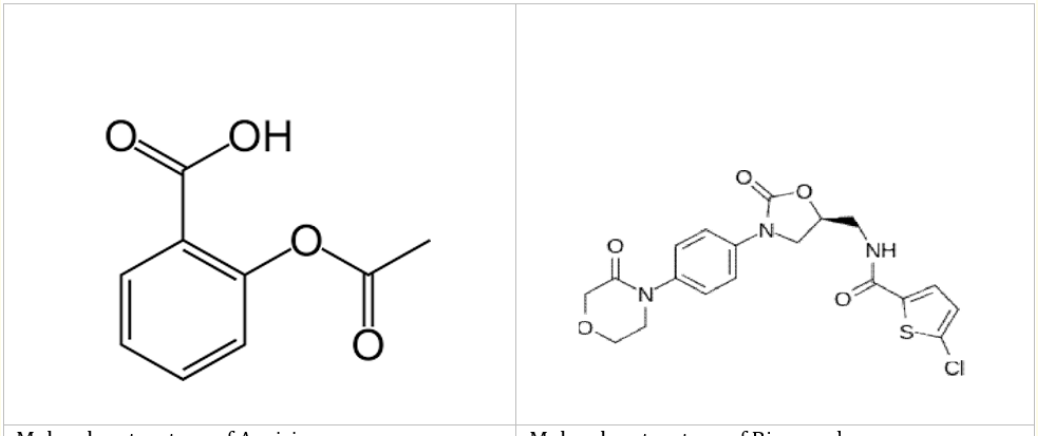
High-Performance Liquid Chromatography (HPLC) has emerged as one of the most widely used analytical techniques for the qualitative and quantitative determination of pharmaceutical compounds. It offers high resolution, sensitivity, and reproducibility, making it suitable for simultaneous estimation of multiple drugs in combination dosage forms or biological matrices. Aspirin (acetylsalicylic acid) is a well-known nonsteroidal anti-inflammatory drug (NSAID) extensively used for its analgesic, antipyretic, and anti-inflammatory properties. It is also employed in low doses as an antiplatelet agent to prevent thrombotic events such as myocardial infarction and ischemic stroke [1]. Due to its widespread clinical use, especially in cardiovascular therapy, there is a significant need for reliable analytical methods to monitor its concentration in pharmaceutical formulations and biological fluids.

Rivaroxaban is a selective, direct Factor Xa inhibitor, used as an oral anticoagulant for the prevention and treatment of venous thromboembolism (VTE), atrial fibrillation-related stroke, and pulmonary embolism [2]. Rivaroxaban has a rapid onset of action and predictable pharmacokinetics, eliminating the need for routine coagulation monitoring, yet it still requires robust analytical methods for quality control and pharmacokinetic studies.

Simultaneous administration of aspirin and rivaroxaban is often indicated in patients with cardiovascular conditions, particularly in those with high thrombotic risk, such as post-acute coronary syndrome patients [3]. Given their co-administration, developing a validated, HPLC method for the simultaneous estimation of aspirin and rivaroxaban is essential for formulation development, quality control, and regulatory compliance. It is then validated in accordance with ICH Q2 (R1) guidelines [4].

The review of the literature indicates that only a few analytical techniques, including HPTLC [5], HPLC [6-8] for Aspirin alone, and HPLC [9-11], are available for the quantification of Aspirin and Rivaroxaban both alone and in combination with other medications in APIs and formulations.

The present study aims to develop and validate a simple, sensitive, and rapid reverse-phase HPLC (RP-HPLC) method for the simultaneous estimation of aspirin and rivaroxaban in bulk and combined dosage forms, in accordance with ICH Q2 (R1) guidelines. The method will be validated for specificity, linearity, accuracy, precision, robustness, and detection limits to ensure its suitability for routine analysis [12-16].



Molecular structure of Aspirin | Molecular structure of Rivaroxaban

Figure 1

Materials and Methods

Chemicals and reagents

Aspirin and rivaroxaban pure drugs were received from Pharma life research lab, Hyderabad. Aspirin and rivaroxaban tablets were obtained from the local market. Acetonitrile HPLC grade was received from Rankem, Water (Milli Q) was produced in-house, and Ammonium Formate, orthophosphoric acid, and Formic Acid of HPLC grade were obtained from analytical reagents.

