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NOVEL METHOD DEVELOPMENT AND VALIDATION OF RIVAROXABAN IN BULK AND PHARMACEUTICAL FORMULATION BY RP-HPLC METHOD

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ABSTRACT

Objective: The Therapeutic Goods Administration approved rivaroxaban in November 2008 to treat and avoid deep vein thrombosis (DVT). It is an oxazolidinone compound with anticoagulant action that functions by preventing the body from producing blood clots. The goal of the current research was to create a novel, straightforward, accurate, reliable, precise, and quick RP-HPLC method and then validate it by the ICH criteria for the determination of Rivaroxaban including both bulk and pharmaceutical dosage forms.

Methods: High-Performance Liquid Chromatography was used to analyze rivaroxaban. Using an INERTSIL C8 column (150x4.6mm, 5m), a mobile phase composed of KH_2PO_4 and methanol in the ratio of (60:40 v/v) at a flow rate of 1 ml per minute, and detection performed at the wavelength of 249nm using a UV/Visible detector, improved drug separation was made possible.

Results: Rivaroxaban's retention time (Rt) was discovered to be 2.667 minutes. The correlation coefficient (r2) of the method's linearity in the 05–30 µg/ml range was found to be 0.9996. According to calculations, the method's LOD and LOQ are 0.005 µg/ml and 0.016 µg/ml, respectively. The precision studies were estimated, the results were calculated as percent RSD values, and it was discovered that they were within 2% of the acceptable range. Rivaroxaban's mean Recovery was determined to be 99.3%, confirming the effectiveness of the procedure.

Conclusion: According to ICH criteria, the designed RP-HPLC technique was verified. Rivaroxaban can be analyzed using the established approach in both its bulk and commercial form.

Keywords: Rivaroxaban, RP-HPLC, deep vein thrombosis (DVT)

1. INTRODUCTION:

The Therapeutic Goods Administration approved Rivaroxaban, a novel oral anticoagulant medication, in November 2008 to avoid venous thromboembolism in those who have had knee or hip replacement surgery **[1]**. Riva (5-chloro-N-(5S)-2-oxo-3-[4-(3-oxo-4-morpholinyl) phenyl)] - carboxamide of 1,3-oxazolidine-5-ylmethyl-2-thiophene) **(Figure 1)** is an oral anticoagulant used to prevent thromboembolism in patients following surgery. It is a highly effective and selective inhibitor of factor Xa **[2]**. Riva, an oral anticoagulant based on oxazolidinone, has a molecular weight of 435.882 g mol1 and a molecular formula of $C_{19}H_{18}ClN_3O_5S$. In acetone, it is barely soluble, yet it dissolves freely in methanol. It functions by preventing the body from producing blood clots. Riva is prescribed to persons with non-valvular atrial fibrillation to assist avoid strokes or life-threatening blood clots. Riva is sold as Xarelto® and comes in tablets with a dose of 2.5, 10, 15, and 20 mg **[3].** Riva is an oxazolidinone analog that has been created especially to block Factor Xa which is both unbound and bound to the prothrombinase complex. Inhibition of factor Xa prevents both intrinsic and external pathways of the blood clotting cascade from working properly, preventing the production of thrombin and the growth of thrombi **[4].** Riva binds directly to the enzymatic domain of factor Xa, inhibiting the thrombus from forming and stopping the extrinsic and intrinsic coagulation cascade pathways from being amplified **[5]**.

2. MATERIALS AND METHODS

2.1 Instrumentation

A Make Waters Alliance e2695 HPLC system with an autosampler and Model No. 2489 The development and validation of the approach utilized a UV/visible detector. The analysis was completed using INERTSIL with dimensions of (C8, 150 x 4.6 mm, 5 µm) at room temperature. Utilizing empower3 software, the data was compiled and evaluated.

2.2 Chemicals and Reagents

The Symed labs, Hyderabad, India, supplied a free sample of the drug rivaroxaban. From Sd Fine-Chem Limited in India, we obtain NaH_2PO_4, Na_2HPO_4, K_2HPO_4, KH_2PO_4, HCl, NaOH, and H_2O_2, Methanol, and ACN, both of HPLC grade, were purchased from "Merck Specialties Ltd.," an HPLC rating company. I bought Rivaban 20 mg tablets at a nearby market. All other substances and reagents have been of analytical grade.

