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Analytical method development and validation of rivaroxaban in bulk and pharmaceutical dosage form by using RP-HPLC

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***Abstract*---**A simple, specific, accurate, and precise reverse phase high performance liquid chromatography (RP-HPLC0 method was developed and validated to analyse Rivaroxaban bulk dosage form. Using Sun Q C_{18} HPLC column separation was carried out. This was maintained at ambient temperature. During separation mobile phase consist of Acetonitrile: Buffer(sodium acetate buffer) 80:20 was delivered at a flow rate of 1mL/min. Using UV detector analysis was carried out at the wavelength 249 nm. RP-HPLC method was validated by using various parameter like system suitability, linearity, specificity, precision, limit of detection, limit of quantification and robustness. The RP-HPLC method was found to be linear over the concentration range from 5-30 µg/mL (r^2 =0.994). Retention time for bulk rivaroxaban was found to be 3.69 min. LOQ of method was 1.331 µg/mL and LOD 0.439 µg/mL. Thus the developed RP-HPLC method

was found to be robust which can be applied for the regular analysis of Rivaroxaban in the bulk as well as pharmaceutical dosage form.

***Keywords*---**rivaroxaban, RP-HPLC, $C_{18,}$ acetonitrile, sodium acetate buffer.

Introduction

Rivaroxaban (RIV- fig 1) is an oral anticoagulant. It is potent and sensitive direct Inhibitor of factor Xa. Factor Xa is the active component of the prothrombinase complex that catalyses conversion of prothrombin (factor II) to thrombin (factor II A). It prevents the venous thromboembolism in adult patients after total hip replacement or total knee replacement surgery (Eswardu, 2020) The chemical name of Rivaroxaban is 5-chloro-N-[{(5S)-2-oxo-3-[4-(3-oxo-4-morpholinyl)phenyl]-1,3-oxazolidine-5-yl}methyl)-2 thiophene carboxamide(FDA, 2014). It is an odorless, non-hygroscopic, white to yellowish powder and pure (s)enantiomer The molecular formula of RIV is $C_{19}H_{18}ClN_3O_5S$.Rivaroxaban is invented and manufactured by Bayer and it is marketed as Xarelto. Each marketed preparation of Xarelto contains 10 mg, 15 mg, and 20 mg of Rivaroxaban. The Croscarmellose sodium, Hypromellose, Lactose, Monohydrate, Magnesium stearate, Microcrystalline cellulose, and Sodium lauryl sulfate are the inactive Ingredients of Xarelto. The most common adverse reaction are bleeding complications, in that including major bleeding event, fainting, muscles spasm and itching have been have been reported. The introduction of Rivaroxaban in a surgical department represent a standard changes. It can not administered preoperatively but can be administered postoperatively (Jebaliya, 2015)

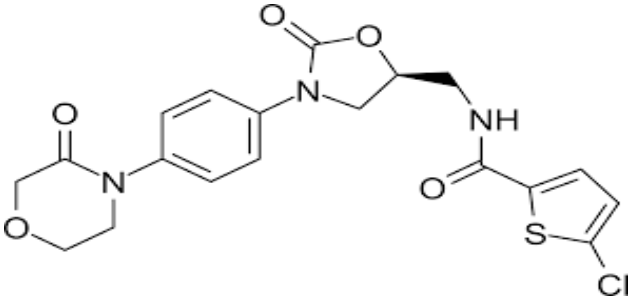


Fig 1-Chemical structure of Rivaroxaban

Method And Materials

- Chemicals and Reagents
  Pure sample of Rivaroxaban was procured from Swapnroop Drugs and pharmaceuticals Pvt. LTD, Aurangabad. Acetonitrile (HPLC Grade), Sodium Acetate (AR Grade), Methanol (HPLC Grade), HPLC grade water. All chemicals and reagents that is Methanol, Acetonitrile (ACN), Phosphoric acid, were purchased from LOBA CHEME PVT. LTD., Mumbai.
- Instrumentation

The analysis was performed by using chromatographic system, Shimadzu HPLC comprised of a Model PU 2080 Plus Intelligent HPLC pump, Rheodyne sample injection port with 50µl loop JASCO UV-2075 UV-VIS detector and running on Borwin chromatography software (version 1.50) with a SUN Q C18 Column having 250 ×4.6 mm internal diameter , 5µ particle size. Shimadzu (model AY-120) Electronic was used for weighting purpose. Sonicator: PRAMA solutions for laboratory for sonication purpose .