Preparation of standard solution

Accurately weigh and transfer 15 mg of Aspirin into 10 ml volumetric flask, 20 mg of Rivaroxaban working standard into another 10 ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Add 1 ml of Rivaroxaban solution in to Aspirin 10ml volumetric flask and dilute up to the mark with diluent. (Stock solution).

Further pipette 1 ml of the above stock solutions into a 10 ml volumetric flask and dilute upto the mark with diluent. (150 ppm of Aspirin, 20 ppm of Rivaroxaban).

Preparation of sample solution

Accurately weighed and transfer 22.6 mg of Aspirin and 5 mg Rivaroxaban sample into a 10 mL clean dry volumetric flask add Diluent and sonicate it up to 30 mins to dissolve, and centrifuge for 30min. to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.45 micron Injection filter (Sample Stock solution).

Further pipette 1 ml of the above stock solutions into 10 ml volumetric flask and dilute up to the mark with diluents. (150 ppm of Aspirin, 20 ppm a of Rivaroxaban).

Inject 10 *µL of the standard, sample into the chromatographic* system and measure the areas for Aspirin and Rivaroxaban peaks and calculate the %Assay by using the formulae.

Preparation of diluent (Mobile Phase)

Mobile phase was prepared by mixing Acetonitrile and Ammonium FormatepH-3.0/OPA taken in the ratio50:50. It was filtered through 0.45µ membrane filters to remove the impurities which may interfere in the final chromatogram.

Method validation

Specificity

Specificity of an analytical method is ability to measure specifically the analyte of interest without interference from blank and known impurities. For this purpose blank chromatogram, standard chromatogram and sample chromatogram were recorded. The chromatogram of blank shows no response at the retention times of drugs which confirms the response of drugs was specific.

Linearity

Preparation of stock solution

Accurately weigh and transfer 15 mg of Aspirin into 10 ml volumetric flask, 20 mg of Rivaroxaban working standard into another 10 ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Add 1 ml of Rivaroxaban solution in to Aspirin 10 ml volumetric flask and dilute up to the mark with diluent. From the above stock solution 37.5, 75, 112.50, 150, 187.50, 225 ppm of Aspirin, 5, 10, 15, 20, 25, 30 ppm of Rivaroxaban were prepared using the diluents.

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Range

The Range of an analytical method is the interval between the upper and lower levels of analyte (including these levels) that have been demonstrated with precision, accuracy and linearity.

Preparation accuracy sample solutions

For preparation of 50% solution (With respect to target Assay concentration)

Accurately weigh and transfer 22.6 mg of Aspirin and 5mg of Rivaroxaban sample into a 10 ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.5 ml of the above stock solutions into a 10 ml volumetric flask and dilute upto the mark with diluent. (75 ppm of Aspirin 10 ppm of Rivaroxaban).

For preparation of 100% solution (With respect to target Assay concentration)

Accurately weigh and transfer 22.6 mg of Aspirin and 5 mg of Rivaroxaban sample into a 10 ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 1 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent. (150 ppm of Aspirin and 20 ppm of Rivaroxaban).

For preparation of 150% solution (With respect to target Assay concentration)

Accurately weigh and transfer 22.6 mg of Aspirin and 5 mg of Rivaroxaban sample into a 10 ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 1.5 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent. (225 ppm of Aspirin 30 ppm of Rivaroxaban).

Inject the standard solution, Accuracy-50%, Accuracy-100% and Accuracy-150% solutions. The %Recovery for each level should be between 98.0 to 102.0%.

Precision

Precision is the degree of repeatability of an analytical method under normal operation conditions. System precision is checked by using standard chemical substance to ensure that the analytical system is working properly. In this peak area and %of drug of six determinations is measured and % RSD should be calculated.

In method precision, a homogenous sample of single batch should be analyzed 6 times. This indicates whether a method is giving constant results for a single batch. In this analyze the sample six times and calculate the % RSD.

The precision of the instrument was checked by repeatedly injecting (n = 6) solutions of 150 ppm of Aspirin, 20 ppm of Rivaroxaban).

