IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 18, Issue 12 Ser. I (December. 2025), PP 37-49
www.iosrjournals.org

Stability-Indicating Comprehensive Chromatographic Method Development For The Estimation Of Rivaroxaban-Related Substances In Bulk Materials And Pharmaceutical Formulations Dosages

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Abstract:
Aim: *This study aims to develop and validate a stability-indicating comprehensive, reliable, cost-effective, rapid, robust reverse-phase liquid chromatographic (RP-HPLC) method to quantify impurities related to Rivaroxaban in drug substances and pharmaceutical formulations dosages.*
Material and Method: *The method efficiently elutes all impurities with good resolution using a C18 with 3 µm particle size column. Simple mobile phase gradient program is used for separation of Rivaroxaban and its impurities at a flow rate of 1 mL/min, where Mobile Phase A and B are consists of diluted orthophosphoric acid and 100% acetonitrile respectively. A 5µL sample solution is injected through a YMC ODSA C18 column, and the eluted compounds are monitored using a DAD/VWD detector set to a wavelength of 250 nm. This straightforward analytical procedure was validated in accordance to ICH guidelines.*
Result: *The analytical optimized method validation demonstrated the method's sensitivity, selectivity, specificity, precision, accuracy, and linearity over concentration ranges of 0.08-0.60ppm for known and unknown Rivaroxaban impurities. Robustness tests were conducted with minor modifications to flow rate, column temperature, and wavelength, The robustness results observed within acceptable limits. The solution stability study proved stable up to 72 hours. Additionally, the method was evaluated under various forced stress conditions, revealing significant degradation under alkaline conditions.*
Conclusion: *The validation results indicate that this method is novel, straightforward, fast, cost-effective, sensitive, robust, rugged, and time-efficient. The forced degradation data confirm its capability as a stability-indicating method for its intended applications.*
Keywords: *Stability-indicating, Rivaroxaban, tablets, RP-HPLC, related substances, impurities.*

Date of Submission: 07-12-2025 Date of Acceptance: 17-12-2025

I. Introduction:

Rivaroxaban belongs to the anticoagulant drug family and is chemically identified as "5-chloro-N-(5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl) phenyl]. -1,3-oxazolidin-5-yl-m-thiophene-2-carbinoxamide" [1]. they are available in tablet form and is administered orally. Rivaroxaban, developed by Bayer under the brand name Xarelto, is an oxazolidinone derivative that inhibits free Factor Xa, which is part of the prothrombinase complex [2]. This highly selective, direct Factor Xa inhibitor works rapidly to interrupt blood clot formation, particularly preventing clotting during hip or knee replacement surgery [1-3]. Refer to Fig. 1 for the chemical structure of Rivaroxaban.

A review of the literature shows that limited methods are available for quantifying Rivaroxaban, either alone or in combination with other drugs [9-17]. Various techniques such as spectrophotometry [4], technique such as RP-HPLC[5-16], U-HPLC[17], HP-TLC[18, 19], and LC-MS[20-24] have been reported for quantifying Rivaroxaban in tablet form and human serum. Some methods are also available for analysing related impurities using RP-HPLC [25-26], However, the run time for impurity elution is excessively long.

The official USP-NF monograph for Rivaroxaban [27] includes a related substance analysis method using a gradient program with a run time of 40 minutes. The elution occurs on a C18 column (15 cm, 3 mm, 3.5 µm) at 60°C, using a buffer made from potassium dihydrogen phosphate and sodium hexane sulfonate. However, the preparation process of this buffer is time-consuming, and the ion-pairing reagent requires an extended period for column saturation. Based on current literature, there is a lack of a simple, rapid, economic, and time-efficient chromatographic method to separate and quantify of Rivaroxaban-related impurities in both drug substances and products.

This research focuses on developing and validating a fast, reliable, cost-effective, rapid, robust RP-HPLC method for the identifying and quantifying of Rivaroxaban-related impurities in bulk and pharmaceutical finish dosages, which is essential for ensuring product quality and safety.

The new method effectively separates Rivaroxaban-related impurities, including USP Impurities B, D, E, F, G, H, and in-house impurities such as Impurity C, Rivaroxaban open ring acid compound, and Rivaroxaban de-carbonyl compound, within 18 minutes with optimal peak resolution. This method uses a C18 YMC ODSA column (length 10 cm x dimeter 0.46 cm, particle size 3 µm). The mobole phase is employs a highly diluted 0.07% orthophosphoric acid (OPA) solution instead of an complex buffer system or ion-pairing reagent. The diluted OPA solution minimizes the risk of buffer deposition, thus extending the column's lifespan. This method is applicable to the analysis of APIs and tablet dosage forms.

