Available online at **www.joac.info**

ISSN: 2278-1862

Journal of Applicable Chemistry

2019, 8 (4): 1870-1879

(International Peer Reviewed Journal)

Advancement and Validation of an Innovative Stability Indicating RP-HPLC Assay Method of Rivaroxaban in Tablet Formulation

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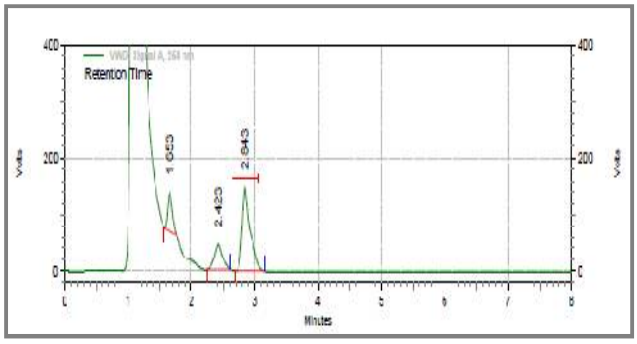
Accepted on 3rd June, 2019

ABSTRACT

An innovative RP-HPLC assay procedure has been established for rivaroxaban in pharmaceutical dosage forms. Outstanding segregation of the rivaroxaban was accomplished on Thermo BDS, C8, 150 x 4.6, 5µm column using the mobile phase (potassium dihydrogen phosphate buffer (pH 2.5±0.05) and acetonitrile in the ratio of 50:50 %v/v) at a flow rate of 1.0ml/min with UV detection of 254nm in ambient temperature. The recommended RP-HPLC procedure had viably separated rivaroxaban from its degradation products, making it stability-indicating. A calibration graph was obtained over six different concentrations in the range of 8.0-48µg ml^{-1} illustrating regression equation of $y=1103652.16x-10146$ ($r^2= 0.9999$) for rivaroxaban admitting a superb relationship. Reasonableness of this system for the quantitative determination of the rivaroxaban was demonstrated by validation as per the prerequisites of International Conference on Harmonization (ICH) guidelines.

Graphical Abstract



Chromatogram of rivaroxaban in peroxide degradation.

Keywords: Rivaroxaban, Stability Indicating, Method Validation, HPLC Estimation.

INTRODUCTION

Rivaroxaban (s)-5-chlor-n-{2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-lmethyl}-thiophen-2-carbamid (Figure 1), is an innovative, oral, direct factor xa inhibitor affirmed for the treatment of profound vein thrombosis (DVT) and aspiratory embolism (PE) [**1-10**]. It works by

inhibiting platelet activation and fibrin clot formation by means of direct and reversible inhibition of factor xa in both the intrinsic and extrinsic coagulation pathways. In mix with ibuprofen, it is utilized for lessening the danger of major cardiovascular occasions in patients with chronic coronary artery disease or peripheral artery disease.

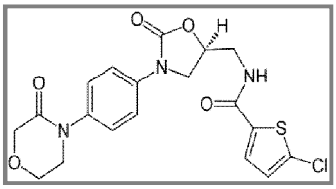


Figure 1. Chemical Structure of Rivaroxaban.

Two brands of generics of rivaroxaban are available in local pharmacy that is formulated for oral administration. In the current study for one commercial formulation of rivaroxaban in brand name of Ixarola (Cadila Healthcare Ltd; Dosage strengths-20 mg) was used.

Not many HPLC strategies have been accounted for in the writing for the assay of rivaroxaban in pharmaceutical dosage forms [**11-13**]. To the best of our insight, the reported analytical methods resulted in the impediment of long partition times, tedious and required costly extraction methods. It was therefore, felt necessary to develop a new rapid, stability-indicating HPLC assay method for rivaroxabanin oral dosage forms. The present paper portrays the improvement and approval of a novel and stability indicating RP-HPLC assay method for rivaroxaban in oral dosage forms.

MATERIALS AND METHODS

Instrumentation: The present analysis was performed on Agilent 1120 HPLC System was provided with a Agilent 1120 series consisting 2 channels. Auto sampler with one rack it has 100 vials. Auto injector has capacity to inject 0.1 µL to 100 µL. UV-Vis Detector. Thermostat column compartment connected it has a capacity to maintain 4°C to 80°C column temperature. Data acquisition was done by using Ezchrome Elite software. Shimadzu Automatic Digital Analytical Balance (ATX124) and Systronics digital pH meter were used for weighing and pH measurements respectively.