Robustness

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

The flow rate was varied at 0.9 ml/min to 1.1 ml/min.

Standard solution 150 ppm of Aspirin, 20 ppm of Rivaroxaban was prepared and analysed using the varied flow rates along with method flow rate.

On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.

The variation of Organic Phase ratio.

Standard solution of 150 ppm of Aspirin, 20 ppm of Rivaroxaban was prepared and analysed using the varied in mobile phase ratio.

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) limit of quantification (LOQ) of the drug carry was calculated using the following equation as per international conference harmonization (ICH) guidelines.

LOD = 3.3 X σ/S

LOQ = 10 X σ/S

Results and Discussion

The chromatographic analysis was performed using an Zorbax C18(150x4.6 mm, 3.5µ) with a mobile phase consisting of Acetonitrile and Ammonium Formate pH- 3.0/OPA(50:50). The flow rate was set at 1 ml/min, and the detector wavelength was 269 nm. The column temperature was maintained at 25°C, and an injection volume of 10 µL was used. The total run time for each analysis was 6.0 minutes. Both peaks have good resolution, tailing factor, Theoretical plate count, and resolution. The total runtime for each validation parameter was set to 6 minutes.

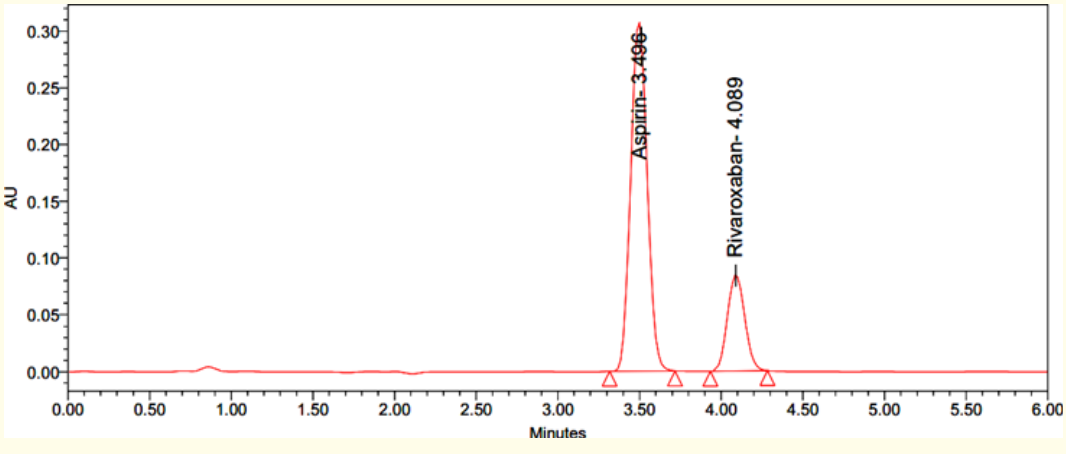


Figure 2: Optimized Chromatogram.

With the optimized chromatographic conditions, the Aspirin peak was observed at 3.496 min with peak area 2148402, tailing factor 1.06 Rivaroxaban peaks was observed at 4.089 min, with peak area 301043, tailing factor 1.09 and resolution 3.53.

Method validation

The following parameters were studied to validate the HPLC method for the determination of Aspirin and Rivaroxaban as per the protocol and demonstrate that the method is appropriate for its intended use. All the validation parameters were carried out according to ICH.

Specificity

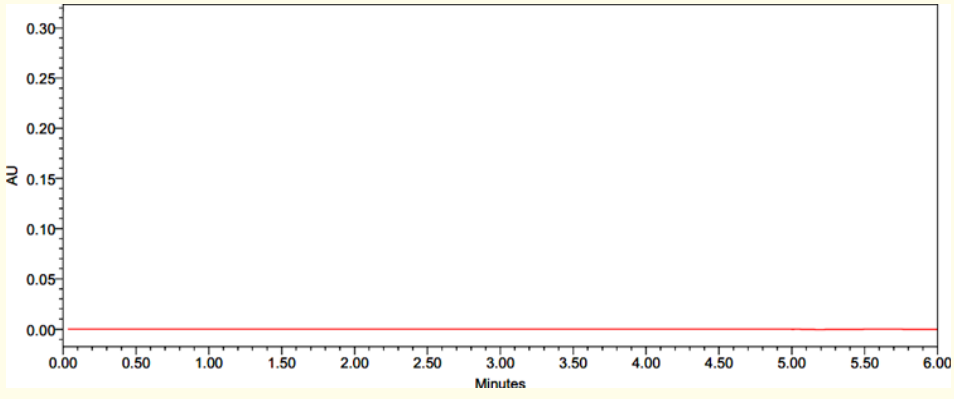


Figure 3: Chromatogram of blank.

