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A VALIDATED RP-HPLC METHOD FOR ESTIMATION OF RIVAROXABAN IN BULK AND TABLET DOSAGE FORM

Vaishali K.[*1], Sowmya H. G.[2], Naveen Kumar G. S.[3]

[1]2nd Year M Pharma, Student of Department of Pharmaceutical Analysis, Bharathi College of Pharmacy, Bharathinagara, Mandya, Karnataka, India -571422.

[2]Associate Professor of Department of Pharmaceutical Analysis, Bharathi College of Pharmacy, Bharathinagara, Mandya, Karnataka, India -571422.

[3]Professor and HOD of Department of Pharmaceutical Analysis, Bharathi College of Pharmacy, Bharathinagara, Mandya, Karnataka, India -571422.

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***Corresponding author:**

***Vaishali K.**

2nd Year M Pharma, Student of Department of Pharmaceutical Analysis, Bharathi College of Pharmacy, Bharathinagara, Mandya, Karnataka, India -571422.

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 Rivaroxaban in pharmaceutical dosage form. Chromatographic separation of Rivaroxaban was achieved on Prominence LC-20A Quaternary Gradient HPLC system, by using Shimpack C-18 (5µm, 4.6 x 250mm) column and the mobile phase containing Acetonitrile and Phosphate Buffer with pH of 4.5 in a 50:50 v/v ratio. The flow rate was 1.0ml/min; detection was carried out by absorption at 235nm using a UV detector at ambient temperature. LOD and LOQ were found to be 0.7497µg/ml and 2.272µg/ml respectively and retention time was found to be 4.848mins. The % Recovery was found to be 100.70-101.37%. The number of theoretical plates and tailing factor for Rivaroxaban were not less than 2000 and 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 Rivaroxaban.

KEYWORDS: Rivaroxaban, High performance liquid chromatography, Method development, Validation.

INTRODUCTION

Rivaroxaban is an anti-clotting medication that acts at a vital stage in the clotting process to prevent blood clots from forming. It is also used of safeguarding persons with atrial fibrillation who do not have heart valve impairment from strokes or severe blood clots. It works by suppressing the activity of a natural substance that assists in the production of blood clots. Rivaroxaban is an enantiomer in its most pure form.[1]

It's an odourless, non-hygroscopic powder that ranges from white to yellowish in appearance. It dissolves in organic solvents such as methanol, DMSO, and acetonitrile. Rivaroxaban, also known as (S)-5-chloro-N-((2-oxo-3-(4-(3-oxomorpholino)phenyl)oxazolidin-5-yl)methyl)thiophene-2-carboxamide is a new, oral, selective, and very effective direct Factor Xa inhibitor. Factor Xa is a critical component of the blood coagulation cascade, which causes thrombin activation and clotting. Rivaroxaban's chemical formula is $C_{19}H_{18}ClN_3O_5S$ and its molecular weight is 435.881g/mol. Its melting point varies from 228°C to 232°C.[2]

In recent years, direct oral anticoagulants that target a single coagulation factor (e.g. Factor Xa or thrombin)

have been developed to address limitations of traditional anticoagulants. Factor Xa is essential for blood clotting and is activated by both the intrinsic and extrinsic coagulation pathways. Factor Xa directly transforms prothrombin to thrombin via the prothrombinase complex, causing fibrin clot formation and platelet activation by thrombin.[3] Rivaroxaban works by blocking free FXa, FXa attached to prothrombinase, and FXa linked to a clot in a concentration-dependent manner. Adenosine diphosphate, collagen, and thrombin cause platelets to aggregate, although it has no direct effect on this process.[4]

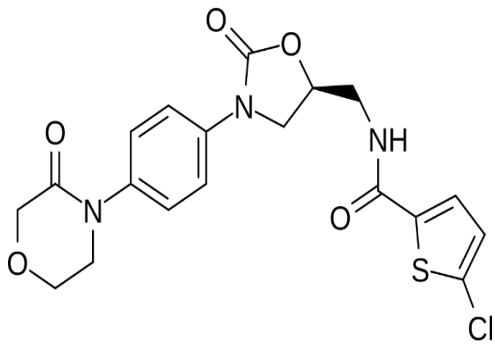

Figure 1: Chemical Structure of Rivaroxaban.

