RP-HPLC Method Development and Validation of Rivaroxaban in Bulk and Tablet Dosage Form

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Abstract:

The fewer spectrophotometric and HPLC methods have been reported for determination of Rivaroxaban in tablet dosage forms. Hence, in the present study, a sensitive, suitable and cost effective reversed-phase high performance liquid chromatography method was developed and validated for the determination of Rivaroxaban in bulk and tablet dosage forms.

In RP-HPLC method, the analyte were resolved by using isocratic system, Methanol and Water (90:10 v/v) was used as mobile phase, at a flow rate of 1.0 ml/min, on HPLC system containing UV- detector with Workstation Software and C18 column (250 x 4.6 mm; 5µm). The detection was carried out at 249 nm. The method gave the good resolution and suitable retention time i.e. 4.4 min for Rivaroxaban. The results of analysis in the method were validated in terms of accuracy, precision, specificity, linearity, limit of detection, limit of quantification and robustness. A simple and precise method was developed for the assay of Rivaroxaban from tablet formulation.

The method does not require use of expensive reagent and also less time consuming, it can be performed routinely in industry for routine analysis of marketed product of Rivaroxaban.

Keywords: RP-HPLC, Rivaroxaban, Methanol, Validation.

Introduction:

Rivaroxaban is 5-chloro-N-({(5S)-2-oxo-3-[4-(3- oxomorpholin-4-yl) phenyl]-1,3-oxazolidin-5yl}methyl) thiophene-2-carbinoxamide. In November 2008, the Therapeutic Goods Administration approved new oral anticoagulant drug Rivaroxaban for the prevention of venous thrombosis in patients having knee or hip replacement [1]. Rivaroxaban is available in India in the brand name of Xarelto® as a tablet [2]. It blocks the amplification of the intrinsic and extrinsic pathway of coagulation cascade by binding directly to the catalytic pocket of factor Xa and thereby preventing the formation of thrombus. It has molecular formula of $C_{19}H_{18}ClN_3O_5S$ and molecular mass of 435.881 g/mol [3] Figure 1.

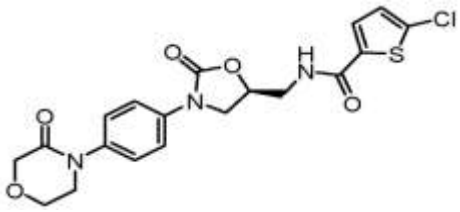

Figure 1: Structure of Rivaroxaban

There is no official monograph available for Rivaroxaban or drug product in any pharmacopoeia [1]. Literature survey suggests that fewer studies had been carried out on Rivaroxaban on RP-HPLC. Hence, the present study attempts to develop a simple, rapid and economical RP-HPLC method for the estimation of Rivaroxaban in its tablet dosage form.

Material and Methods

Chemicals:

All the chemicals used were of analytical grade and the solvents were of HPLC grade which has procured from Pharmatech Solutions, Nashik. Rivaroxaban API was obtained as gift sample from Inventia Healthcare Ltd. Tablet Xarelto® was procured from local market.

Instruments:

HPLC analyses were performed on HPLC 3000 Series system with UV-3000-M detector. Separations were carried out on a Cosmosil C18 (250 x 4.6mm i.d, 5µm) Column. The flow rate was 1.0 ml/min while using isocratic elution with Methanol: Water (90:10 v/v) mixture. Injection volume was 20 µL and UV detection was performed at 249nm. Peak identity was confirmed by comparing retention time.

Preparation of Standard Stock Solution:

The standard stock solution of Rivaroxaban (1000 µg/ml) was prepared in Methanol: Water (90:10 v/v) mixture. The working standard solutions (10.0, 20.0, 30.0, 40.0 and 50.0 µg/ml) were prepared by diluting the stock solution in mobile phase solution.

Preparation of Sample Solution:

A total number of 20 tablets were weighed and the average weight was calculated. From the powdered tablets quantity equivalent to 10mg was taken and it was dissolved in to methanol, sonicated for 30min, then final volume was made up with mobile phase. The solution was filtered using 0.45 µm membrane filter paper, resulting solution was diluted with mobile phase to required concentration with appropriate dilutions.

Method Development

Different ratios of mobile phases were tried for the separation and resolution. The various method optimization procedures were carried out and compared with system suitability parameters. The choice of wavelength 249nm was considered satisfactory, permitting the detection of the drugs with adequate sensitivity.