This novel RP-HPLC method ensures accurate separation and quantification of Rivaroxaban-related impurities, making it highly effective for evaluating the quality of drug substances and pharmaceutical products.

This method is simple, rapid, cost-effective, accuracy, reproducibility, robustness and efficient to analysed shelf-life samples. this method represents a valuable tool for pharmaceutical analysis, contributing to the consistent quality of Rivaroxaban in both bulk and finished forms.

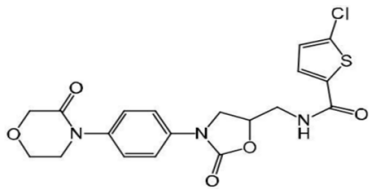

Figure 1: Rivaroxaban dug Structure

II. Materials And Method:

Chemicals, Reagents, Standards and Samples:

Rivaroxaban tablets were Purchased from a commercial store. Rivaroxaban standard, placebo sample and its Impurities standard were gifted by medley pharmaceutical Ltd, Andheri.

HPLC grade chemicals and reagents used in this study 85% pure Orthophosphoric acid, Acetonitrile, methanol, water are arranged by Chemcluse lifescience Pvt Ltd.

Instrumentation method and chromatographic condition:

Optimization of Chromatographic parameters for separation of Rivaroxaban and their impurities was achieved by using a Thermo Scientific Dionex Ultimate 3000 HPLC system with chrome Leon 7.2.10 ES software for data acquisition and data execution.

Optimized chromatographic method involves the RP-HPLC with DAD/VWD. The simple gradient elution program to separation was achieved at a flow rate of 1.0 ml/min (refer table 1), The column was employed as C18 YMC ODSA (dimension 10cm x 0.46cm, 3µm particle size) with column thermostat temperature set at 40°C. The sample were injected 5µL volume using an auto sampler with set temperature at 25°C. Detection were monitoring of eluted compounds using the DAD/VWD detector at wavelength 250nm. Rivaroxaban standard analysis was done by using optimized method and checked system suitability criteria to complies as per guideline refer table 2.

Table 1: Gradient Program:

Time (Min.)	0	13	16	18
Mobile Phase (MP)-A (%)	92	49	80	92
Mobile Phase (MP)-B (%)	8	51	20	8

Mobile phase solution:

Separately Mobile Phase-A and B, consisted of diluted orthophosphoric acid in proportion of 3.5 mL in 5-liter water and 100% acetonitrile.

Diluent and Blank solution:

The diluent was combination of MP-A: MP-B in a 40:60(v/v) ratio.

Rivaroxaban Standard stock Solution:

An accurately weighed and transferred 10mg of Rivaroxaban standard into a 250ml volumetric flask, added approximately 150 mL of diluent to dissolve the standard, followed by dilution to the mark with diluent. The standard solution was mixed thoroughly. (final concentration 40ppm)

Rivaroxaban Impurity Stock Solution:

A 2mg amount of each Rivaroxaban Impurities (USP monograph Impurities-B, D, E, F, G, H, J, and inhouse impurities C, Open ring acid and De-carbonyl) standards individually weighed and transferred into a respective 50ml volumetric flasks, added approximately 30 mL of diluent to dissolve the standard, followed by dilution to the mark with diluent. The standard solution was mixed thoroughly. (final individual impurities conc. 40ppm).

Final Standard Preparation:

A prepared the final standard mixture, 1 mL each of the Rivaroxaban standard stock solution and the individual impurity stock solutions were transferred into a 100 mL volumetric flask. The solution mixture was diluted to the mark with diluent. The mixture of standards solution was mixed thoroughly. (final Conc. Rivaroxaban and their impurities is 0.4µg/ml)

Rivaroxaban Tablet solution:

An accurately weighed and transferred portion of triturated Rivaroxaban tablets corresponding to 20mg Rivaroxaban in a 100 mL volumetric flask. Added approximately 75 mL of diluent and sonicated the mixture upto 15 minutes with occasional shaking. The solution mixture was diluted to the mark with diluent. and The sample solution was mixed thoroughly. Filtered the sample solution by using 0.45micron nylon syringe filter. (Conc.: 200ppm)

Placebo of Rivaroxaban solution:

An accurately weighted and transferred placebo sample corresponding to 20mg Rivaroxaban in a 100 mL volumetric flask. Added approximately 75 mL of diluent and sonicated the mixture upto 15 minutes with occasional shaking. The solution mixture was diluted to the mark with diluent. and The sample solution was mixed thoroughly. Filtered the sample solution by using 0.45micron nylon syringe filter.