According to a literature review, few analytical techniques have been published for determining Rivaroxaban in pure medication and pharmaceutical dosage forms employing UV[5-8], HPLC[9-19], RP-HPLC[20-22], and HPTLC.[23-25] The current effort aims to develop and verify a new Reverse phase-High performance liquid chromatography for estimating Rivaroxaban in tablet and bulk dose form that is quick, easy, accurate, and specific.

MATERIALS AND METHODS

Apparatus and Software

Chromatographic separation was performed on a Prominence LC-20A Quaternary Gradient HPLC system as the instrument model and column used is Shimpack C-18 5µm, 4.6 x 250mm.

Chemicals and Reagents

Rivaroxaban pure form was obtained as gifted sample from gift sample by Medreich limited(R&D Centre), Bengaluru and pharmaceutical dosage form Rivaroxaban 20 tablets (Xarelto) labelled claim 20mg from Bayer zydus pharma private limited. Acetonitrile, Sodium Dihydrogen Orthophosphate and water obtained from Bharathi college of pharmacy Bharathinagara, K.M. Doddi, Maddur TQ & Mandya dist. India. All the Chemicals used in this investigation were HPLC grade.

Selection of mobile phase

Based on sample solubility, stability and suitability various mobile phase compositions were tried to get a good resolution and sharp peaks. The standard solution was run in different mobile phases. From the various mobile phases Acetonitrile and Phosphate Buffer in a 50:50 v/v ratio with detection wavelength of 235 nm, since it gave sharp peak with good symmetry within limits.

Preparation of mobile phase

Mobile phase was prepared by mixing Acetonitrile and Phosphate Buffer with pH of 4.5 in a 50:50 v/v ratio. It was filtered through FCP-305µ membrane filter to remove the impurities which may interfere in the final chromatogram.

Preparation of standard stock solution

Accurately weigh and transfer 100mg of Rivaroxaban working standard into a 100ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 1ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. (100ppm of Rivaroxaban).

Preparation of sample solution

Accurately weighed and transfer 100mg of Rivaroxaban sample into a 100ml clean dry volumetric flask add diluent and sonicate it up to 30min 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 0.2µ Whatman Uniflo Nylon filter (Stock solution). Further pipette 1ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent (100ppm of Rivaroxaban).

Flow rate selection

Different flow rates were studied. A flow rate of 1.0ml/min gave an optimal signal to noise ratio with a reasonable separation time.

Validation of Analytical Method

The method is validated according to the ICH guidelines; Validation of an analytical method is the process to establish by laboratory studies that the performance characteristic of the method meets the requirements for the intended analytical application. Performance characteristics are expressed in terms of Analytical parameters.

System suitability
20µl of the standard solution was injected under optimized chromatographic conditions to evaluate the suitability of system. Parameters such as number of theoretical plates (N), tailing factor (T), retention time (tr), asymmetry and area were determined. The obtained values indicate good performance of system.

Solution stability
In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24hr at room temperature. The results show that for solutions, the retention time and peak area of Rivaroxaban remained almost unchanged (% RSD less than 2.0).

Specificity
Specificity of the HPLC method was checked for interference of impurities, degradants or excipients in the analysis of sample solution and was determined by injecting a volume of 20µl of sample solution and the chromatogram was recorded. There is no interference of impurities, excipient peak on the peak of Rivaroxaban, indicating the high specificity of method.

Linearity and Range
The linearity of the method was demonstrated over the concentration range of 20- 10µg/ml of the target concentration. Aliquots of 20,40,60,80 and 120µg/ml were prepared from above prepared stock solution. Different concentrations of the pure drug were injected into the chromatographic system. Calibration curve of Rivaroxaban was constructed by plotting peak area v/s applied concentration of Rivaroxaban. The obtained results shown an excellent correlation between peak area and concentration of pure drug within the concentration range & it has shown in Fig: 6.0. The correlation coefficient for the average area at each level versus concentration of analyte was calculated and is presented in Table and their calibration parameters were shown in Table:

Precision
The precision of the analytical method was determined by intra-day and inter- day precision Table:, respectively the sample solution was prepared as per the test method. In intra-day precision, the same concentration of sample solution was injected 6 times in the same day and in inter-day precision, injecting six solutions of same concentration for six different days in a week. The average and standard deviation of mean area were taken and %RSD was calculated and reported. %RSD values were within the limits and the method was found to be precise.