Result and Discussion

Method Validation

System Suitability

The chromatographic conditions for the estimation of Rivaroxaban were discussed in Table 1. Rivaroxaban standard drug solution was injected into HPLC system for six times, and checked for the system suitability parameters like tailing factor, number of theoretical plates (N) and % Relative Standard Deviation of areas for six injections of standard Rivaroxaban solution was calculated. The results were shown in Table 2.

Sr. No.	Chromatographic Parameters	Chromatographic Conditions
1	Mode of separation	Binary elution
2	Mobile Phase	Methanol: Water (90:10 v/v)
3	Column	Cosmosil C18 (250mm x 4.6 i.d, 5µm)
4	Flow rate	1.0 ml/min
5	Detection wavelength	249nm
6	Injection volume	20 µl
7	Run Time	7.16 min
8	Pressure	9-10MPa

Table 1: Optimized chromatographic conditions

Sr. No.	System Suitability Parameters	Results	Acceptance Criteria
1	Tailing Factor	1.07	NMT 2.0%
2	Number of Theoretical Plates	8822	NLT 2000
3	% RSD of areas for six injections of standard solution	0.053	NMT 2.0%

Table 2: System Suitability Testing Parameters Results

Linearity

The linearity of an analytical procedure is its ability to obtain test results, which are directly proportional to the concentration of analyte in the sample. Calibration curve was constructed for Rivaroxaban standards by plotting the concentrations versus peak area ratios as shown in Figure 2. The graph proved that the method was linear up to 50 µg/ml. Five different standard solutions within the linear range containing 10.0, 20.0, 30.0, 40.0 and 50.0 µg/ml were prepared and

injected into HPLC system. The linearity was evaluated by linear regression analysis and the regression equations were calculated from the calibration graphs, along with the standard deviations of the slope (m) and intercept(y) of the calibration curve. The results were shown in Table 3.

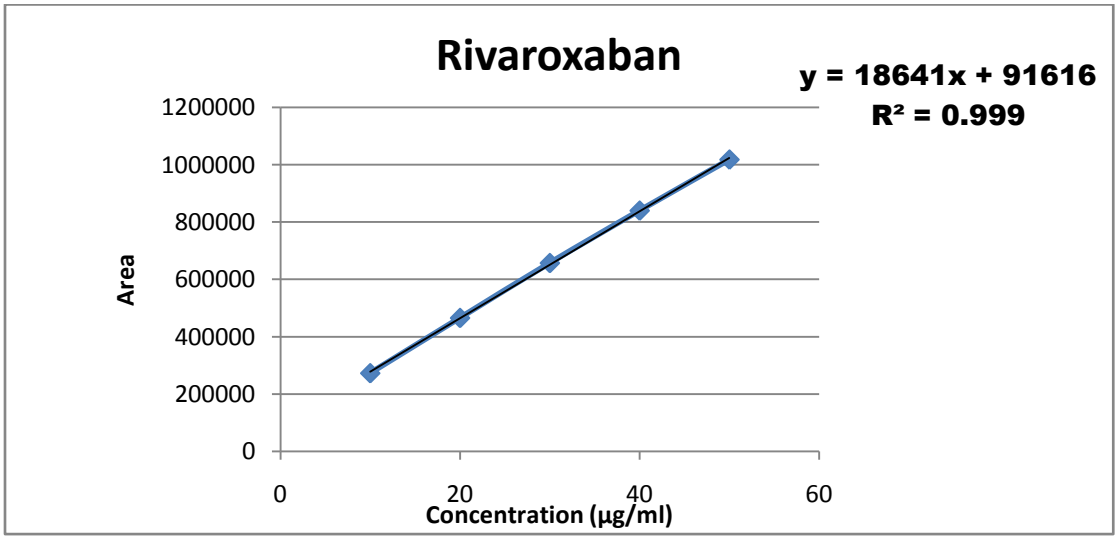

Figure 2: Linearity Curve of Rivaroxaban

Sr. No.	Concentration (µg/ml)	Peak area (N=5)
1	10.0	273114
2	20.0	465783
3	30.0	657239
4	40.0	840075
5	50.0	1018018
Correlation Coefficient (R^2)		0.999
Slope		18641
Intercept		91616