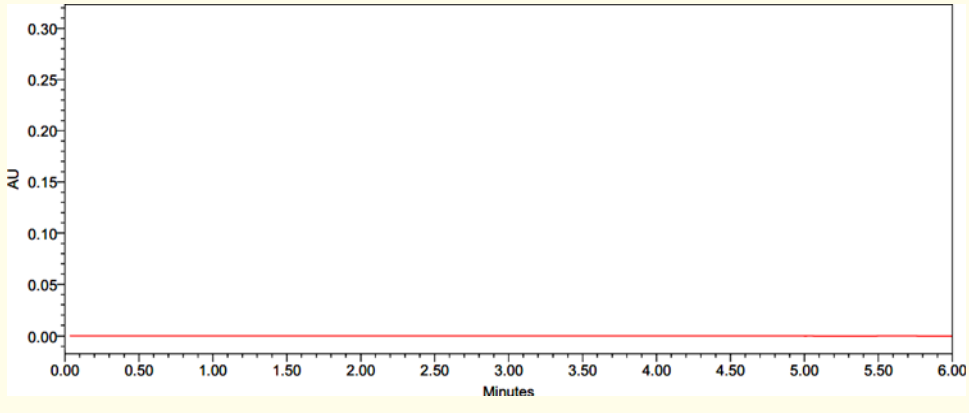


Figure 4: Chromatogram of placebo.

Linearity

Six linear concentrations of Aspirin (37.50–225 µg/ml) and Rivaroxaban (5 -30 µg/ml) were injected and duplicated. Average areas were mentioned in table No. 1, and the linearity equations obtained for Aspirin was y = 13981.84x+26341.75, and for Rivaroxaban, was y =15273.63x+1960.00. The correlation coefficient obtained was 0.999 for the two drugs.

Precision

Method precision

The %RSD (Relative Standard Deviation) for Aspirin and Rivaroxaban was calculated using six replicate injections. The mean area for Aspirin was 2136525 with a standard deviation (S.D) of 8138.98, resulting in a %RSD of 0.381%. Similarly, the mean area for Rivaroxaban was 302124, with a standard deviation of 1232.18,

S. No.	Aspirin		Rivaroxaban	
	Conc.(µg/ml)	Peak area	Conc.(µg/ml)	Peak area
1	37.50	558639	5.00	82746
2	75.00	1085735	10.00	151773
3	112.50	1624721	15.00	231448
4	150.00	2132381	20.00	305091
5	187.50	2629283	25.00	388697
6	225.00	3164333	30.00	457696
Regression equation	y=13981.84x+26341.75		y=15273.63x+1960.00	
Slope	13981.84		15273.63	
Intercept	26341.75		1960.00	
R^2	0.99987		0.99979	

Table 1: Linearity for Aspirin and Rivaroxaban.

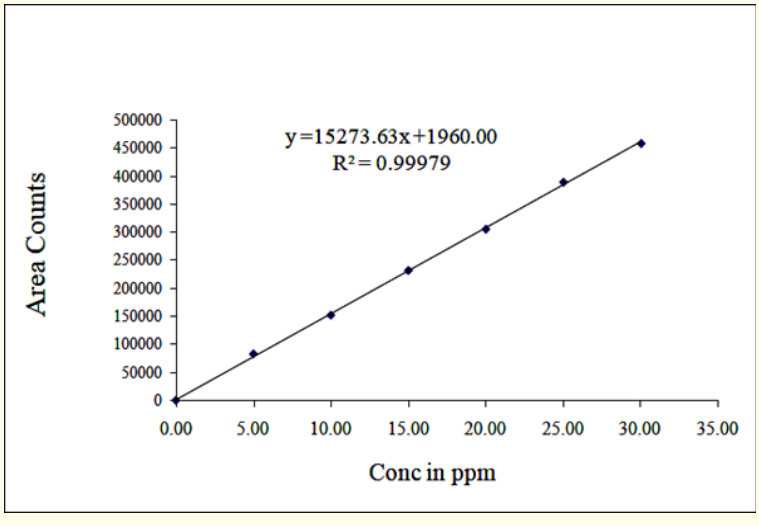


Figure 5: Calibration curve of Rivaroxaban.