Accuracy
The accuracy of the method was determined by recovery studies by the determination of % mean recovery of the drug at three different levels (80%, 100% and 120%). At each level, three determinations were performed. A known amount of standard pure drug was added to pre analyzed tablet powder and the sample was then analyzed by developed method. Results of recovery studies were reported, the observed data were within the range, which indicates good recovery values.

Robustness
Robustness is a measure of capacity of a method to remain unaffected by small but deliberate variations in the method conditions, and is indications of the reliability of the method. A method is robust, if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at by changing parameters like change in Flow rate of the Mobile phase and change in organic phase, and the results were shown in Table. The method has no effect on the retention time and chromatographic response of the 6 solutions indicating that the method was robust.

Limit of detection
Limit of detection is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. The results of LOD were shown in Table.

Limit of quantitation
Limit of quantitation is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably Quantitate. The LOQ can also be calculating based on the LOD strength, the LOD values were multiplied by three times to get LOQ. The results of LOQ were shown in Table.

RESULTS AND DISCUSSION

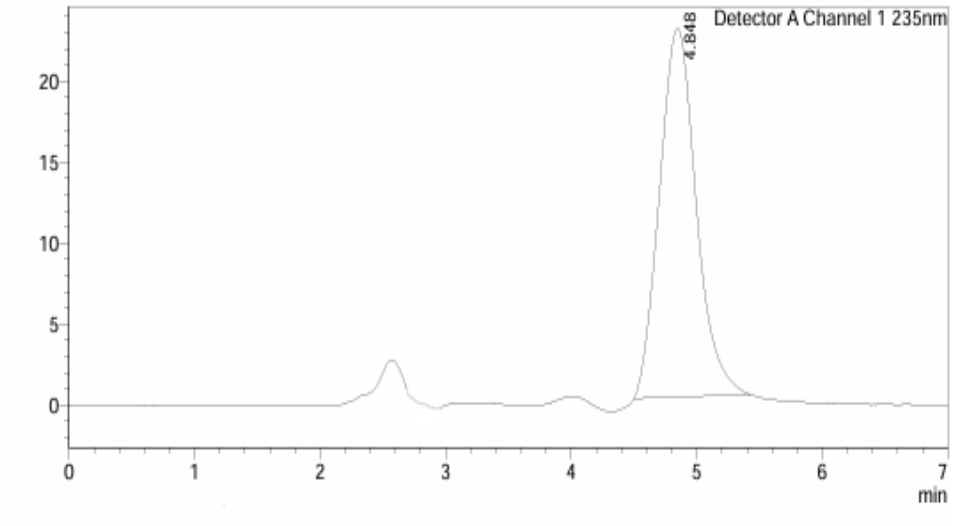

Fig. 1: Chromatogram of Rivaroxaban.

Table 1: Optimized chromatographic conditions.

Optimized conditions	Values
Column	Shimpack C-18 (5µm, 4.6 x 250mm)
Mobile phase	Acetonitrile and phosphate buffer with pH of 4.5 in a 50:50 v/v ratio
Flow rate	l.0 ml/min
Injection volume	20µl
Wavelength	235nm
Temperature	40ºC
Retention time	4.848min
Run time	10min
Elution	Isocratic

Table 1.1: System suitability studies of Rivaroxaban by RP-HPLC method.

System suitability Parameters	Acceptance criteria	Results
Tailing factor	T ≤ 2	1.163
Theoretical plates	N ≥ 2000	2531
Retention time	-	4.848
Area	-	916277