2.3 Preparation of Solutions

2.3.1 Preparation of buffer (0.1M KH_2PO_4 buffer)

Accurately weigh 13.609g of KH_2PO_4 in a 1000 ml volumetric flask, dissolve it, and

add water to make it the proper volume. Then, filter the solution through a 0.45micron membrane filter and sonicate it for 10 minutes.

2.3.2 Preparation of mobile phase

400 ml of methanol and 600 ml of KH_2PO_4 buffer were combined and degassed in an ultrasonic water bath for 10 minutes before being filtered through a 0.45 m filter while under vacuum. **Preparation of diluent**

The diluent was the mobile phase.

2.3.4 Preparation Rivaroxaban standard stock solution

20 mg of Rivaroxaban working standard, accurately weighed, was put into a 100 ml clean, dry volumetric flask. Diluent (mobile phase) was then added, and 30 ml was sonicated for 30 minutes before the remaining volume was made up with diluent. Utilizing diluents, the aforementioned standard stock solution was suitably diluted to yield various Rivaroxaban concentrations.

2.3.5 Preparation of Rivaroxaban sample solution

Twenty tablets of Riva were taken in the mortar and crushed into a fine powder (Each tablet contains 20mg of Riva).106.44 mg of Riva sample equivalent to 20 mg was transferred into a 100ml clean and dried volumetric flask, then diluent (mobile phase) was added, thoroughly dissolved using a sonicator, and the volume was restored using the diluent. The sample solution mentioned above was filtered. by using a 0.45µm filter then 1ml of the filtrate was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent.

2.3.6 Selection of wavelength for method development

Rivaroxaban was produced as a stock solution at a concentration of 100 µg/ml, and subsequent methanol serial dilutions were made. By scanning the abovementioned typical drug sample between 200 and 400 nm, the wavelength was determined. The findings of the scan revealed that the absorbance peaked at 249 nm. As a result, the detection wavelength for the RP-HPLC analysis of Riva was chosen to be 249 nm. appeared in **(Figure 2)**.

2.3.7 Method development

The chromatographic method was developed by performing the trials considering the response as a/c to United States Pharmacopoeia (USP) plate count and tailing factor. The variables considered for conducting experiments were column type and mobile phase composition. The wavelength (flow rate (1ml/min), and injection volume (10µl) are kept constant throughout the trails. The experimental chromatographic conditions for method development have shown in **(Table 1)**.