- Chromatographic conditions
  Rivaroxaban was analysed with SUN Q C_{18} column (250 × 4.6 i.d, 5µ particle size) for the chromatographic separation and column was maintained at ambient temperature. The mobile phase was composed of a mixture of acetonitrile and sodium acetate buffer in the ratio of 80 :20 v/v and it was delivered at a flow rate of 1ml/min and detection was monitored at 249 nm with UV-VIS detector. Acetonitrile was used as diluent. Injection volume was 50µl loop. The run time of Rivaroxaban was found to be 3.69 min.

Table 1: Optimized chromatographic condition

Parameters	Conditions used
Column	Sun Q C18 (250 x 4.6 mm, 5 µ)
Mobile Phase	ACN: BUFFER (Sodium Acetate Buffer) (80:20)
RT (retention time)	3.697 ± 0.135 min
Flow Rate	1 ml/min
Sample Injector	50 µl loop
Detection Wavelength	249 nm
Column Temperature	Ambient

Preparation of standard stock solution

Standard stock solution of Rivaroxaban (RVX) was prepared by dissolving 10 mg of the RVX in 10 ml of acetonitrile to get concentration of 1000 µg/ml. From the standard stock solution, 1 ml solution was pipetted out to into 10 ml volumetric flask and volume made using acetonitrile to get concentration 100 µg/ml. Further dilutions were prepared using stock solution. The retention time (RT) of RVX was 3.692 min.

Selection of analytical wavelength

The standard stock solution (1000 µg/ml) of Rivaroxaban was prepared in acetonitrile and further dilutions were prepared using mobile phase and solution was scanned over the range of 200-400 nm and the spectra was obtained. It was observed that the 10 µg/ml RVX solution showed maximum absorbance at 249 nm. Representative UV spectrum of Rivaroxaban (Figure 1)

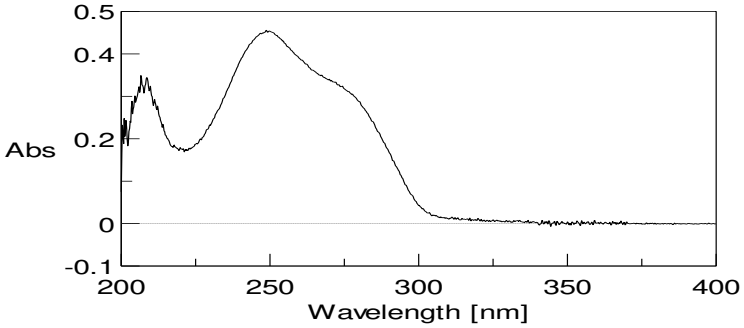
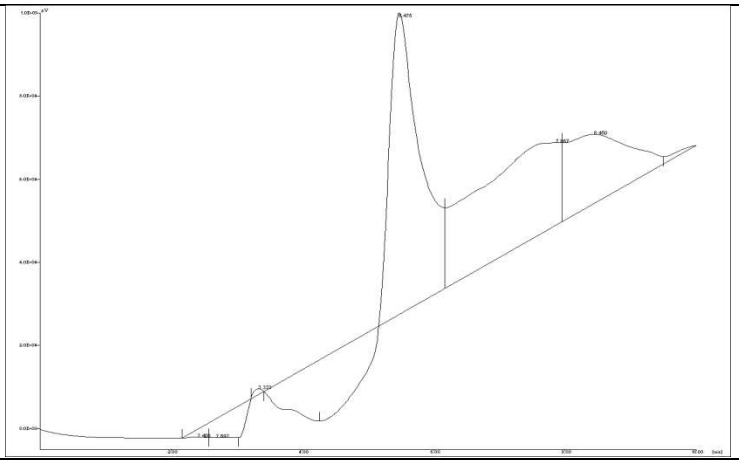


