Quantitative Estimation of Rivaroxaban in Bulk and Pharmaceutical Formulation using High-Pressure Liquid Chromatography in Reverse Phase

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ABSTRACT

Rivaroxaban is prescribed to patients with nonvalvular atrial fibrillation to reduce the risk of stroke and blood clots in blood vessels. It is applied to the management of vascular blood clots. Goal: To create a method for estimating the dosage of Rivaroxaban in tablets and bulk using RP-HPLC that is straightforward, specific, accurate, and exact. The C18 column was used to provide the optimal circumstances. Acetonitrile: water (60:40) with pH adjusted to 4 with formic acid. Flow rate maintained at 1.0 ml/min. λ_{max} was detected at 249.5nm. The retention time for Rivaroxaban was found at 2.44min. Linearity was obtained in the range of 10-45µg/ml. Recovery studies were performed and %Recovery was found to be 99.91±0.1.%RSD was found to be within the limits i.e., less than 2. The correlation coefficient from the linearity plot was found to be 0.999. By considering all the results obtained, the method can be successfully applied for estimating Rivaroxaban in bulk and tablet dosage by RP-HPLC without interfering with any of the excipients and commonly used substances.

Key Words: *Method development, RP-HPLC, Rivaroxaban, Retention time, Validation*

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INTRODUCTION

Rivaroxaban has the following chemical formula: C19H18ClN3O5S. Its molecular weight is 435.89 g/mol, and its IUPAC nomenclature is 5-chloro-N-[(5S) [4-(3-oxomorpholin -4-yl) phenyl]-2-oxo-3- Thiophene-2-carboxamide, or [-1, 3-oxazolidin-5-yl]methylthiophene [1-3]. It belongs to a class of an anticoagulant [4]. It is used in the treatment of deep venous thrombosis. In patients with atrial fibrillation, it is used to prevent blood clots, systemic embolism, and stroke. With oral bioavailability, it is a highly selective Xa inhibitor. It prevents both thrombin formation and the development of thrombi. It has no effects on platelets, but it does not inhibit thrombin (activated Factor II). Ticagrelor is an antagonist that prevents platelet aggregation of the P2Y12 receptor of thrombotic events in myocardial infarction with ST-elevation or acute coronary syndrome [5-7]. **Figure 1** shows the structure of Rivaroxaban. Xarelto is the brand name of Rivaroxaban available in the market which is used as an anticoagulant for thinning the blood to prevent and treat blood clots [8]. There is a need to develop a simple, precise, and economic method for the estimation of Rivaroxaban in bulk and a formulation by RP-HPLC [9, 10].

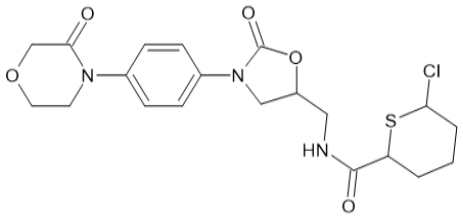

Figure 1. Rivaroxaban Chemical structure.

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Drug profile
Category: Anti-coagulant
Molecular weight: 435.89g/mol
Molecular Formula: $C_{19}H_{18}ClN_3O_5S$
Solubility: Freely soluble in DMSO, soluble in methanol, and Acetonitrile. Insoluble in water
pKa: 13.6

MATERIALS AND METHODS

Apparatus & instrument
RP-HPL(Shimadzu LC-20AD), software LC solution, digital analytical balance, and Ultrasonic water bath were used. Pipettes, beakers, measuring cylinders, and Volumetric flasks were used.

Chemicals and materials
A Pharma company gave a sample of standard Rivaroxaban as a gift. 10mg Rivaroxaban (Xarelto) tablets were purchased from a nearby shop. The other substances utilized were all of HPLC quality.

Instrumentation and chromatographic conditions
HPLC (Shimadzu with binary pump LC 20AD) and the column employed with PHENOMENEX C18 and the sample was injected using an anodyne injector and it was detected using UV detector and it is connected to software called LC solutions, vacuum filter, Sonicator, and analytical weighing balance were used. Acetonitrile: Water (60:40) is taken as an isocratic mobile phase, which is flowing through the column. 1.0 ml/min was the flow rate that is maintained. A Phenomenex C18 (250mm x 4.6 mm, 5µ) was used as the stationary phase. λ_{max} was detected at 249.5nm shown in **Figure 2**.

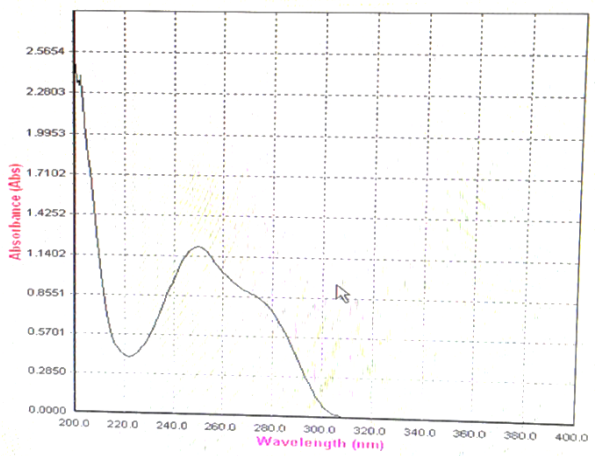

Figure 2. λ_{max} of rivaroxaban

Selection of diluent
For the preparation of standard and sample solutions, the diluent used should be compatible and should not affect the retention and resolution of the analyte. After performing various trials with HPLC-grade Acetonitrile: HPLC grade Distilled Water (60:40) was selected as diluent.