III. Method Validation:

Method Validation:

Following the ICH Guidelines [29], the validation process aims to ensure its suitability for the intended purpose. The analytical procedure was validated in line with ICH standards to evaluate the method's performance, based on analytical parameters that meet the predefined acceptance criteria for the method's application. The analysis was carried out using the optimal and chromatographic procedure.

Specificity: The specificity test confirmed that chromatographic peaks must be clearly distinguishable, with no interference from placebo, diluent and impurities. Impurities should not co-elute with the Rivaroxaban peak or other impurity peaks. The peak purity for both impurities and active analytes must not be less than 950.

Limit of Detection (LOD) and Limit of Quantification (LOQ): The LoD is denoted that the smallest detectable amount of an impurity, although quantification is not required. The LoQ is the smallest quantifiable amount of impurity with acceptable recovery and reproducibility.

Linearity: Linearity of the analytical method demonstrates a direct relationship between analyte concentration and detector response over a specified range. The Linearity was assessed through the regression, slope, percentage y-intercept, and correlation coefficient (r^2), evaluated on five concentration levels from LoQ (0.08ppm) to 150% (0.6ppm) for both known and unknown impurities. The relative response factor (RRF) was calculated based on establish linearity.

Accuracy: Accuracy was assessed using spiked known analyte concentrations in the sample. For known impurities, accuracy was demonstrated through impurity spiking tests. Triplicate samples were prepared and analysed at different levels, including LoQ, 100%, and 200% spiked sample solution. Recovery of each level and the mean recovery for the known and unknown impurities were calculated.

Precision: Precision, or repeatability, determine the consistency of results across multiple analyses of the numerous samples were prepared homogeneous under optimal conditions. Precision testing was carried out on six individual impurity spiking test solutions at known impurity concentrations.

Robustness: The method's robustness was assessed by intentionally varying critical chromatographic parameters, such as flow rate, column temperature, and detection wavelength., to assess their impact on the analysis of Rivaroxaban and its impurities. These variations of optimized condition were planned to simulate potential of

minor change that might happen during regular laboratory testing.

Filter Compatibility: Filter compatibility studies were evaluated to assess analyte loss due to adsorption by the membrane filter during filtration. The study compared results obtained from centrifuged spiked test solutions with spiked test solutions passed through various membrane syringe filters. The difference between the centrifuge and filtered sample results was calculated.

Solution Stability: Solution Stability studies on spiked sample solutions stored at room temperature over time confirmed that the Rivaroxaban test solution remained stable, with no significant changes in analyte concentrations.

Force degradation study: A forced degradation (FD) study is a crucial method used to assess and confirm the specificity of an analytical procedure. It helps identify potential degradation products, contributing to the understanding of degradation pathways and the inherent stability of the molecules involved. In FD studies, blank, placebo, and sample solutions are intentionally subjected to degradation under controlled stress conditions, with the mass balance calculated to monitor the process. The following stress conditions were applied to optimize degradation behaviour:

Acid degradation:

An accurately weighed and transferred portion of triturated Rivaroxaban tablets corresponding to 20mg Rivaroxaban in a 100 mL volumetric flask. Added approximately 50 mL of diluent and sonicated the mixture upto 15 minutes with occasional shaking. Then added 5mL 1N diluted HCl (hydrochloric acid) and mixed. left flask at room temperature for 24 hours. After the 24-hour period, added 1N NaoH (sodium hydroxide) to neutralized sample solution. The solution mixture was diluted to the mark with diluent, mixture was well-mixed. Filtered the sample solution by using 0.45micron nylon syringe filter. (Conc.: 200ppm)

Base degradation:

An accurately weighed and transferred portion of triturated Rivaroxaban tablets corresponding to 20mg Rivaroxaban in a 100 mL volumetric flask. Added approximately 50 mL of diluent and sonicated the mixture upto 15 minutes with occasional shaking. Then added 2.5mL 1N diluted NaoH (sodium hydroxide) and mixed. left flask at room temperature for 24 hours. After the 24-hour period, added 1N diluted HCl (hydrochloric acid) to neutralized sample solution. The solution mixture was diluted to the mark with diluent, mixture was well-mixed. Filtered the sample solution by using 0.45micron nylon syringe filter. (Conc.: 200ppm)