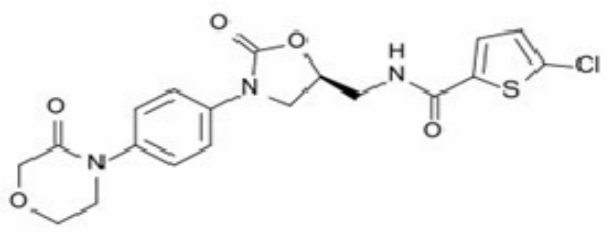

Figure 1: Chemical Structure of Rivaroxaban

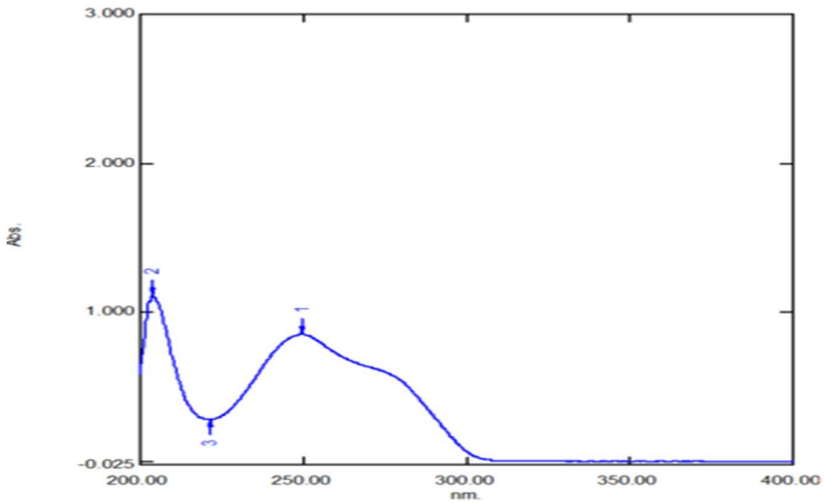

Figure 2: UV spectra of Rivaroxaban

Table 1: Experimental conditions of method development

Trial no	Mobile phase composition	Column used	Flow rate (ml/min)	Injection volume (µl)	Observations
1	NaH_2PO_4:Acetonitrile (60:40v/v)	SUPELCO, C_{18}, 250×4.6mm, 5µm	1	10	The peak shape was not good and theoretical plates are not within the limits
2	NaH_2PO_4:Acetonitrile (60:40v/v)	ACE, C_{18}, 250×4.6mm, 5µm	1	10	Retention time is more and plate counts are not within the limits
3	KH_2PO_4:Methanol (75:25v/v)	INTERSIL C_8, 150×4.6mm, 5µm	1	10	Peak shape is good but Rt is more
4	KH_2PO_4:Methanol (70:30v/v)	INTERSIL C_8, 150×4.6mm, 5µm	1	10	Rt is more
5	KH_2PO_4:Methanol (65:35v/v)	INTERSIL C_8, 150×4.6mm, 5µm	1	10	Rt is more
6	KH_2PO_4:Methanol (60:40v/v)	INERTSIL C_8, 150×4.6mm, 5µm	1	10	The peak shape is good and all parameters are within the limits.

2.4 Construction of Calibration Curve

The standard stock solution was diluted to get the various concentrations of 5, 10, 15, 20, 25, and 30 g/ml using diluent (mobile phase). The chromatograms were captured under ideal chromatographic circumstances. From the chromatograms, at varied concentration levels, the mean peak areas were calculated. The mean peak areas of the corresponding concentrations were then used to build the linear plot.

2.5 Method Validation

According to ICH criteria, the developed technique was validated for the following

parameters: robustness, system appropriateness, linearity, accuracy, precision, detection, and quantitation limits **[6]**.

2.5.1 Selectivity (Specificity)

The ability to quantify the desired analyte in a complicated combination is known as specificity **[7]**. Specificity was demonstrated by spiking a pure specific concentration of a drug with appropriate levels of impurities. A marketed formulation of Rivaroxaban (Rivaban 20) from Local Pharma was taken and specificity was determined in 5 replicates at a concentration of 100 µg/mL and percentage RSD (Relative Standard Deviation) was calculated.

2.5.2 System Suitability

System appropriateness is described as the evaluation of a system to assure system performance before or during the investigation of unknowns **[8]**. By using six replicate analyses of Rivaroxaban at a concentration of 100 g/ml, the system appropriateness was evaluated. The acceptability standard for the peak area and retention durations for rivaroxaban was 2% (% RSD).

2.5.3 Linearity and Range

The capacity to generate test results that are inversely proportional to the analyte concentration is known as linearity **[9]**. 5 injections of different concentrations of Rivaroxaban (05, 10, 15, 20, 25, 30 g/ml) were used to test for linearity. Plots of the average peak areas vs concentrations were made. The least square regression method was utilized to evaluate the linearity using linear regression analysis. A correlation coefficient (r2) value greater than 0.9999 is typically seen as evidence of a good fit between the data and the regression line.

2.5.4 Accuracy

The proximity between the expected value and the value discovered expresses how accurate an analytical process is. It is calculated by figuring out the analyte's percentage recovery (%R). In this instance, consecutive analysis (n = 3) for three distinct concentrations (50 g/ml, 100 g/ml, and 150 g/ml) of the standard Rivaroxaban solution was carried out using the established method to assess the accuracy of the approach. The results of the experiment were analyzed statistically using the equation [% Recovery = (Recovered conc /Injected conc) x 100] to assess the recovery and validity of the developed approach. To be acceptable, the mean recovery must be between 90% and 110%.