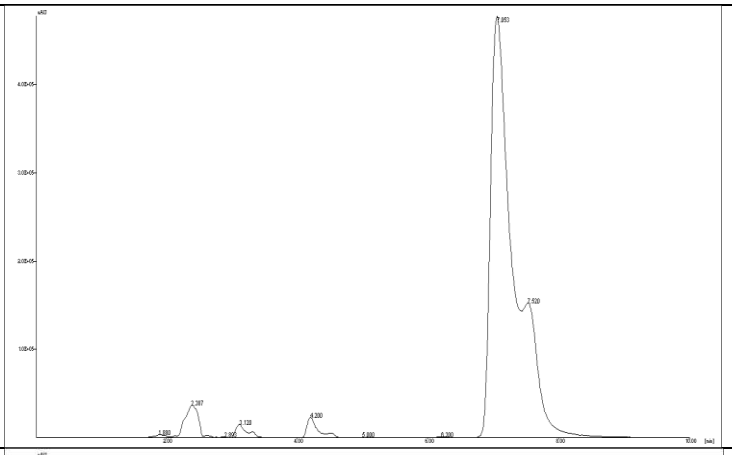
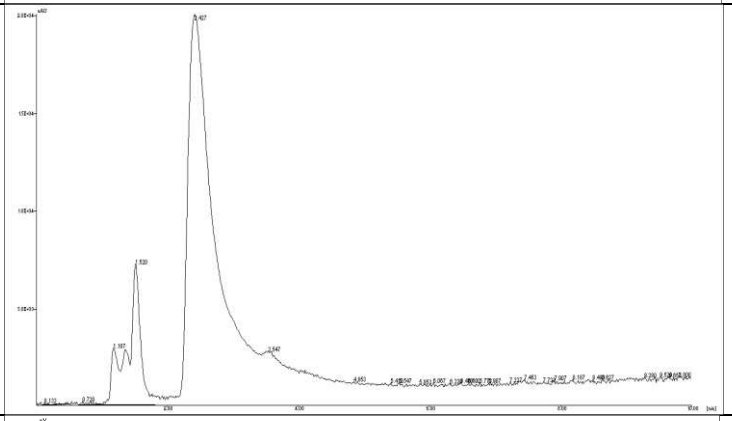
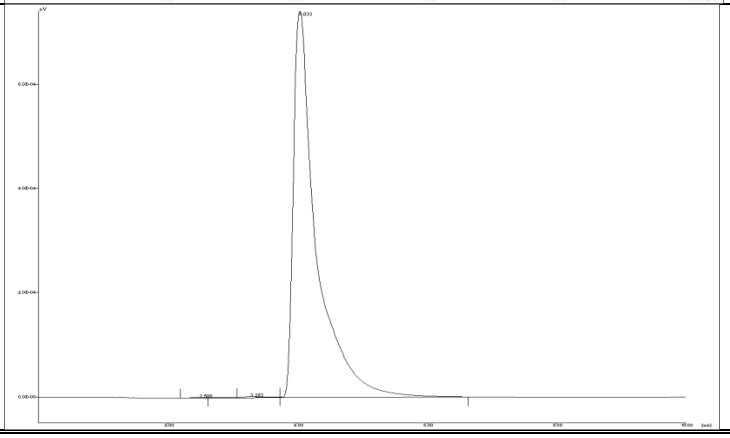
Fig 2: UV- Spectrum of Rivaroxaban 10 µg/ml

