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Rivaroxaban – Analytical Method Development and Validation

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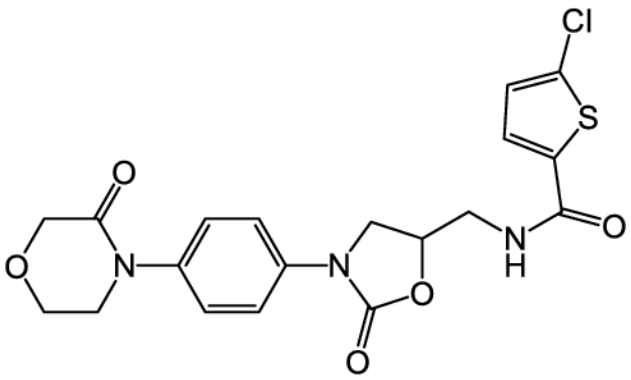
Abstract: A simple, specific, accurate, and precise reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the quantitation of Rivaroxaban in bulk drug and pharmaceutical dosage form. The quantification is carried out with a HiQSil C18 (250 mm x 4.6 mm, 5 µm) column maintained at room temperature with mobile phase consisted of a mixture of methanol and water in the ratio of 65:35 v/v delivered at a flow rate of 1.4 ml/min and effluents were monitored at 249 nm through JASCO UV-4075 UV-VIS detector. The retention time of Rivaroxaban was found to be 3.12 min. The method was validated as per ICH guidelines, parameters like linearity, accuracy, precision, specificity, limit of detection, limit of quantitation and robustness. The linearity was in the range of 5 – 30 µg/ml with correlation coefficient of 0.9995. The recovery of Rivaroxaban was found to be 100%. Limit of detection and limit of quantitation were found to be 1.12 µg/ml and 2.02 µg/ml respectively. The proposed method was successfully applied for the quantitative determination of Rivaroxaban in tablet dosage form in quality control testing laboratories.

Introduction

Rivaroxaban is an oral anticoagulant and direct factor Xa inhibitor which is used in the prevention of stroke and venous embolism in patients with chronic atrial fibrillation, as well as treatment and prevention of deep venous thromboses and pulmonary embolism. Rivaroxaban has been associated with a low rate of serum enzyme elevations during treatment and with rare instances of clinically apparent liver injury with jaundice. Chemically it is 5-Chloro-N-[[(5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl) phenyl]-1,3-oxazolindin-5-yl] methyl] thiophene-2-carboxamide. It is an empirical formula $C_{19}H_{18}ClN_3O_5S$ and molecular weight of 435.882 g/mol. [1]



Rivaroxaban competitively inhibits free and clot bound factor Xa. Factor Xa is needed to activate prothrombin (factor II) to thrombin (factor IIa). Thrombin is a serine protease that is required to activate fibrinogen to fibrin, which is the loose meshwork that completes the clotting process. Since one molecule of factor Xa can generate

more than 1000 molecules of thrombin, selective inhibitors of factor Xa are profoundly useful in terminating the amplification of thrombin generation. The action of Rivaroxaban is irreversible. [2]

Literature survey revealed that few analytical methods have been reported for estimation of Rivaroxaban individually or in combination with other drugs. The reported methods are spectrophotometric, RP-HPLC and HPTLC methods. The present study was aimed to develop a simple, sensitive, rapid and precise RP-HPLC method for estimation of Rivaroxaban. The analytical method was validated according to ICH validation parameters. [3]

Analytical method development

Rivaroxaban API

Determination of Lambda maximum

Preparation of stock solution of Rivaroxaban

Rivaroxaban (25mg) in a 50 ml volumetric flask and 25 ml of methanol to it and it was vortexed (Eltek) for 2 minutes. This was the main stock accounting for concentrations of 1000 µg/ml. A diluted solution was used to scan in UV-Spectrophotometer in the range of 200-400 nm, taking methanol as blank.

The lambda maximum for Rivaroxaban was found to be 249 nm.

Instrumentation and chromatographic conditions

HPLC system was used JASCO system equipped with model PU 4180 RHPLC pump, Rheodyne sample injector port (20 µl), JASCO UV-4075 UV-VIS detector and ChromNAV CFR chromatography software (version 2.0). Separation was carried out on HiQSil C18 (250 mm x 4.6 mm, 5 µm) column using methanol: water (65:35 v/v) as mobile phase at flow rate of 1.4 ml/min. Samples were injected using Rheodyne injector with 20 µl loop, detection was carried out at 249 nm. All weighing were done on Shimadzu balance (Model AY-120).