2.5.5 Precision (Repeatability)

Using the devised method and six replicate analyses of the standard Rivaroxaban solution at a concentration of 100 g/mL, the precision was computed. Repeatability describes the accuracy over a brief period while using the same operating conditions

[10]. The precision was reported as % RSD, and it was discovered to be less than 2%, demonstrating the system's good precision by Ich.

2.5.6 Robustness

Robustness is an evaluation of the accuracy of analysis to purposeful changes in technique parameters. The analytical conditions should be appropriately regulated or a warning should be included in the method **[10]**. It is the measurement of fluctuations in analytical conditions. In the current study, robustness was tested by allowing a small intentional change in composition (5%) in the mobile phase solvents, a temperature change (2°C), a change in injection flow rate (10%), and a change in buffer's pH in the mobile phase solvent. RSD was calculated as a percentage of the mean.

LOD is the minimum concentration in a sample that, given the specified experimental conditions, can be detected but not always measured. The lowest concentration of an analyte that may be identified with reasonable accuracy and precision is known as the LOQ. The formulas were used to determine these two parameters.

$$LOD = \frac{3.3\sigma}{S}$$

$$LOQ = \frac{10\sigma}{S}$$

Where S = slope of the calibration curve.

σ= standard deviation of response (peak area)

3. RESULTS AND DISCUSSION

3.1 Development of HPLC Method

The blank mobile phase is shown by chromatogram 3.1A, while Rivaroxaban, which has a retention time of 2.667 minutes and no interfering peaks, is represented by chromatogram 3.1B. This demonstrates how specialized the developed HPLC process is.

3.2 System Suitability

Five repeat analyses of Rivaroxaban at a concentration of 100 g/ml were used to evaluate the applicability of the system. According to **Table 2**, the acceptable range for the peak area and retention durations for Rivaroxaban was 2% relative standard deviation (% RSD).

3.3 Linearity

From the stock solution, chromatograms of 05, 10, 15, 20, 25, and 30 g/ml solutions were prepared and recorded. The average peak areas of their corresponding concentrations were determined from the obtained chromatograms, and the linearity plot was built using those mean peak areas. It was discovered that the correlation coefficient (R2) was 0.9996. Rivaroxaban's linearity data are displayed in **(Table 5)** and **(Figure 4)** displays the calibration plot.

3.4 Accuracy

A recovery study was performed to check the accuracy of the method at three levels

50%, 100%, and 150%. A recovery study was carried out 3 times for each level and the percentage recovery and % relative standard deviation is calculated. The mean recovery of Rivaroxaban was found to be 99.93% **(Table 3)**.

4. ROBUSTNESS STUDY RESULTS OF THE DEVELOPED METHOD FOR RIVAROXABAN

4.1 Precision

Precision Results are tabulated in **(Table 4)**. Precision was expressed as percentage relative standard deviation (% RSD), which was found to be less than 2% depicting satisfactory precision of the system.

4.2 Robustness

Robustness was tested by allowing small variations in the mobile phase's composition (5%), temperature (2°C), flow rate (10%), and buffer's pH (0.1). RSD was calculated, and the findings are shown in **(Tables 6, 7, 8, and 9)**. An analytical procedure's robustness, which measures its ability to be unaffected by little but intentional changes in method parameters, gives a clue as to how reliable it will be in typical conditions. The temperature, flow velocity, and pH of the buffer were determined to be less than 2% of RSD when taking into account a modest change in composition.

4.3 LOD and LOQ

The results showed that the LOD and LOQ were 0.005 g/ml and 0.016 g/ml, respectively. The developed method's sensitivity is expressed by the LOQ.