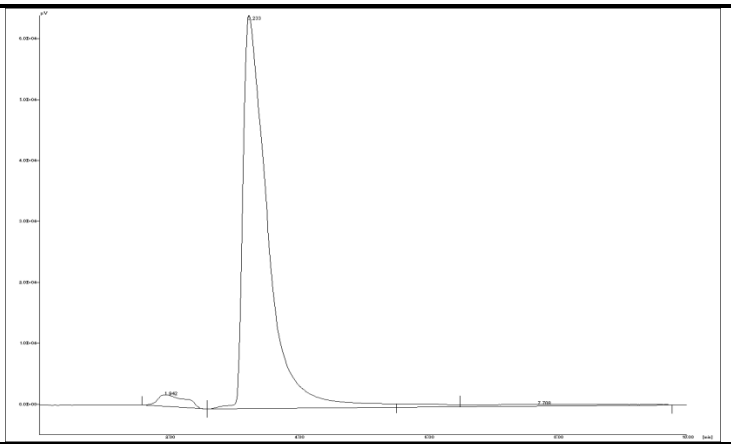
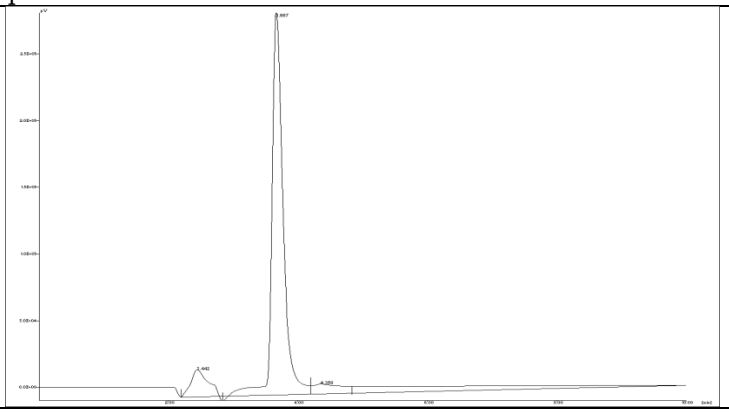
Mobile phase optimization

To achieve optimum chromatographic conditions various mobile phases like Acetonitrile: water, Methanol: water were tried at different proportions (70:30, 80:20, 90:10) initially but did not lead to appropriate peak shape. Sodium acetate buffer (pH-5) was subsequently tried with Acetonitrile, Using Acetonitrile: Sodium acetate buffer (pH-5) (80:20 v/v) a good peak shape was obtained with appropriate system suitability parameters. Table 1 shows all the optimized chromatographic conditions.

Table 2: Trials for mobile phase of Rivaroxaban

Sr.No.	Column and M.P.	Observation	Chromatogram (230 nm)
1.	Sun Q C18 (250 x 4.6 mm, 5 µ) ACN: Water (70:30v/v)	Peak was not found.	

2.	Sun Q C18 (250 x 4.6 mm, 5 µ) ACN: Water (80:20 v/v)	Peak Tailing observed	
3.	Sun Q C18 (250 x 4.6 mm, 5 µ) Methanol: Water (90:10 v/v)	Peak Tailing	
4.	Sun Q C18 Methanol: Water (70:30v/v)	Peak tailing was observed	
Trial for Disodium Hydrogen Phosphate Buffer			

5.	Sun Q C18 ACN: BUFFER (Disodium Hydrogen Phosphate- 10 mM) (80:20 v/v)	Peak Tailing	
Buffer change – to Sodium Acetate Buffer pH- 5			
6.	Sun Q C18 ACN: BUFFER (Sodium Acetate Buffer) (pH-5) (80:20 v/v)	Peak was observed having good shape with RT-3.69 min.	