Chemicals and Solvents: Potassium dihydrogen ortho phosphate, Ortho Phosphoric acid (AR-Grade), Triethyl amine (AR- Grade), Hydrochloric acid (AR- Grade), Sodium hydroxide (AR-Grade), and Hydrogen peroxide (AR-Grade) and Acetonitrile of HPLC grade used were purchased from Merck Chemicals Ltd., Ahmadabad, India. High purity water was prepared by using Milli-Q Plus water purification system (Millipore USA). Rivaroxaban (API-99.9% Pure) was provided by Cadila Healthcare Ltd, as gratis sample and its commercial tablets in the brand name Ixarola-20 mg was purchased from local Mediplus pharmacy.

Preparation of Mobile Phase: Mix potassium dihydrogen phosphate buffer (pH 2.5±0.05) and acetonitrile in the ratio of 50:50 %v/v degassed and filtered through 0.45 µ membrane filter.

Buffer preparation: Weigh and dissolve 6.8 g of potassium dihydrogen phosphate in 1000 mL of water, add 1.0 mL triethyl amine mix well. Adjust pH with ortho phosphoric acid to 2.5±0.05.

Diluent: Mobile phase is used as diluent in the present study.

Preparation of stock and working standard solutions: Standard stock solution of concentration 100 µg mL^{-1} was prepared by transferring 10 mg of rivaroxaban in 100 mL clean and dry volumetric flask containing 50 mL of diluent, sonicated for 2 min and later the solution was made up to the mark using the same diluent. The stock solution was kept at 4°C that it is stable at least one month. Linearity test solutions for the assay procedure of rivaroxaban were prepared from the above stock

solution at 6 concentration levels from 25% to 150% (8.0-48 µg mL^{-1}) of assay concentration respectively.

Preparation of Sample Solution: Ten tablets of Ixarola-20 mg were weighed and grounded to fine powder. A measure of powder equivalent to 10 mg of rivaroxaban was transferred to a 100 mL volumetric flask and added 70 mL of diluent and sonicated for 30 min. The final volume was made up with the same diluent to obtain sample stock solution of concentration 100 µg mL^{-1} of rivaroxaban. Filter the above sample solution through 0.45 µ membrane filter.

From the above arrangement, pipette out various aliquots into discrete 10 mL volumetric flasks, diluted with the diluent to acquire concentrations of 8.0-48 µg mL^{-1} of rivaroxaban individually, 20 µL of these solutions were injected in prescribed HPLC system under described conditions.

RESULTS AND DISCUSSION

Method development: To build up a productive and basic RP-HPLC procedure for the investigation of the rivaroxaban in pure and in its tablet dosage forms, different primer tests were directed utilizing various parameters, for example, mobile phase composition, wavelength of detection, type of the column, column temperature and pH of mobile phase respectively.

Preliminary development trials with columns of different types, configurations and from different manufacturers revealed good separation of rivaroxaban was achieved with analytical column, Thermo BDS, C8, 150 x 4.6, 5µm. 254 nm was selected as the optimum wavelength for detection, as such best detector response for rivaroxaban was obtained.

Secondly, different composition of mobile phases containing a mixture of potassium dihydrogen phosphate buffer (pH 2.5±0.05) and acetonitrile was tried and the best results were achieved on using the mixture of potassium dihydrogen phosphate buffer (pH 2.5±0.05) and acetonitrile in the ratio of 50:50 %v/v as mobile phase. Using the above mentioned column and mobile phase at a flow rate of 1.0 mL min^{-1}, resulted in the elution of rivaroxaban at the retention time of 2.884 min respectively. Under the described experimental conditions the analyte peak area of rivaroxaban was well defined and free from tailing.

Chromatographic conditions: From the method developmental studies the assay of rivaroxaban in pharmaceutical forms was touted by employing isocratic column, Thermo BDS, C8, 150 x 4.6, 5 µm, mobile phase of potassium dihydrogen phosphate buffer (pH 2.5±0.05) and acetonitrile in the ratio of 60:40 %v/v at a flow rate of 1.0 mL min^{-1} in ambient temperature. Spectral analysis was carried using UV detector at a wavelength of 254 nm. A sample volume of 20 µL fixed loop at ambient temperature was material during the analysis (Table 1 and Figure 2).