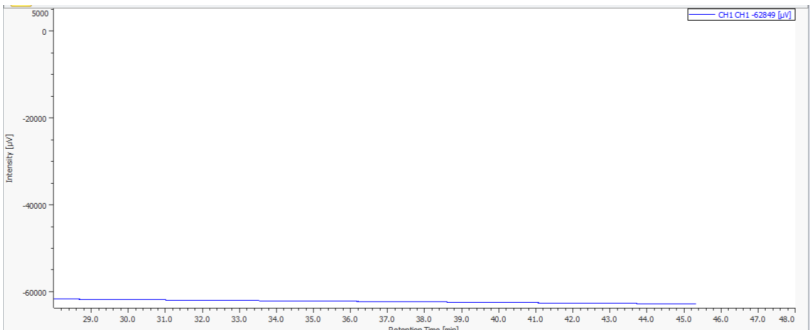


Fig. 1 HPLC chromatogram of blank

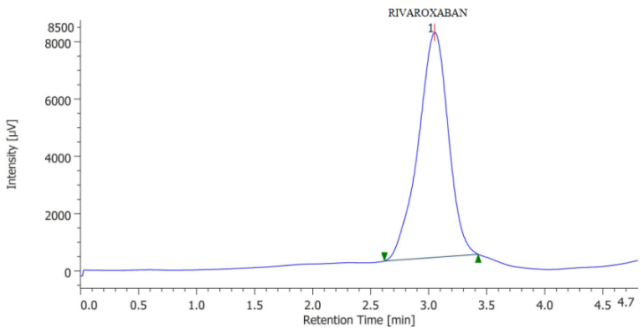


Fig. 2 HPLC chromatogram of standard Rivaroxaban

The retention time was found to be 3.12 with distinct peak.

Materials and methods

Material

Rivaroxaban standard is procured as a gift sample from current science. Chemicals utilized for method development are of HPLC grade includes methanol, water were purchased from Merck (India) Ltd.

Preparation of mobile phase

The preparation of mobile phase was done by mixing methanol with HPLC grade water in the ratio of 65:35. Removal of gases were carried out in ultrasonic water bath for 30 minutes. Filtered the solution through 0.45 µ filter.

Diluent preparation

Mobile phase used as diluents.

Preparation of standard stock solution

25 mg of Rivaroxaban standard was transferred into 50 ml volumetric flask, dissolved and make up to volume with mobile phase to get 1000 µg/ml. Further dilution was done by transferring 1 ml of the above solution into a 10 ml volumetric flask and make up to volume with mobile phase and performed the subsequent dilutions.

Preparation of test solution

25 mg equivalent of Rivaroxaban API standard was transferred into 50 ml volumetric flask, dissolved and make up to volume with mobile phase 1000 µg/ml. Further dilution was done by transferring 1 ml of the above solution into a 10 ml volumetric flask and make up to volume with mobile phase and performed the subsequent dilutions.

Selection of analytical wavelength

It is the characteristic of a compound which helps to provide the electronic structure of the compound or analyte. The structural analysis of Rivaroxaban was carried out under UV ranging from 200-400 nm using the standard solution.

Method validation

Linearity

The linearity of the developed method was studied over the concentration ranges between 5-30 µg/ml. The aliquots of 5, 10, 15, 20, 25 and 30 µg/ml were prepared by diluting standard stock solution of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml with mobile phase. The obtained concentrations were injected into the chromatographic system. Calibration curve of Rivaroxaban was constructed by plotting peak area versus used concentration of Rivaroxaban. To assure the concentration range studied is linear the regression equation and correlation coefficient were evaluated.

Accuracy

Accuracy was carried out by % recovery studies at three different concentration levels. To the pre-analysed sample solution of Rivaroxaban, a known amount of standard drug powder of Rivaroxaban was added to 80, 100, 120 % level.

Precision method

By studying the changes in the inter-day and intra-day determined the precision of the method. In the intra-day studies, six repeated injections of standard solution were made and % RSD were calculated. In the inter-day

variation studies, six repeated injections of standard solution were made for six consecutive days and % RSD were calculated.