Method development

To saturate the column, mobile phase was pumped for about 30 min to get a stable base line. A series of dilutions were prepared from the standard stock solutions using mobile phase to get the concentrations of 5-30 µg/ml for RVX. Each concentration was injected 6 times to the HPLC system. The parameters noted after every injection were peak area, retention time, asymmetry factor and theoretical plates. Calibration curve was plotted by taking average peak area on Y-axis and concentration on X-axis, Regression equation was calculated from the calibration curve, this regression equation is used to calculate the content of RVX. Optimized Chromatographic Conditions are given in Table 2 and system suitability parameters are given in Table 3

Preparation of Acetate Buffer PH- 5 as per IP

Dissolve 13.6 g of sodium acetate and 6 ml of glacial acetic acid in sufficient water to produce 1000 ml. Adjust the PH if necessary with orthophosphoric acid.

Method validation [24]

Validation of proposed analytical method involves linearity and range, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and robustness study. It was validated according to ICH Q2 (R1) guideline.

System Suitability

System suitability is an integral part of chromatographic system. The calculation and comparison of verified resolution, capacity factor, tailing factor, theoretical plate count with standard specification of system.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. The determination of the excipients effect and other additives which are present in formulation can be determined by using analytical method i.e. specificity.

Linearity

The linearity was determined by analysing six solutions over the concentration range of 5-30 µg/ml. Evaluation of drug was performed with UV-VIS detector at 249 nm, peak area was recorded for all the peaks. The correlation coefficient of Rivaroxaban was 0.994.

Accuracy

Accuracy of the analytical method is the capability of method to determine the correct assay value. Accuracy of analytical method can be determined by adding the known amount of standard drug to placebo preparation and then same shall be analysed by proposed method. The accuracy of the method was determined by calculating recovery of API by method of standard addition.

Precision

The closeness of agreement between a series of measurements obtained from multiple sampling of similar homogenous sample under the prescribed condition. Precision was determined by injecting the 6 injections of standard preparation.

Limit od detection and Limit of quantification

LOD is the lowest concentration of analyte that gives a detectable response where the LOQ is lowest concentration of analyte that can quantified with a specified level of accuracy and precision.

Robustness

The robustness of analytical method is a measurement of its capacity to remain unaffected by small changes but deliberate changes in procedure parameters and provides an indication of its reliability during normal usages.

Results & Discussion

Identification of pure drug (Rivaroxaban)

Identification and confirmation of pure drug (RIV) was carried out by observing obtained spectra. It showed characteristics peak at 1735.99 (-C=O stretching); 1280 (-C-O stretching); 1219.78 (-C-N stretching); 1512 (-C=C stretching); 1280 (-C=S stretching). These peaks value were in accordance with previously reported spectra of Rivaroxaban. (fig.3)

Fig.3 FTIR spectra of pure RIV

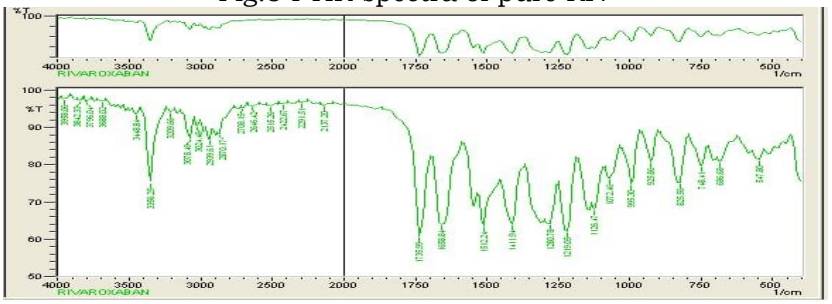


Table 3: System suitability parameters

Name	RT (Min) Mean ± % RSD	Concentration (µg/ml)	Area	No of Theoretical Plates (N)	Asymmetry
RVX	3.698 ± 0.135	10	1119208.3	2648	1.08