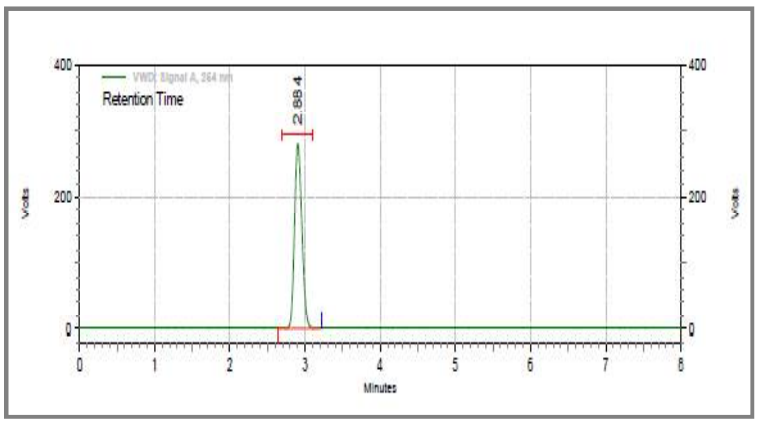


Figure 2. Ratified Chromatogram of rivaroxaban

Table 1. Optimized Chromatography conditions

Parameter	Results
Mobile phase	Potassium dihydrogen phosphate buffer (pH 2.5±0.05): Acetonitrile in the 50:50 (%v/v)
Pump mode	Isocratic
pH	2.5
Column	Thermo BDS, C8, 150 x 4.6, 5µm
Column Temperature	Ambient
Wavelength	254 nm
Injection Volume	20 µL
Flow rate	1.0 mL min^{-1}
Run time	8 min
Retention Time	2.884 min
Area	32273092

Forced degradation studies: The forced degradation or stress studies of rivaroxaban (API) were implemented in presence of excipients to show the stability indicating property of the present proposed RP-HPLC method.

Acid hydrolysis: Transfer sample quantitatively equivalent to 20mg of rivaroxaban into 50 mL RB flask add 25 mL of freshly prepared 0.1 N HCl. Leave it for 12 h. After 12 h filter the solution through filter paper neutralize the solution with suitable Base. Dilute 0.8 ml of filtrate to 10 mL with mobile phase (Diluent).

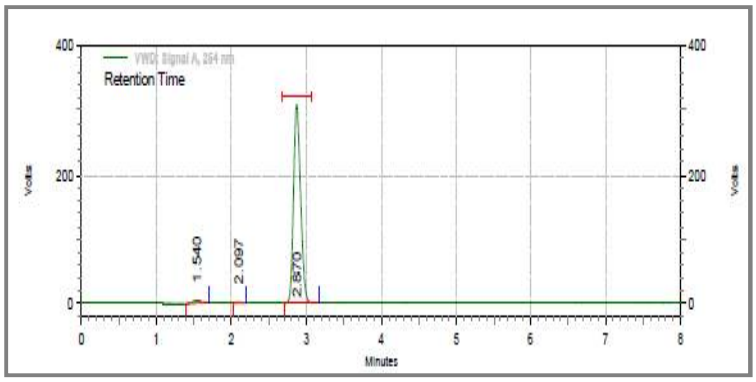


Figure 3. Chromatogram of rivaroxaban in acid degradation.

Base hydrolysis: Transfer sample quantitatively equivalent to 20 mg of rivaroxaban into 50 mL RB flask add 25 mL of freshly prepared 0.1NaoH. Leave it for 12 h. After 12 h filter the solution through filter paper neutralize the solution with suitable acid. Dilute 0.8 mL of filtrate to 10 mL with mobile phase (Diluent).

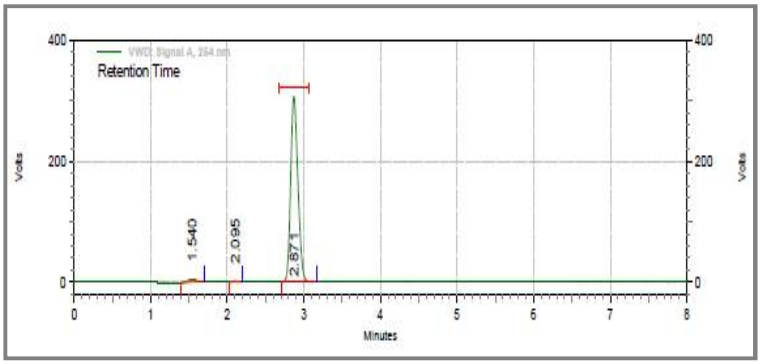


Figure 4. Chromatogram of rivaroxaban in base degradation.