Oxidation degradation:

An accurately weighed and transferred portion of triturated Rivaroxaban tablets corresponding to 20mg Rivaroxaban in a 100 mL volumetric flask. Added approximately 50 mL of diluent and sonicated the mixture upto 15 minutes with occasional shaking. Then added 5 mL of 30% H_2O_2 (hydrogen peroxide) and mixed. left flask at room temperature for 24 hours. After the 24-hour period, solution mixture was diluted to the mark with diluent, mixture was well-mixed. Filtered the sample solution by using 0.45micron nylon syringe filter. (Conc.: 200ppm)

Thermal degradation:

The Rivaroxaban tablets was placed in a petri dish and exposed to a temperature of 105°C for 24 hours in a vacuum oven. After the exposure period, the sample was prepared following the procedure described in section 2.8. placebo were similar treated and prepared sample in the same manner.

Photolytic Degradation:

The Rivaroxaban tablets was placed in a petri dish and exposed at 1.2million lux-hrs. and 200W Hrs./m2 lights in photolytic compartment. After the exposure period, the sample was prepared following the procedure described in section 2.8. placebo were similar treated and prepared sample in the same manner.

IV. Results And Discusion

Method development:

The goal was to obtained a simple a simple comprehensive, cost-effective, reliable, and efficient quantitative analytical method for the rivaroxaban-related substances using a YMC pack C18 column. During the development of the RP-HPLC method, various mobile phase combinations of buffer solutions, and different column dimensions and stationary phases were to use for optimize the separation of Rivaroxaban impurities within an acceptable run time. After several trials, all analytes were successfully separated within a short run time

at simple gradient chromatographic procedure. Details of the optimized method are provided in Section 2.2.

Method validation:

The experiment of the RP-HPLC method was validated followed by ICH guidelines to demonstrate that the method is appropriate for its application. A newly developed comprehensive RP- HPLC was validated for quantifying Rivaroxaban-related impurities in both Rivaroxaban-related impurities in APIs and finished products. The validation experiment covered parameters such as specificity, accuracy, precision (repeatability and ruggedness), linearity, robustness, and forced degradation. The results for each parameter are summarized below.

Specificity

The method demonstrated well separation of all analytes, with no interfering peaks observed at the elution time of the Rivaroxaban and their impurities analyte. Peak purity was within acceptable limits. For specificity results, refer to Figures 2a, 2b, 2c, 2d and Table 2.

Table 2: System Suitability - Mean Results
Limits: %RSD ≤ 5%, Asymmetry ≤ 2, Theoretical plates ≥ 2000, Peak purity ≥950.

Analyte	% RSD of area (n=6)	RRT (n=6)	Mean Asymmetry (n=6)	Mean Theoretical Plate (n=6)	Peak Purity	
					Standard	Sample
Rivaroxaban	1.15	1.00	1.13	76047	995	1000
Imp B	1.02	0.45	1.02	18408	991	992
Imp D	1.16	0.64	1.08	67137	997	998
Imp E	1.22	0.76	1.09	55028	995	997
Open Ring	2.45	0.78	1.08	72636	986	987
Decarbonyl	2.21	0.84	1.07	81085	991	992
Imp C	0.55	0.87	1.11	68247	995	990
Imp G	1.63	0.94	1.04	66139	992	997
Imp F	1.36	0.97	1.26	39750	984	982
Imp H	1.61	1.19	1.13	78113	991	991
Imp J	1.42	1.34	1.31	66262	953	952