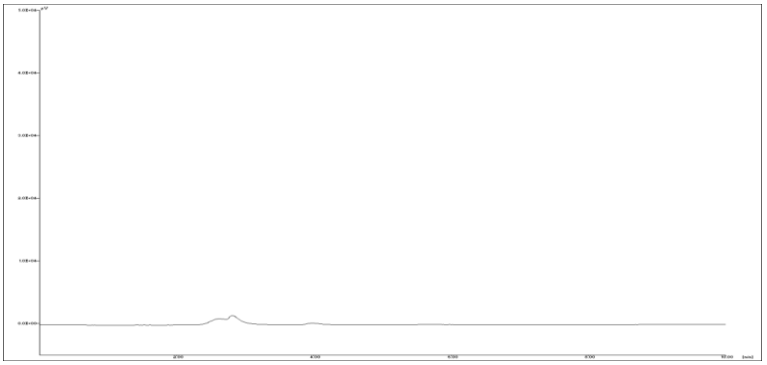


Figure 3:(a) Chromatogram of Mobile phase

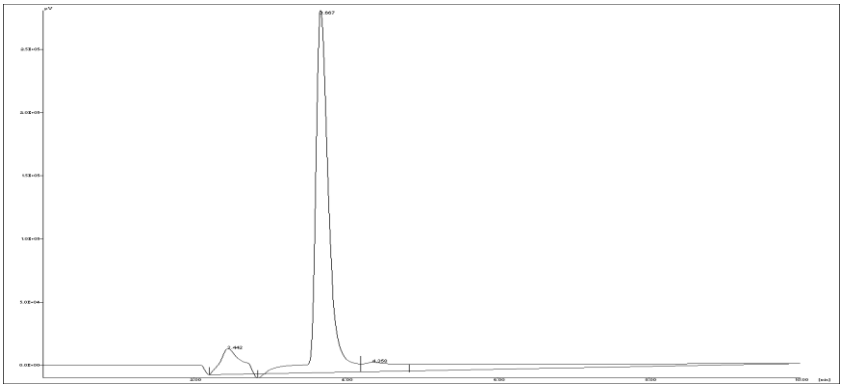


Figure 3:(b) Chromatogram of Standard Rivaroxaban - 3.67 min

Specificity

The specificity of the method was ascertained by peak purity profile studies. The peak purity values were found to be more than 0.998, indicating the no interference of any other peak of degradation product, impurity or matrix.

Linearity and Range

From the standard stock solution (1000 µg/ml) of RVX, solution was prepared containing 100 µg/ml of RVX in acetonitrile. This solution was further used to prepare range of solutions containing six different concentrations in mobile phase. The linearity (relationship between peak area and concentration) was determined by analysing six solutions over the concentration range of 5-30 µg/ml. Linearity curve and overlain of the 6 concentration are represented in Figure 3 (a) and 3 (b) and results are depicted in Table 3

Table 4: Linearity data for RVX

CONC (µg/ml)	Area 1	Area 2	Area 3	Area 4	Area 5	Area 6	Average	SD	%RSD
5	504735.6	512463.1	516931.8	499876.7	510325	501249	507596.87	6728.26	1.33
10	1119208.3	1113621.1	1112311.1	1097658.3	1095671	1088546	1104502.63	12164.14	1.10
15	2020912.1	2010401.2	1968736.5	1992545.5	1973697	2050123	2002735.88	30782.23	1.54
20	2871275.7	2907437.8	2936541.9	2898743.2	2903698	2825632	2890554.77	38016.57	1.32
25	4025228.3	4012287.1	3987542.3	3957895.6	3960015	3925613	3978096.88	37334.06	0.94
30	4762884.3	4621364.7	4595124.5	4698754.7	4702365	4699365	4679976.37	61218.77	1.31