Oxidation (Peroxide): Transfer sample quantitatively equivalent to 20 mg of rivaroxaban into 50 mL RB flask add 25 mL of freshly prepared 3.0% Hydrogen Peroxide. Leave it for 12 h. After 12 h filter the solution through filter paper neutralize the solution with suitable Base. Dilute 0.8 mL of filtrate to 10 mL with mobile phase (Diluent).

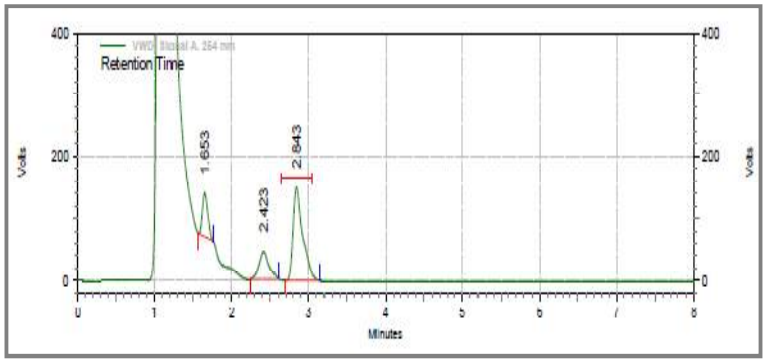


Figure 5. Chromatogram of rivaroxaban in peroxide degradation.

Heat exposure: Transfer sample quantitatively equivalent to 20 mg of rivaroxaban on to clean and dry Petri dish spread it throughout the plate. Place the petri dish in an oven which is maintaining at 100°C for 12 h. After 12 h transfer contents in to 50 mL volumetric flask add 25 mL of diluent and sonicate it for 10 minutes and dilute volume with diluent. Further, filter the solution through filter paper. Dilute 0.8 mL of filtrate to 10 mL with mobile phase (Diluent).

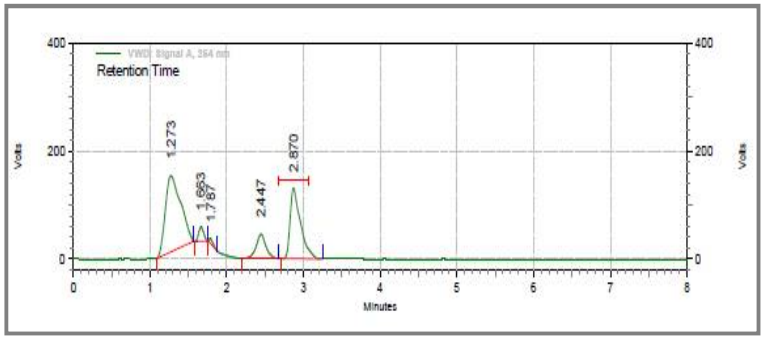


Figure 6. Chromatogram of rivaroxaban in thermal degradation.

UV Exposure: Transfer sample quantitatively equivalent to 20 mg of rivaroxaban on to clean and dry Petri dish spread it throughout the plate. Place the plate in UV Chamber for 12 h. After 12 h transfer contents in to 50 mL volumetric flask add 25 mL of diluent and sonicate it for 10 min and dilute to volume with diluent. Further filter the solution through filter paper. Dilute 0.8 mL of filtrate to 10 mL with mobile phase (Diluent).

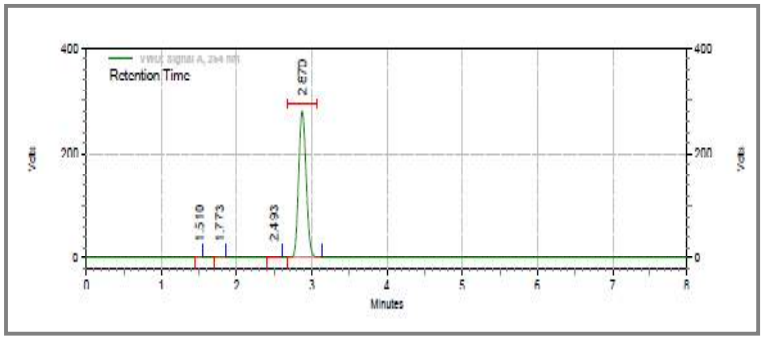


Figure 7. Chromatogram of rivaroxaban in photo degradation.