2. Specificity

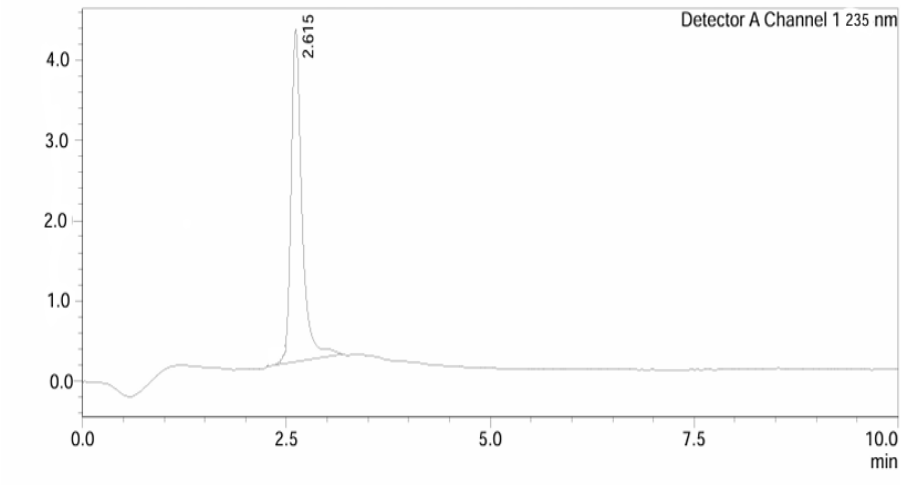

Fig. 2: Chromatogram of Blank.

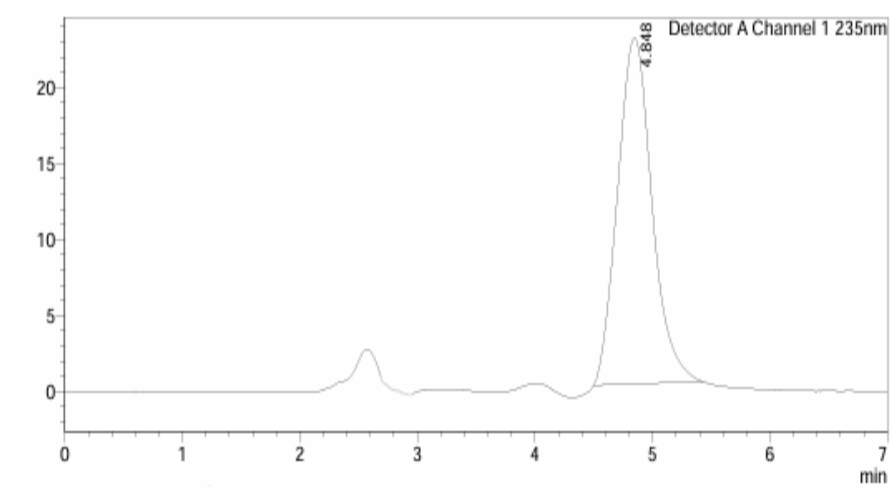

Fig. 3: Chromatogram of sample.

Table 1.2: Calibration data of Rivaroxaban by RP-HPLC method.

Concentration (µg/ml)	Peak area* (mv)
20	124804
40	185720
60	237745
80	301996
100	359226
120	418939