Standard solution preparation
The standard solution was made by properly weighing 10 mg of standard rivaroxaban and pouring it into a 10 ml volumetric flask. The medication was dissolved with small volumes of diluent before being made up to a volume of 10 ml with diluent to achieve a concentration of 1000µg/ml.

Working standard solution preparation
Different dilutions were made by pipetting appropriate volumes from 1000µg/ml. 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, and 4.5ml were pipetted from 1000µg/ml to get the 10, 15, 20, 25, 30, 35, 40, 45µg/ml concentration

Sample solution preparation
10 tablets were taken and weighed to calculate the average weight of each. Equivalent weight was calculated and tables were powdered. Weight equivalent to 10mg was taken in a 10ml volumetric flask, then small amounts of diluents were added to dissolve the powder. Then sonicate in an ultrasonic water bath for 15 minutes, then filter the solution, and makeup to 10ml with diluent to get the concentration of 1000µg/ml. From this 0.1ml was pipetted and taken in a 10ml Volumetric flask and the volume was made up to mark with diluent. (10µg/ml)

Optimization of parameters
Different mobile phases and different ratios were tried for all the solvents but Rivaroxaban was found to obtain a peak at 2.4mins using Acetonitrile: Water (60:40), flowing through the column. The flow was kept constant at 1 ml/min. The C18 column (250mm x 4.6mm, 5µ) is the stationary phase that is employed. λ_{max} was detected at 249.5nm. The standard optimized Chromatogram of Rivaroxaban is shown in **Figure 3**. Optimized chromatographic conditions are shown in **Table 1**.

Table 1. Optimized chromatographic conditions

Column	PhenomenexC18 250mm x 4.6 mm, 5µ.
Mobile phase	Acetonitrile: Water (60:40)
Flow rate	1.0 ml/min
Injection Volume	20.0µL
Detector	UV 249.5nm
Pump mode	Isocratic
Retention time	2.499min
Run time:	10min

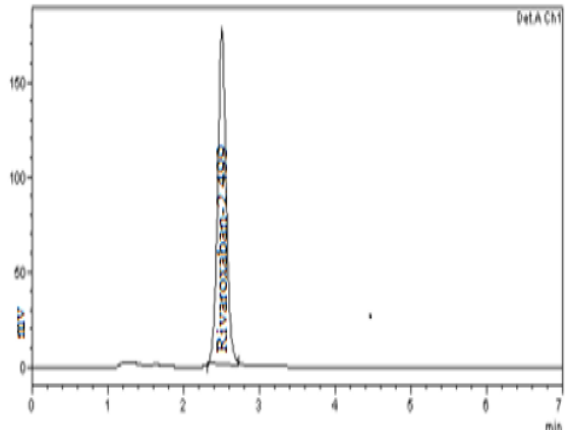

Figure 3. Rivaroxaban optimized chromatogram

RESULTS AND DISCUSSION

Validation parameters

ICH guidelines Q2 (R1) is used for validating an analytical method.

Specificity

Specificity in simple terms means when the diluent or blank is injected in HPLC it should not show any peaks at the retention times of drugs. Then the method is said to be specific.

Diluent was injected and seen for peaks then the 10µg/ml standard solution was injected and the peaks were seen at retention times.

System suitability

System suitability was determined by injecting standard solutions 5 times and the area was measured for all the injections. %RSD was calculated. %RSD should be within the limits.

1 ml was pipetted from 100µg/ml into a 10 ml volumetric flask, and the diluent was adjusted to the mark to create a 10ppm standard solution.

Precision

Precision was assessed by injecting Rivaroxaban 100 µg/ml concentration of 6 replicate injections into the HPLC system. %RSD was determined.

Intra-day precision

Six standard solutions of 10ppm of rivaroxaban were injected and the % Amount found was calculated and the %RSD was found to be 0.0003036%.

Inter-day precision

Six standard solutions of 10ppm of rivaroxaban were injected and the % Amount found was calculated and the %RSD was found to be 0.00025808%.

Linearity

Linearity is determined by injecting a standard solution in a range of 10-45µg/ml, and the response was noted, and the calibration curve was produced using the concentrations on the X-axis and the peak areas on the Y-axis to calculate the R^2 value. The calibration plot is shown in **Figure 4**.