Table 2. Results of stress degradation studies by the proposed method

Condition	No. of degradation Products obtained	Rt	% Degraded	% assay Recovered	Tf	Tp
Acid	02	2.870	1.78	98.30	1.26	4164
Base	02	2.871	1.70	98.22	1.72	4134
Peroxide	02	2.843	35.75	64.25	1.65	2655
Thermal	04	2.870	62.02	32.98	1.90	2127
Photolytic	03	2.879	0.15	99.84	1.19	3831

Rivaroxaban assay was dropped to 98.30%, 98.22%, 64.25%, 32.98% and 99.84% in acid, alkali hydrolysis, peroxide oxide, thermal and photolytic stress. In all the above portrayed stress conditions the additional peaks are well separated from rivaroxaban peak revealing there is no impedance from degradants, encouraging error-free quantification of rivaroxaban. The chromatograms obtained in acid, alkali hydrolysis, peroxide oxidation, thermal and photolytic degradation are depicted in figures 3-7 and the results obtained are summarized in table 2.

Method validation: The method was validated according to International Council for Harmonization Q2 (R1) guidelines [**14**] for validation of analytical procedures in order to determine the specificity, linearity, limit of detection, limit of quantification, accuracy, precision, robustness and ruggedness respectively.

System Suitability: To ascertain the system suitability of the recommended RP-HPLC method, a number of parameters such as relative retention, theoretical plates, peak asymmetry and peak areas of rivaroxaban have been calculated with the observed reading and the results are recorded in table 3 and in figure 2 respectively.

Table 3. System suitability conditions by the proposed method

Sample	Retention time	Area	Tailing factor	Theoretical plates
Solifenacin succinate	5.535 min	76976	1.24	3754

Specificity: Standard, blank and formulation solutions were prepared and were analyzed in the optimized method conditions. Base line obtained for the resultant chromatograms were compared and confirmed that no chromatographic interference was observed at the retention time of rivaroxaban in the analysis of blank solution. In standard and formulation solution, similar retention time was observed, no other additional detections were found revealing specificity of the developed RP-HPLC method table 4.

Table 4. Results of specificity studies

S.No.	Sample Name	RT	Area
1	Blank	-	-
2	Rivaroxaban Standard	2.884	32273092
3	Placebo	-	-
4	Sample	2.869	32590949

Linearity: For linearity study working standard solution s of rivaroxaban was prepared with mobile phase from the stock solution in the concentration range of 8, 16, 24, 32, 40 and 48 µg mL respectively. These solutions were infused in triplicates into the HPLC system and the resulting chromatograms were recorded. A standard calibration plot for rivaroxaban was constructed by plotting their response ratios of the peak area of the analyte against their respective concentrations. Method of least square analysis was carried out for getting the slope, intercept and correlation coefficient values. The linearity of rivaroxaban were found to be in the range of 8.0-48 µg mL^{-1} with

correlation coefficient greater than 0.9999, revealing an excellent correlation between the peak area and analyte concentration respectively. The results of linearity studies were given in table 5 and the calibration curve so obtained was presented in figure 8 reciprocally**.**

Table 5. Results of Linearity by the proposed method

Level	Concentration (µg mL^{-1})	Peak area
Level–1(25%)	8.0	8870945
Level–2(50%)	16.0	17522961
Level–3(75%)	24.0	26449809
Level–4(100%)	32.0	34845778
Level–5(125%)	40.0	44013050
Level–6(150%)	48.0	53102219
Range: 8.0 to 48 µg mL^{-1}		Intercept:-10146, Slope:110365.16 Correlation coefficient: 0.9999