Limit of Detection and Limit of Quantitation

Based on the standard deviation of response of the calibration curve the LOD and LOQ of the drug was determined separately.

Robustness

Robustness of the method was tested by small but deliberate variations of flow rate, mobile phase composition and wavelength.

Results and discussion

Selection of wavelength maxima

The solution of Rivaroxaban was scanned between ranges 200-400 nm. UV spectra of the drug show maximum absorbance at 249 nm.

Method development

The proposed chromatographic method was found to be suitable for effective separation of Rivaroxaban with good resolution, peak shape given in the figure. The mobile phase composed of methanol: water in ratio of 65:35 % v/v, at flow rate of 1.4 ml/min was selected as it gave well resolved peaks of standard Rivaroxaban. The optimum wavelength 249nm selected for detection and quantitation.

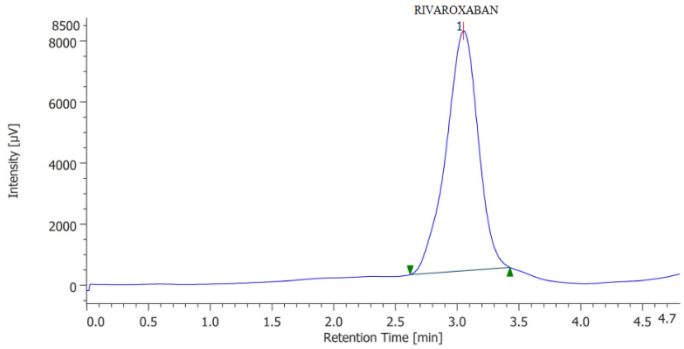


Fig. 3 HPLC chromatogram with resolved peak of Rivaroxaban

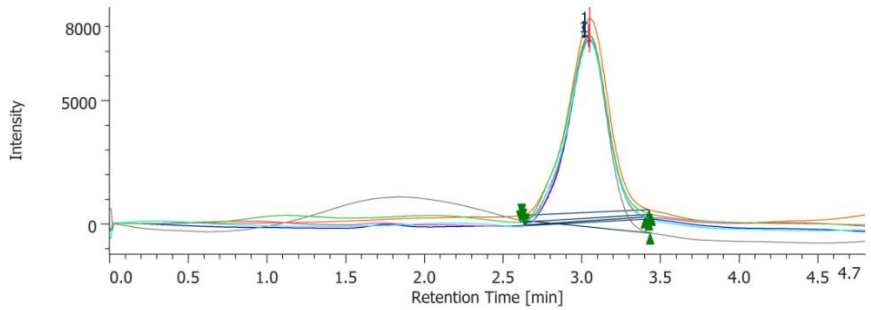


Fig. 4 HPLC chromatogram with resolved peak of Rivaroxaban (Overlay)

Method validation

Linearity

The calibration curves were found to be linear for the concentration range of 5-30 ppm. The standard working curve equation for drug was found to be y = 3713.9x – 109601 with correlation coefficient value r^2 = 0.9995. The results of linearity are given in table and figure.