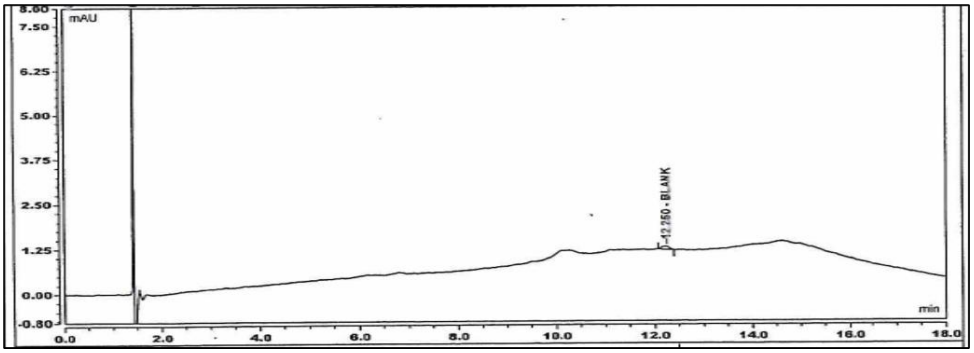

Fig. 2a Observed Chromatogram-blank

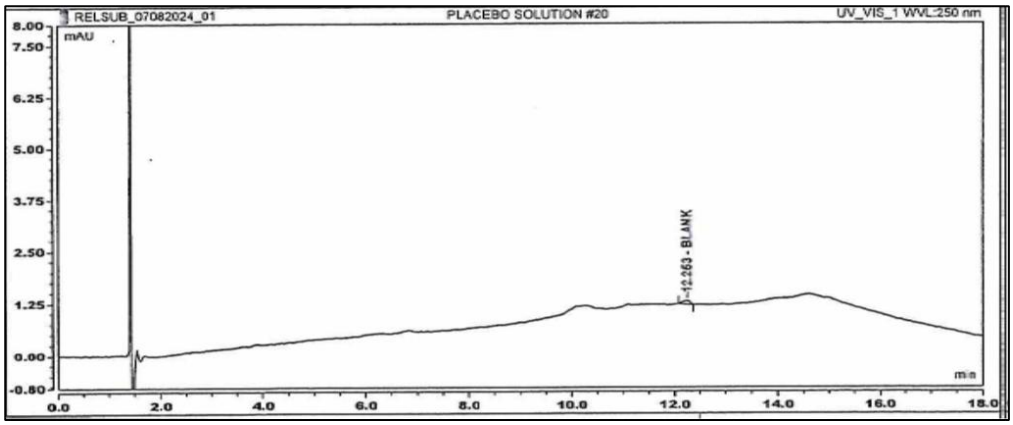

Fig.2b Observed Chromatogram–Placebo

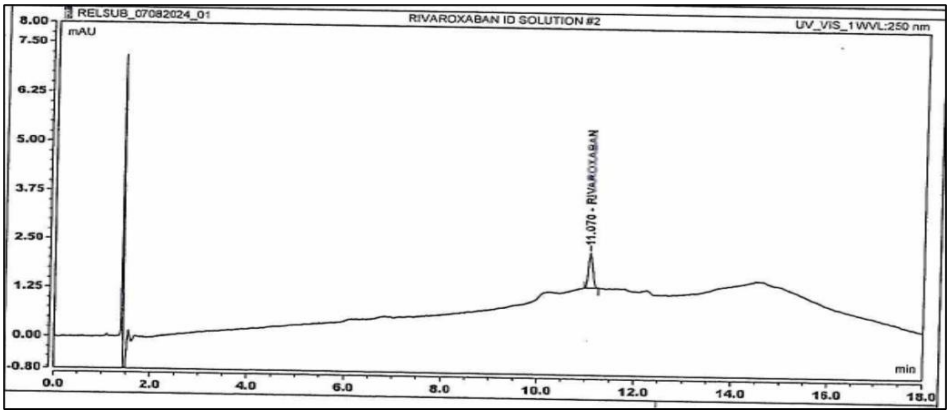

Fig.2c observed Chromatogram- Standard

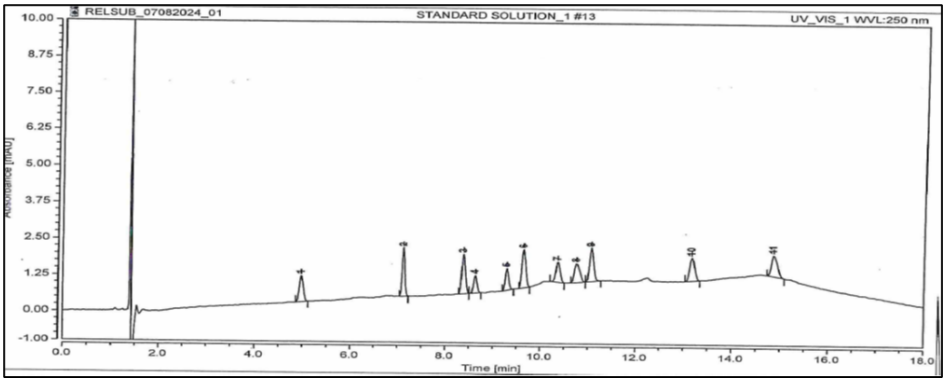

Fig.2c observed Chromatogram-Spiked Standard

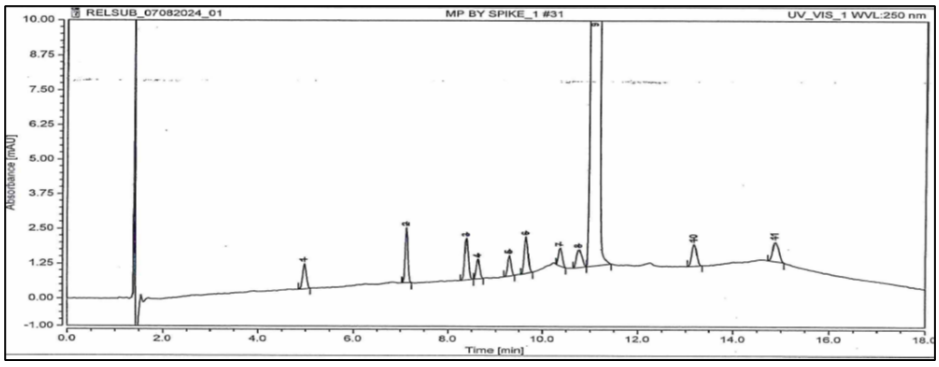

Fig.2d Observed Chromatogram-spiked test sample

LOD and LOQ solution:

In the sensitivity parameter analysis, the signal-to-noise (S/N) ratios for LOD and LOQ and %RSD for the precision of LOQ solution (n=6) for all analytes, met the acceptance criteria. This indicates that the method is highly sensitive. Refer to Figures 3a and 3b for representative chromatograms, and Table 3 for detailed LOD-LOQ results.