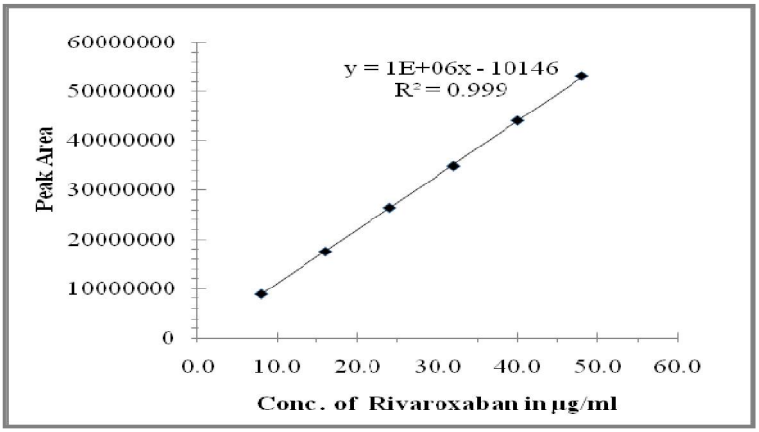


Figure 8. Calibration curve of Rivaroxaban.

LOD and LOQ: The LOD and LOQ for rivaroxaban were determined at a signal to-noise ratio of 3:1and10:1, respectively, by injecting a series of dilute solutions with known concentrations. The LOD and LOQ values for rivaroxaban by the recommended method were 0.587 and 1.95 µg mL^{-1} that indicated good sensitivity of the developed RP-HPLC method (Table 5).

Table 6. Limit of detection and limit of quantification by the proposed method

Parameter	Measured Value µg mL^{-1}
Limit of Quantification	0.54
Limit of Detection	1.95

Precision: The precision of the recommended method was demonstrated by intra-day variation studies. In intra-day studies, six repeated injections of one standard solution at 100% concentration level of rivaroxaban were made and response factor of the drug peak, and the % RSD were calculated. In the inter-day variation studies, six repeated injections of standard solutions of rivaroxaban were injected and the response factor of the drug peak, the % RSD was calculated. From the results it was concluded that the recommended HPLC method found to be precise. Results of the precision studies are presented in table 7.

Accuracy: Recovery studies were conducted by analyzing known amounts of pure drug added to each of the previously analyzed formulation and the total amount of drug within the linearity limits. 50%,

Table 7. Results of precision studies by the proposed method

Rivaroxaban			
S No	**Name**	**RT**	**Area**
1	System Precision-1	2.883	32269499
2	System Precision-2	2.873	32282600
3	System Precision-3	2.903	32273012
4	System Precision-4	2.863	32305850
5	System Precision-5	2.87	32349216
6	System Precision-6	2.89	32259384
	*Avg	2.880	32289927
	*Std Dev	0.015	33041.51
	*%RSD	0.509	0.102

*Mean of six estimations

100% and 150% recoveries were tested for standard drug solution and the peak areas were compared with the linearity results. Results of recovery studies were given in table 8 revealing that the proposed RP-HPLC method is accurate for determination of rivaroxaban.

Table 8. Results of recovery studies by the proposed method

Spike Level	Target Conc.(µg mL^{-1})	Spiked Conc.(µg mL^{-1})	Final Conc.(µg mL^{-1})	Conc. Obtained	% Assay
				25.79	99.19
50%	16		26	25.51	97.73
				25.86	99.46
		10		41.92	99.80
100%	32		42	41.73	99.35
				41.80	99.52
				57.89	99.81
150%	48		58	57.86	99.75
				58.08	100.10

Robustness: The vigor of the recommended method, the trial conditions {effect of change in flow rate ± 0.2 mL min^{-1} (0.8 and 1.2 mL min^{-1}), and wavelength ± 2.0 (252 nm and 256 nm)} were purposely changed. Amid the examination, other chromatographic conditions were kept equivalent to the test segment and the goals of rivaroxaban were assessed. From these investigations it was seen that slight intentional changes did not have any huge change on the magnitude of the peak or on chromatographic appropriateness of this strategy, inferring that the technique is very strong. The consequences of potency were given in table 9.

Table 9. Robustness results by the proposed method

S. No.	Parameter	RT	Area
1	Standard	2.867	32589420
2	Robustness-Flow-1	3.613	40649273
3	Robustness-Flow-2	2.387	27171044
4	Robustness-nm-1	2.880	33410364
5	Robustness-nm-2	2.877	31385702

Ruggedness: Inter-day precision at day to day variation was deliberated to express the ruggedness of the recommended method. Standard drug solution of rivaroxaban at concentration of 20 µg mL^{-1} was prepared and injected into cited HPLC system six times. The peak area response was recorded and % RSD was determined. Results of the ruggedness were deduced in the table 10.