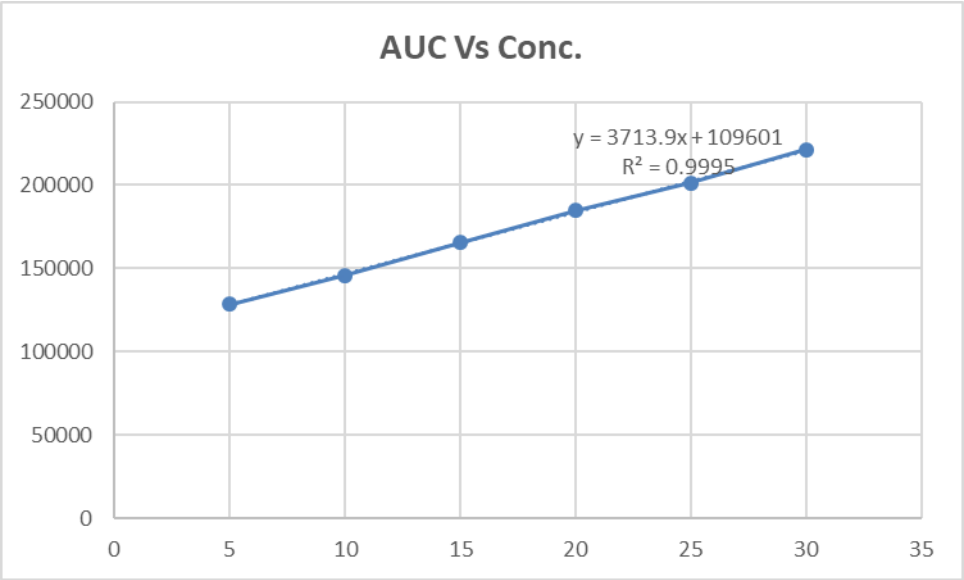


Fig. 5 Linearity curve of standard Rivaroxaban

Table 1 Linearity data of Rivaroxaban

Concentration µg/ml	Area
5	128652
10	145848
15	165478
20	184752
25	201356
30	221478

Recovery studies

The mean % recovery at 80, 100, 120 % of the test concentration along with its statistical validation for drug Rivaroxaban given in table. The % recovery at 80, 100, and 120 % was found to be 100.45, 101.11, and 101.95. It was confirmed that the developed method was accurate as the percent recovery was in the range of 100%.

Table 2 Recovery data of Rivaroxaban

Level %	Drug conc.(mg)	Amt. recovered(mg)	% Recovery
80 %	8	7.98	99.75
100 %	10	10.21	102.01
120 %	2	12.23	101.92

Precision

The repeatability of sample application and measurement of peak area was expressed in terms of % RSD and was found to be less than 2.0 %. The results of precision studies are shown in table.

Table 3 Precision study (intra-day) of Rivaroxaban

Conc µg/ml	Area	AVG	% RSD
10	145578	146218.33	0.78352378
	145536		
	147541		
15	162345	162354.67	0.0339622
	162414		
	162305		
20	184512	184515.33	0.03309658
	184456		
	184578		

Conc – concentration; AVG – average; RSD – relative standard deviation

Table 4 Precision study (inter-day) of Rivaroxaban

Conc µg/ml	Area	AVG	% RSD
10	142356	142175.33	0.12056024
	142015		
	142155		
15	162587	162588.67	0.02615465
	162632		
	162547		
20	184512	184486.67	0.02910095
	184425		
	184523		

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

This data showed that the sensitivity of method to determine the drug Rivaroxaban. The minimum concentration level at which the analyte can be reliable detected (LOD) and quantified (LOQ) were found to be 1.12 and 2.02 µg/ml respectively.

Robustness

Robustness of method was measured by multiple injection of a homogenous sample containing Rivaroxaban by changing flow rate 1.2 ml/min and 1.6ml/min, mobile phase composition methanol: water ratio 64:36 and 66:34, wavelength i.e. 248 nm and 250 nm. The method was found to be robust in the range of deliberate changes made.

Table 5 Robustness study with change in flow rate of Rivaroxaban

Flow rate ml/min	Conc. µg/ml	Area	AVG	% RSD
1.2	20	184755	184664	0.04798
1.2		184659		
1.2		184578		
1.6	20	185326	185232.3	0.05496
1.6		185247		
1.6		185124		

Table 6 Robustness study with change in concentration of mobile phase of Rivaroxaban

Mobile phase (methanol: water)	Conc µg/ml	Area	AVG	% RSD
64:36	20	184756	185009.7	0.3259
64:36		185698		
64:36		184575		
66:34	20	182658	184002	0.63399
66:34		184752		
66:34		184596		

Table 7 Robustness study with change wavelength of Rivaroxaban

Wavelength nm	Conc. µg/ml	Area	AVG	% RSD
248	20	182623	184555.7	0.90855
248		185421		
248		185623		
250	20	184578	184926	0.32547
250		185621		
250		184579		

Conclusion

In the present study, an attempt was made to develop a simple, accurate, selective and sensitive HPLC method of Rivaroxaban in pharmaceutical analysis. This method was validated for selectivity, accuracy, linearity, precision (inter-day and intra-day), sensitivity, robustness and ruggedness in accordance with ICH guidelines. A simple mobile phase without preparation of any buffer solution and a short run time are advantageous and make this method suitable for routine analysis of large number of samples per day. [4]

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