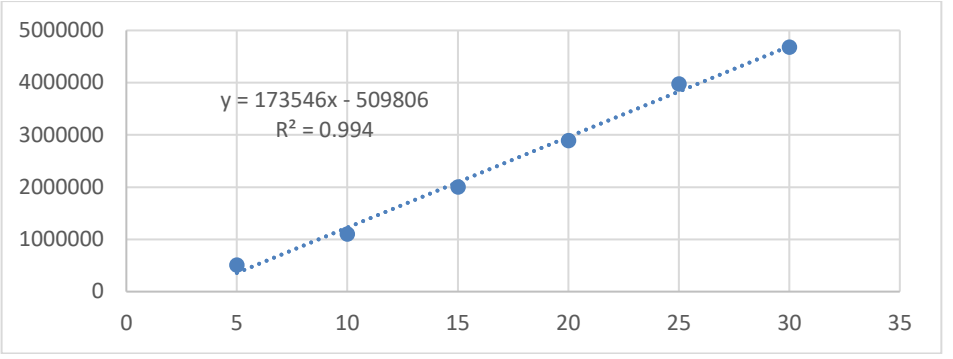


Figure 4 a) Calibration curve for RVX (5- 30 µg/ml)

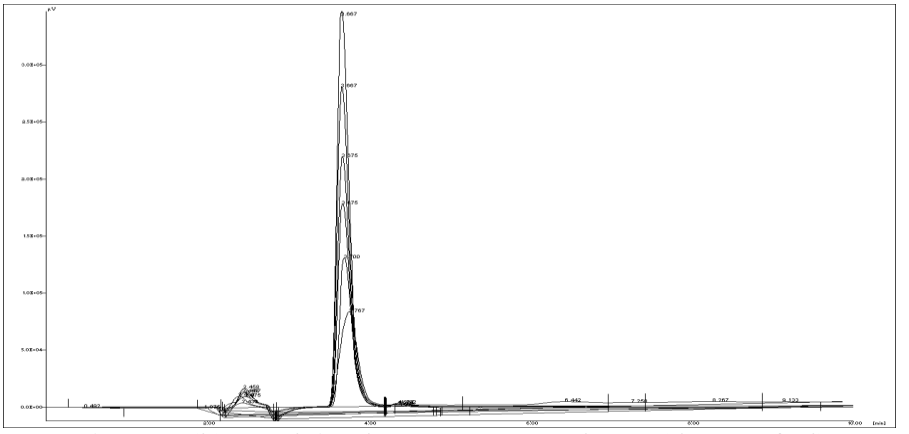


Figure 4 (b) Overlain chromatograms of RVX (5-30 µg/ml)

Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the Intra-day studies, 3 replicates of 3 different concentrations were analysed in a day and percentage RSD was calculated. For the inter day variation studies, 3 different concentrations were analysed on 3 consecutive days and percentage RSD were calculated. Results for intra-day and inter day precision are given in Table 4 and 5

Table 5: Intra-day Precision Results

Conc (µg/ml)	Area	Amount recovered (µg/ml)	% Recovery	Average % Recovery	SD	%RSD
10	1211189	9.917	99.167			
10	1222128	9.980	99.797	99.547	0.335	0.336
10	1220036	9.968	99.676			
15	2101069	15.044	100.295			
15	2090100	14.981	99.874	99.911	0.367	0.367
15	2082058	14.935	99.565			
20	2965111	20.023	100.115			

20	2938791	19.871	99.357	99.637	0.416	0.418
20	2941658	19.888	99.439			

Table 6: Inter- day Precision Results

Conc (µg/ml)	Area	Amount recovered (µg/ml)	% Recovery	Average % Recovery	SD	%RSD
10	1210188	9.911	99.109			
10	1212128	9.922	99.221	99.508	0.597	0.600
10	1229037	10.019	100.195			
15	2090067	14.981	99.873			
15	2108100	15.085	100.565	100.040	0.465	0.465
15	2085077	14.952	99.681			
20	2968011	20.040	100.199			
20	2930791	19.825	99.126	99.684	0.537	0.539
20	2951658	19.946	99.728			

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

LOD and LOQ were calculated from the formula

$$LOD = 3.3\ \sigma / S$$

$$LOQ = 10\ \sigma / S,$$

Where σ = standard deviation of y- intercept, S = slope of the calibration curve. The LOD and LOQ were found to be 0.439 µg/ml and 1.331 µg/ml. results are given in Table 7.