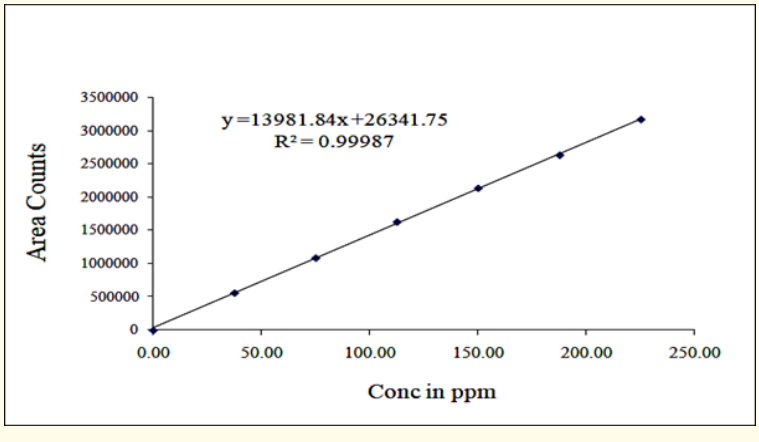


Figure 6: Calibration curve of Aspirin.

giving a %RSD of 0.408%. These low %RSD values indicate high precision and reproducibility of the method for both compounds.

Intermediate precision (Day_ Day Precision)

Intermediate precision of Aspirin and Rivaroxaban based on six replicate injections. The mean area for Aspirin on day-1 and day-2 was 2132504.33, 2148676.33 with a standard deviation (S.D) of 18188.67, 27356.67 resulting in a %RSD of 0.85%, 1.27% respectively. Rivaroxaban mean area on day-1 and day-2 was 303895, 304359.833 with a standard deviation of 1323.91, 3975.8 yielding a %RSD of 0.44%, 1.31% respectively. These results indicate good intermediate precision for both analytes, demonstrating the method's consistency when tested under different conditions or over different days.

Accuracy

Three levels of accuracy samples were prepared by the standard addition method. Triplicate injections were given for each level of accuracy, and the mean Recovery was 100.066% and 99.3% for Aspirin and Rivaroxaban, respectively.

Sensitivity

The Limit of Detection (LOD) for Aspirin was 0.45 µg/ml, and the Limit of Quantitation (LOQ) was 1.5 µg/ml. Rivaroxaban LOD was 0.06 µg/ml, and the LOQ was 0.2 µg/ml. These values indicate the method's ability to detect and quantify deficient concentrations of both analytes with high sensitivity.

Assay

Xarelto, bearing the label claim Aspirin and Rivaroxaban. An assay was performed with the above formulation. The average % Assay for Aspirin and Rivaroxaban obtained was 99.8% and 98.6%, respectively.

Conclusion

The developed HPLC method for the estimation of selected drugs is simple, rapid, accurate, precise, robust and economical. The mobile phase and solvents are simple to prepare and economical, reliable, sensitive and less time consuming.

The sample recoveries were in good agreement with their respective label claims and they suggested non interference of formulation recipients in the estimation and can be used in laboratories for the routine analysis of selected drugs.

Since the system validation parameters of HPLC method used for estimation of selected drugs in pure and have shown satisfactory, accurate and reproducible results (without any interference of recipients) as well, it is deduced that the simple and short proposed methods be most useful for analysis purpose.

The present work concluded that stability indicating assay method by RP-HPLC was simple, accurate, precise, and specific and has no interference with the placebo and degradation products. Hence these can be used for routine analysis of Aspirin and Rivaroxaban.

Conflict of Interest

No conflict of interest exists.

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