Application of the recommended procedure in formulations: The approved method was applied for the determination of rivaroxaban in commercially accessible Ixarola-20 mg tablets. Figure 9 illustrates a typical type of HPLC chromatogram of tablet solution of rivaroxaban at a retention time 2.869 min with no interference of excipients present in tablets. The tablet analysis results were given

Table 10. Ruggedness results by the proposed method

S No	Ruggedness-Day-1		Ruggedness-Day-2	
	RT	Area	RT	Area
1	2.863	32590999	2.863	32305850
2	2.864	32591884	2.864	32304326
3	2.863	32592834	2.863	32304632
4	2.865	32590803	2.864	32305712
5	2.864	32590817	2.865	32305824
6	2.864	32590801	2.864	32305724
*Avg	2.864	32591356	2.864	32305345
*Std Dev	0.001	836.07	0.001	679.64
*%RSD	0.026	0.003	0.026	0.002

**Mean of six estimations; RT=Retention time

in table 11. The results of the assay (n=6) yielded 99.35% (RSD=0.651) of the labeled claim. In comparison with the earlier reported methods [13] no significant difference was found as evident by students-t (1.604) and F-test (2.384). These outcomes showed that the technique is specific for the examination rivaroxaban without the obstruction of the excipients. The low RSD esteem with legitimate precision demonstrated the reasonableness of this technique for the normal investigation of rivaroxaban.

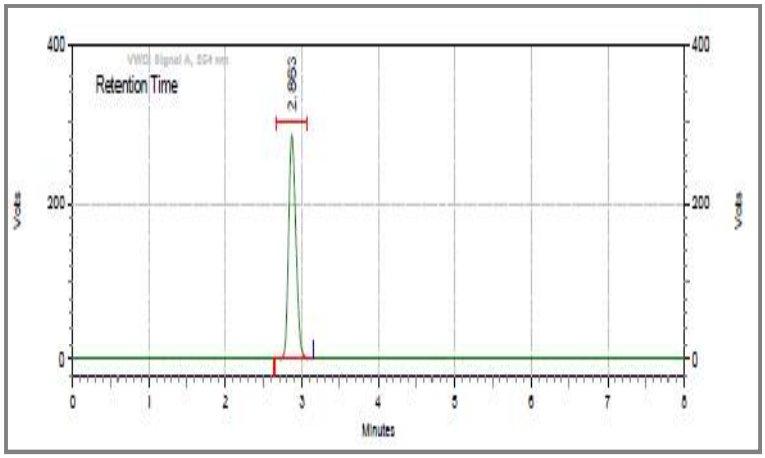


Figure 9. Chromatogram of rivaroxaban in formulation.

Table 11. Results of assay of rivaroxaban in dosage forms

Drug Name [Ixarola-20 mg]	Quantity Label Claim(mg)	**Quantity Found(mg) ± RSD	*% Assay ± RSD	*% Assay ± RSD Reported ethod[13]	t-Test	F-Test
Rivaroxaban	20	19.87 ± 0.421	99.35 ± 0.651	99.74 ± 0.421	1.60	2.384

*Mean of six estimations

APPLICATION

These results of validation studies showed that the recommended procedure is specific for the examination rivaroxaban without the obstruction of the excipients. The low RSD esteem with legitimate precision demonstrated the reasonableness of this technique for the normal investigation of rivaroxaban. The main asset of this recommended RP-HPLC method, i.e, it does not require cumbersome extraction procedure, internal standard, costly detector system, expertise operational personnel and found to be an alternative procedure to that of strategies detailed in the literature.

CONCLUSION

A new stability illustrating RP-HPLC method is developed and validated for the determination of rivaroxaban in bulk and dosage forms on Shimadzu HPLC instrument with Thermo BDS, C8 column, 150 x 4.6, 5 µm as stationary phase with mobile phase of potassium dihydrogen phosphate buffer (pH 2.5±0.05) and acetonitrile in the 50:50 %v/v with UV detection of 254 nm respectively. The peak of rivaroxaban was distinguishable and quantifiable among the peaks of degradation products under tested stress conditions, making the present RP-HPLC method is stability indicating. The measure of rivaroxaban in dosage formulations was found to be in great understanding with label claim with respect to students-t (1.604) and F-test (2.384). The main asset of this recommended RP-HPLC method, i.e, it does not require cumbersome extraction procedure, internal standard, costly detector system, expertise operational personnel and found to be an alternative procedure to that of strategies detailed in the literature.

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