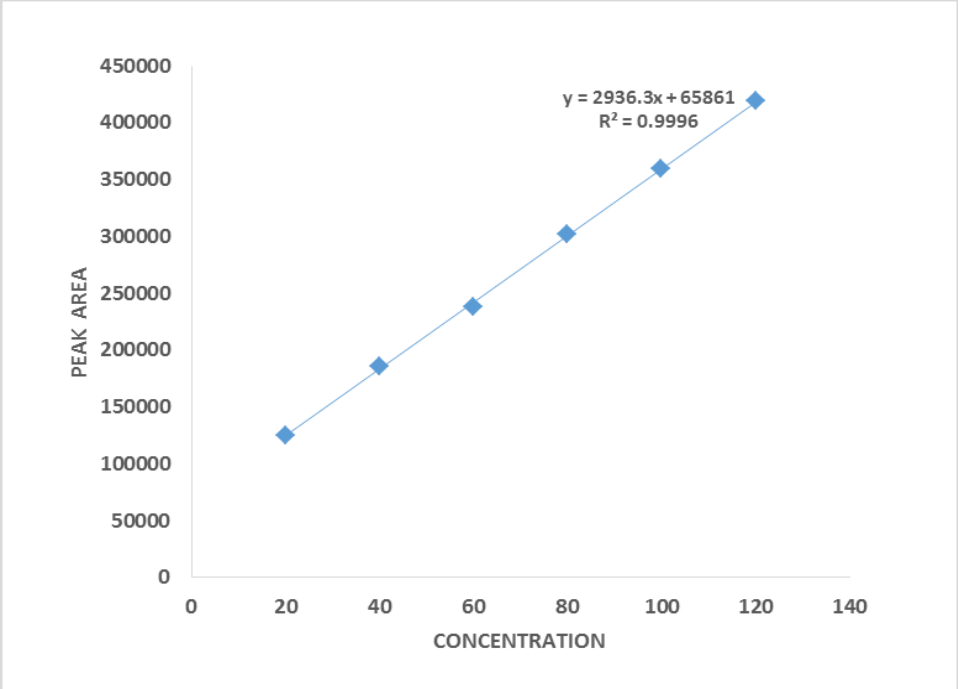

Fig. 4: Calibration curve of Rivaroxaban by RP-HPLC.

Table 1.3: Regression parameters table of Rivaroxaban by RP- HPLC Method.

Optimized conditions	Values
Linearity range(µg/ml)	20-120µg/ml
Regression equation(Y*)	Y = 2936.3x + 65861
Correlation Coefficient (r^2)	0.9996
Slope(a)	2936.3
Intercept(b)	65861

*Y= bX+a, where X is the concentration of compound in mcg/ml and Y is the peak area.

Table 1.4: Intra-day Precision results for Rivaroxaban by RP-HPLC.

SL NO	Concentration (µg/ml)	Area (mv)	Concentration Found (µg/ml)	Mean* (µg/ml)	±SD	%RSD
1	20	131851	19.34	19.22	0.11	0.593
	20	131021	19.07			
	20	131638	19.27			
2	120	428736	119.02	119.37	0.26	0.217
	120	427730	119.34			
	120	426734	119.68			

*Average of three determination

Table 1.5: Inter-day precision results for Rivaroxaban by RP-HPLC.

SL NO	Concentration (µg/ml)	Area (mv)	Concentration Found (µg/ml)	Mean* (µg/ml)	±SD	%RSD
1	20	132851	19.68	19.49	0.21	1.07
	20	131010	19.61			
	20	132438	19.20			
2	120	428520	119.94	118.91	0.89	0.75
	120	424202	118.49			
	120	423686	118.32			

*Average of three determination

Table 1.6: Accuracy results for Rivaroxaban by RP-HPLC.

SL NO	Spiked level	Amount of Standard (µg/ml)	Amount of sample (µg/ml)	Total amount of drug (µg/ml)	Total amount of drug Found (µg/ml)	% Recovery	Mean* %	±SD	%RSD
1	80%	50	40	90	89.25	99.147	100.70	1.352	1.342
					89.11	101.616			
					89.20	101.337			
2	100%	50	50	100	101.10	99.407	100.78	1.188	1.179
					101.15	101.453			
					100.85	101.479			
3	120%	50	60	110	110.50	101.166	101.37	0.545	0.537
					109.60	100.837			
					110.52	102.122			

*Average of three determination

Table 1.7: Robustness results for Rivaroxaban by RP-HPLC.

Parameters	Level	Factor	Mean area ± SD	%RSD
Flow rate(1ml/min±1)	-1	0.9ml/min	135720± 653.1973	0.35
	+1	1.1ml/min	134471±249.4438	0.20
Wavelength (235nm±2)	-2	233nm	131638±734.8469	0.55
	+2	237nm	131790±775.6718	0.58
Column oven temperature (40˚C±1)	-1	39˚C	132801±816.4966	0.61
	+1	41˚C	132495±979.7959	0.73

Table 1.8: Determination of LOD and LOQ results of Rivaroxaban by RP-HPLC.

Sl. No	Parameters	Values
1	LOD (3.3×SD of Intercepts/average of slopes)	0.7497µg/ml
2	LOQ (10×SD of Intercepts/ average of slopes)	2.272µg/ml

**Mean value obtained from six calibration curves.

CONCLUSION

The current analytical method satisfies the acceptance requirements and has been validated in accordance with ICH recommendations. The new analytical approach was shown to be simple, sensitive, accurate, and cost-effective. It may be applied to the regular analysis of Rivaroxaban in pharmaceutical dosage forms and bulk drug.

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