Table 3: Linearity data of Rivaroxaban

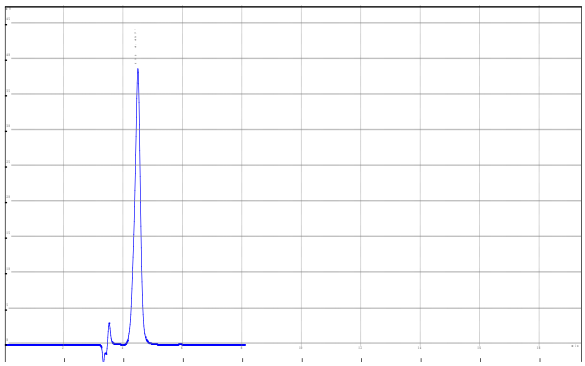

Figure 3: Chromatogram of Rivaroxaban Standard Preparation

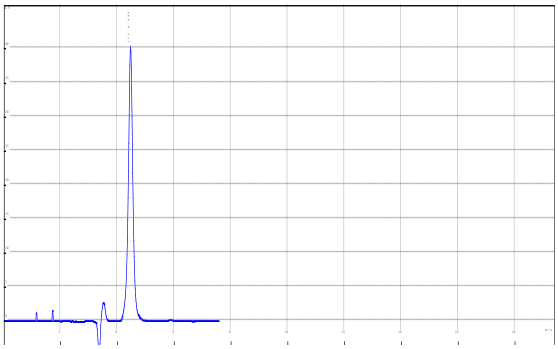

Figure 4: Chromatogram of Rivaroxaban Sample Preparation

Drug	Label Claim (mg/tab)	Area of Standard	Area of sample	% Assay
Xarelto®	15 mg	657239	657490	100.038

Table 4: Assay of Formulation

Accuracy

Accuracy of method was determined by applying the proposed method to synthetic mixture containing known amount of drug to 50%, 100% and 150% of the label claim. The accuracy was then calculated as the percentage of analyte recovered by the assay. The results of the recovery analysis of the recovery analysis are enclosed under Table 5.

Level of addition	Standard added (µg/ml)	Conc. (µg/ml)	Total Conc. (µg/ml)	Area	Drug recovered (µg/ml)	% Recovery
	10	20	30	657600	30.01	100.05

50%	10	20	30	656914	29.98	99.95
	10	20	30	657141	29.99	99.98
100%	20	20	40	839880	39.99	99.97
	20	20	40	839430	39.96	99.92
	20	20	40	839739	39.98	99.96
150%	30	20	50	1012908	49.74	99.49
	30	20	50	1010501	49.63	99.26
	30	20	50	1017509	49.97	99.95

Table 5: Results of Accuracy of Rivaroxaban

Precision

The assay was carried out using proposed method in six replicates. The value of relative standard deviation lie well within the limits, it indicates the sample repeatability of the method enclosed in Table 6.

Sr. No.	**Sample ID Concentration 30 µg/ml**	**Inter-day**	**Intra-day**
1	S1	657239	657239
2	S2	656914	656914
3	S3	657141	657141
4	S4	656656	657699
5	S5	657076	657353
6	S6	657746	657226
Mean		657746	657426
% RSD		0.06%	0.04

Table 6: Results of Inter-day and Intra-day Precision of Rivaroxaban

Robustness

The robustness of the method was determined to check the reliability of an analysis with respect to deliberate variations in method parameters. The typical variations are given below: Variation

in flow rate ±0.1ml/min, variation in wavelength ± 2 nm. The results of robustness are enclosed under Table 7.

Parameter	Concentration	Variation	R.T	Tailing Factor	Theoretical Plates	% RSD
Wavelength Variation	20 µg/ml	247	4.441	1.02	8445	0.1178
		249	4.426	1.01	8508	
		251	4.407	1.00	8317	
Flow rate variation	20 µg/ml	0.9	5.840	1.00	8499	0.0707
		1.0	4.426	1.01	8508	
		1.1	3.516	1.01	8561	

Table 7: Robustness Results

Detection Limit and Quantitation (LOD and LOQ)

LOD and LOQ of the drug were calculated using the following equations according to International Conference on Harmonization (ICH) guidelines. The results of LOD and LOQ are enclosed under Table 8.

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

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

Where, σ = the standard deviation of response

S = the slope of the regression equation

Sr. No.	LOD	LOQ
1	0.0295 (µg/ml)	0.0894 (µg/ml)

Table 8: Results of LOD and LOQ

Conclusion

In this study a sensitive, suitable and cost effective reversed-phase high performance liquid chromatography method was developed and validated for the determination of Rivaroxaban in bulk and tablet dosage form. The proposed method has several advantages, including simple mobile phase, low cost solvent, rapid analysis, simple sample preparation and improved selectivity as well as sensitivity. The regression coefficient (R^2) for each analyte is not less than 0.999 which shows good linearity. The % recovery was in the acceptable range in tablet dosage form. The % RSD was also less than 2% showing high degree of precision of the proposed method.

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