Table 3: Results for LOD & LOQ - Standard Precision and S/N Ratio
Limits: S/N ratio for LOD ≥ 3:1, S/N ratio for LOQ ≥ 10:1, LOQ area precision %RSD ≤ 10%.

Analyte	LOD (µm/mL)	LOD Area (n=3)	LOD S/N	LOD % RSD (n=3)	LOQ (µm/mL)	LOQ Area (n=6)	LOQ S/N	LOQ % RSD (n=6)
Rivaroxaban	0.04	0.655	4.2	6.57	0.08	1.308	21.2	1.53
Imp B	0.04	0.672	4.7	4.90	0.08	1.171	19.7	3.68
Imp D	0.04	0.618	6.1	5.58	0.08	1.273	29.0	2.14
Imp E	0.04	0.809	5.5	5.66	0.08	1.484	24.8	1.84
Open Ring	0.04	0.247	2.3	8.79	0.08	0.576	11.2	6.04
Decarbonyl	0.04	0.354	3.2	9.38	0.08	0.742	13.5	3.55
Imp C	0.04	0.811	5.7	2.29	0.08	1.474	24.8	3.33

Imp G	0.04	0.519	2.7	3.08	0.08	1.175	16.7	5.05
Imp F	0.04	0.493	2.6	8.01	0.08	1.058	12.0	5.05
Imp H	0.04	0.601	3.1	6.53	0.08	1.120	15.1	7.28
Imp J	0.04	0.611	3.4	6.90	0.08	1.204	14.6	3.53