Table 2: System suitability analysis of Rivaroxaban

	SampleName	Peak Name	RT	Area	USP Tailing	USP Plate Count
1	STD-2	RIVAROXABAN	2.656	845410	1.11	4859
2	STD-2	RIVAROXABAN	2.657	844195	1.10	4943
3	STD-2	RIVAROXABAN	2.664	844869	1.08	4897
4	STD-2	RIVAROXABAN	2.657	845482	1.11	4940
5	STD-2	RIVAROXABAN	2.668	845205	1.11	4840
Mean				845032.1		
% RSD				0.1		

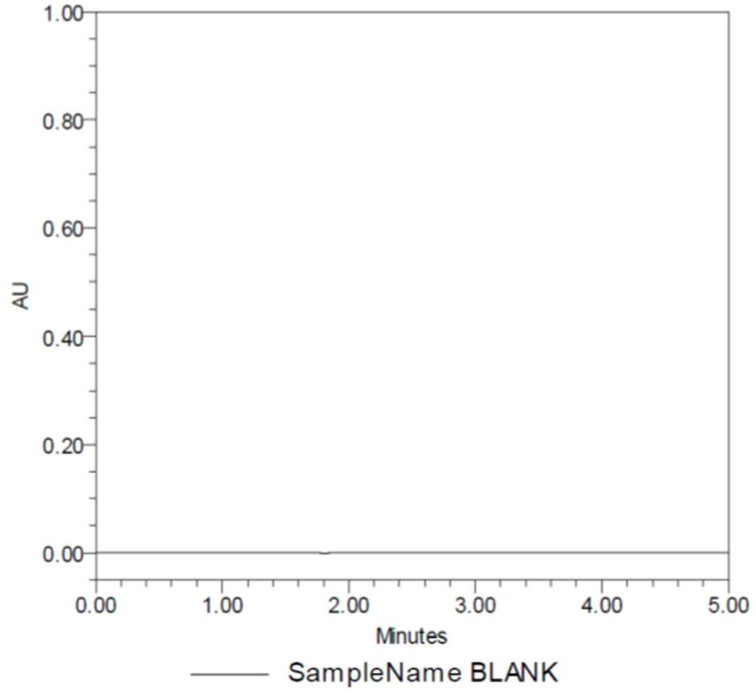

Figure 3.1A: Blank chromatogram

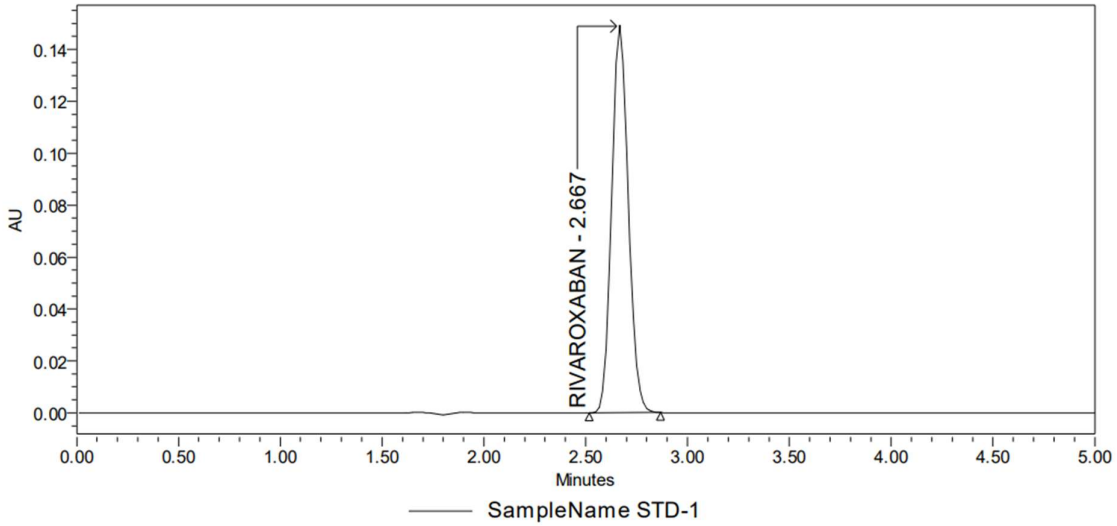

Figure 3.1.B: Standard chromatogram of Rivaroxaban

Table 3: Accuracy or Recovery study results of Rivaroxaban

RRN Spiked Level	Sample RRN Weight	Sample RRN Area	µg/ml RRN added	µg/ml RRN found	% RRN Recovery	%RRN Mean
50%	53.20	420996	9.900	9.92	100.24	100.28
50%	53.20	420891	9.900	9.92	100.22	
50%	53.20	421596	9.900	9.94	100.39	
100%	106.40	843064	19.800	19.87	100.37	100.43
100%	106.40	843498	19.800	19.88	100.42	
100%	106.40	844035	19.800	19.90	100.49	
150%	159.60	1247478	29.700	29.41	99.01	99.09
150%	159.60	1248126	29.700	29.42	99.06	
150%	159.60	1249854	29.700	29.46	99.20	