Table 7: LOD and LOQ Results

Sr. No	Parameter	Using Y- intercept	Using response at lowest conc.
1.	LOD (µg/ml)	0.439	0.128
2.	LOQ (µg/ml)	1.331	0.388

Assay

Assay was carried on marketed tablet formulation (Xarelto 20 mg; Bayer Zydus Pharma; Label Claim: Each tablet contains Rivaroxaban 20 mg). 20 tablets were weighed accurately and triturated to obtain homogenous mixture. From the homogenous mixture, powder weight equivalent to 10 mg of Rivaroxaban was weighed and transferred to the 10 ml of volumetric flask and volume was made with acetonitrile (1000 µg/ml) and it was filtered. This solution was further diluted with mobile phase to final concentration of 10 µg/ml of Rivaroxaban which was injected on HPLC system under optimized conditions. Procedure was repeated for 06 times to obtain the results. The results obtained are summarized in Table 8

Table 8: Assay Results

Sample No.	Concentration µg/ml	Area	Recovered Concentration (µg/ml)	%Recovery	%Average ± %RSD
1	10	1211235	9.917	99.169	99.299 ± 0.802
2	10	1228756	10.018	100.179	
3	10	1208898	9.903	99.034	
4	10	1231753	10.035	100.351	
5	10	1201992	9.864	98.637	
6	10	1198313	9.842	98.425	

Accuracy

Accuracy of the method was determined by method of standard addition method. Standard solution of API to be analysed was added to the sample solution of RVX at 50 %, 100 % and 150 % level. The 3 replicates at each level were evaluated to calculate % recovery. The results obtained are summarized in Table 9

Table 9: Accuracy Results

% Level	Initial amount (µg/ml)	Amount added (µg/ml)	Peak area	% Recovery	Average	% RSD
50	10	5	2100365	100.268	100.397	0.284
	10	5	2098574	100.199		
	10	5	2112232	100.724		
100	10	10	3000351	101.130	100.941	0.288
	10	10	2982153	100.606		
	10	10	2998797	101.086		
150	10	15	3879563	101.169	100.494	0.694
	10	15	3819101	99.775		
	10	15	3852146	100.537		

Robustness

Robustness was performed by doing the small and deliberate changes to developed system. Peak area was checked after doing the changes to Flow Rate, Detection Wavelength and Mobile Phase Ratio. The optimized system is robust as %RSD is below 2 %. The results of the validation proved that the established method comply with validation parameters and data is summarized in Table 10

Table 10: Robustness Results

Sr. No	Parameter		Conc (µg/ml)	% RSD
1.	Flow Rate	+ 0.05 ml/min	10	0.176
		- 0.05 ml/min	10	0.152
2.	Wavelength	+ 1 nm	10	0.332

		- 1 nm	10	0.368
3.	Mobile Phase Ratio (ACN: BUFFER)	(78:22 v/v)	10	0.231
		(82: 18 v/v)	10	0.342

Table 11: Summary of Validation Results

Sr. No	Parameter		RVX
1	Linearity Range		y = 173546 x – 509806 R^2 = 0.994 5 – 30 (µg/ml)
2	Method Precision (%RSD)	Intra Day	0.336
			0.367
			0.418
		Inter Day	0.600
			0.465
			0.539
3	Assay (Mean ± % RSD)		99.299 ± 0.802
4	Accuracy (Mean ± % RSD)	50 %	100.397 ± 0.284
		100%	100.941 ± 0.288
		150%	100.494 ± 0.694
5	LOD		0.439 µg/ml
6	LOQ		1.331 µg/ml
7	Robustness		Robust

Conclusion

By observing current situation, there is need to develop new accurate, rapid, economical method for this drug (RIV) drug formulation or its product, so this method is developed according to ICH guidelines. The developed method was found to be simple, sensitive, specific, accurate, and repeatable for analysis of Rivaroxaban in bulk and pharmaceutical dosage form without any interference from the excipients.

Conflict of interest

The authors declare no conflict of interest.

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