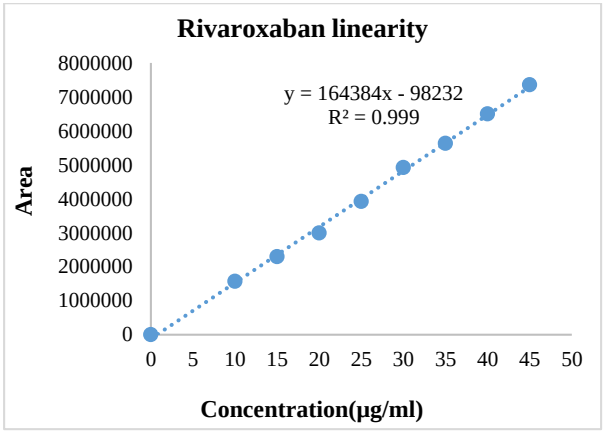

Figure 4. Calibration Plot - Concentration Vs Peak Area

Accuracy

Recovery studies can be used to evaluate the analytical method's accuracy. Three different concentration levels of rivaroxaban standard solution were added to the sample solution (50 percent, 100 percent, and 150 percent). Each concentration level had three duplicates made and injected into the HPLC apparatus. The answer was also noted. percent Recoveries were computed.

Robustness

The proposed technique's robustness can be characterized as a measure of its ability to remain unaffected by tiny but intentional changes in method parameters and offers a clue as to its dependability during typical usage. By injecting three replicates of a 10 g/ml Rivaroxaban solution and adjusting the λ_{max} ±1nmunder chromatographic conditions at 1.0±0.1 ml/min, the robustness method was evaluated. We got chromatograms and determined the percent RSD.

Limit of detection and limit of quantitation

LOD &LOQ were obtained by using the below-given formula:

$$LOD = 3.3 \times SD/slope$$
$$LOQ = 10 \times SD/slope \quad (1)$$

Where,

$SD = Standard\ deviation$

The standard deviation value can be taken from precision and the slope value can be obtained from a linearity graph.

$LOD = 3.3 * \sigma/S$

LOD=3.3*4.7644517/164384

LOD=0.000095646 µg/ml

$LOQ = 10 * \sigma/S$

LOQ=10*4.7644517/164384

LOQ=0.00028983 µg/ml

Assay

Injecting sample and standard solutions separately in HPLC and recording chromatograms to calculate % assay. The chromatogram for the sample solution is shown in **Figure 5**.

$$\%\ assay = \left(\frac{sample\ area}{standard\ area} \times \frac{weight\ of\ standard}{dilution\ of\ standard} \times \frac{dilution\ of\ sample}{weight\ of\ standard} \times \frac{purity}{100} \times \frac{weight\ of\ tablet}{lable\ claim}\right) \times 100$$

$$\%\ \text{Assay} = \left(\frac{1436436}{1569030} \times \frac{10}{10} \times \frac{10}{896} \times \frac{99.5}{100} \times \frac{0.97}{10}\right) \times 100 \quad (2)$$

% Assay =0.990112×100

=99.01%

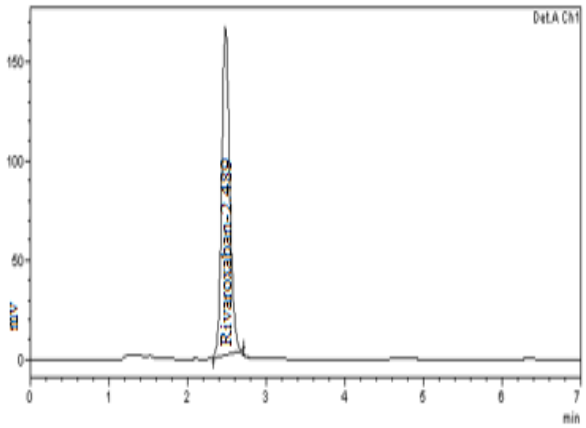

Figure 5. Chromatogram for 10 µg/ml assay sample solution

Table 2 shows the Summary of the results.

Table 2. Summary of results

Parameters	HPLC
Calibration range (mcg/ml)	10-45µg/ml
Optimized wavelength	249.5nm
Retention time	2.499min
Regression equation (Y)	164384x - 98232
Correlation coefficient(r2)	0.999
Intraday Precision (% RSD*)	0.0003036
Interday Precision (% RSD*)	0.00025808
% Recovery	99.8%
Limit of Detection (µg/ ml)	0.000095646µg/mL (95.646ppb)
Limit of Quantitation (µg / ml)	0.00028983 µg/mL (289.83ppb)
% Assay	99.01

CONCLUSION

All the validation parameters results demonstrate that the developed method is specific, simple, rapid, precise, and accurate. The developed method can be used to estimate Rivaroxaban in bulk and tablet dosage form by RP-HPLC without the interference of any of the excipients and other related substances. All the validation parameters were performed according to ICHQ2(R1) guidelines. All the results obtained were within the limits.r^2 and %RSD were found to be 0.999 and less than 2 respectively. The method was found to be linear, precise, and robust. %Assay was found to be 99.01%. Hence for routine analysis of rivaroxaban tablet, this method can be successfully used.

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Ethics statement: None

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