Table 4: Precision study results of Rivaroxaban

S. No	Sample RRN Weight	RRN Sample Area	Precision
1	106.40	844157	Mean = 843780.7
2	106.40	843198	
3	106.40	844443	SD = 481.999
4	106.40	843958	
5	106.40	843534	%RSD= 0.057124
6	106.40	843394	

Table 5: Linearity study results of Rivaroxaban

µg/ml RRN quantity	RRN area	Line equation
10	420501	y = 40835 x + 16908
15	631037	Correlation coefficient
20	843262	R^2 = 0.9996
25	1032659	
30	1240560	

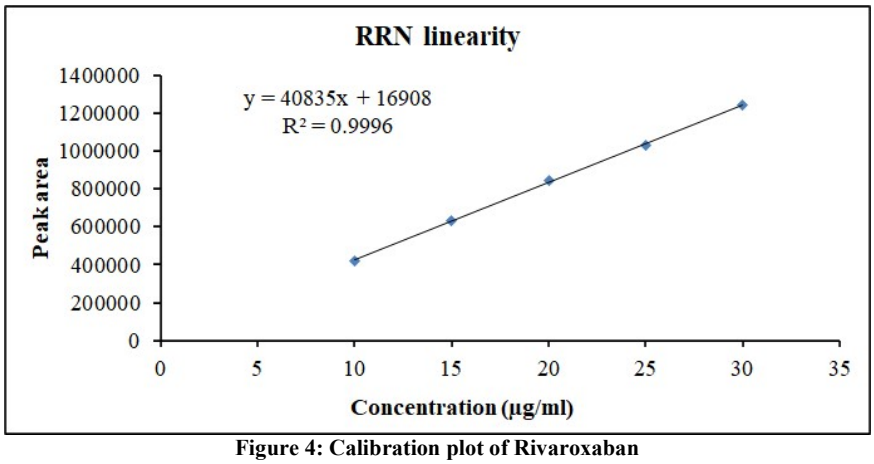

Figure 4: Calibration plot of Rivaroxaban

Table 6: Change in Composition of Mobile phase (±5%)

	SampleName	Peak Name	RT	Area	USP Tailing	USP Plate Count
1	COMP-1	RIVAROXABAN	2.227	707489	1.05	4084
2	COMP-2	RIVAROXABAN	2.930	944369	1.09	5518

Table 7: Change in Temperature (±2°C)

	SampleName	Peak Name	RT	Area	USP Tailing	USP Plate Count
1	TEMP-1	RIVAROXABAN	2.430	772871	1.07	4465
2	TEMP-2	RIVAROXABAN	2.930	944369	1.09	5518

Table 8: Change in Flow rate (±10%)

	SampleName	Peak Name	RT	Area	USP Tailing	USP Plate Count
1	FLOW-1	RIVAROXABAN	2.227	707489	1.05	4084
2	FLOW-2	RIVAROXABAN	3.285	1063007	1.10	6359

Table 9: Change in buffer's pH (±0.1)

	SampleName	Peak Name	RT	Area	USP Tailing	USP Plate Count
1	pH-1	RIVAROXABAN	2.656	845157	1.11	4826
2	pH-2	RIVAROXABAN	2.667	845198	1.10	4885

4.4 CONCLUSION

The results of the developed RP-HPLC method met all the validation parameters criteria. The % RSD of precision was found to be less than 2%. The retention time of Rivaroxaban was found to be 2.667 min. The mean recovery of Rivaroxaban was found to be 99.93%. LOQ confirms the sensitivity of the method. So, it is concluded that the newly created RP-HPLC method was straightforward, precise, quick, and accurate. It can be applied to the additional analysis of formulations and bulk Rivaroxaban.

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