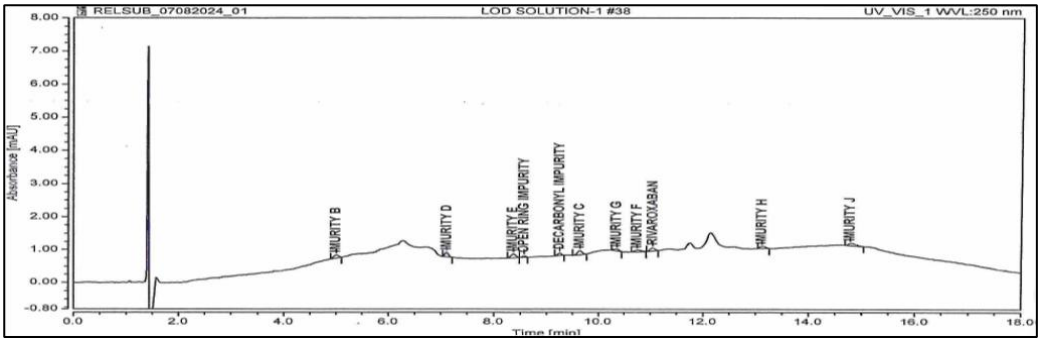

Fig.3a Observed Chromatogram- LOD

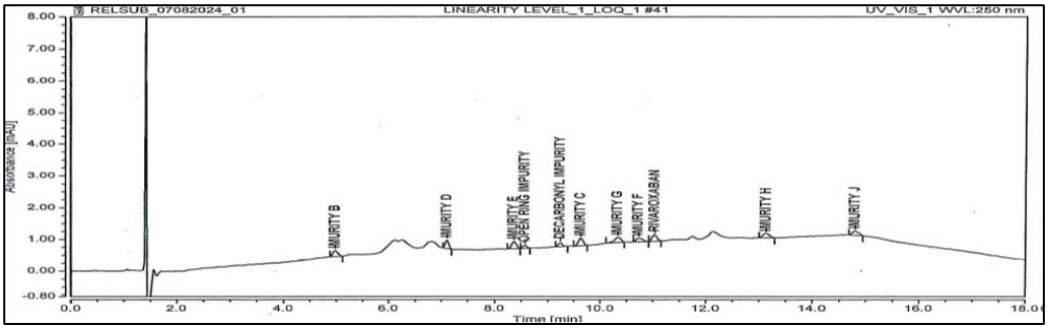

Fig.3b Observed Chromatogram- LOQ

Linearity

The method showed linearity for Rivaroxaban in the concentration range of 0.05 ppm (LOQ) to 2.0 ppm (200%) and for Rivaroxaban impurities from 0.125 ppm (LOQ) to 5.0 ppm (200%). The correlation coefficient (r^2) for Rivaroxaban and its impurities was within acceptable limits. Detailed linearity and range results can be found in Table 4 and for linearity plot refer Figures 4a to 4g.

Table 4: Linearity Results

Limits: $r^2 \geq 0.98$, % Y-Intercept ≤ ± 25%, RRF (Relative Response Factor), and slope information

Analyte	Linearity range (µg/mL)	R- Square value	Slop	% Y Intercept	RRF
Rivaroxaban	0.08-0.60	0.9996	17.045	-0.291	1.00
Imp B	0.08-0.60	0.9993	12.697	0.966	1.34
Imp D	0.08-0.60	0.9997	17.256	-0.789	0.99
Imp E	0.08-0.60	0.9995	18.678	-0.769	0.91
Open Ring	0.08-0.60	0.9996	7.436	-1.308	2.29
Decarbonyl	0.08-0.60	0.9999	9.242	0.205	1.84
Imp C	0.08-0.60	0.9998	18.549	-0.667	0.92
Imp G	0.08-0.60	0.9989	10.671	4.125	1.60
Imp F	0.08-0.60	0.9991	13.913	-3.175	1.23
Imp H	0.08-0.60	0.9993	14.278	-0.120	1.19
Imp J	0.08-0.60	0.9994	15.979	-1.002	1.07

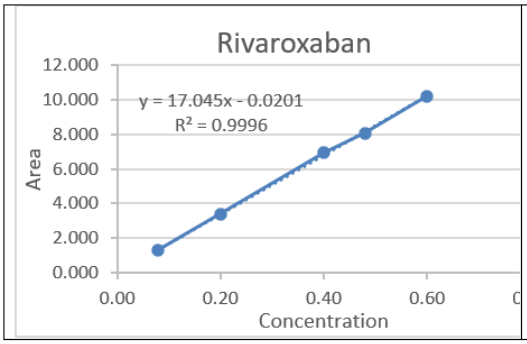

Fig.4a Linearity Graph-Rivaroxaban

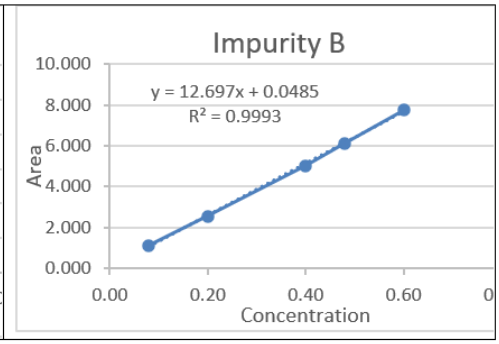

Fig.4b Linearity Graph-Impurity -B

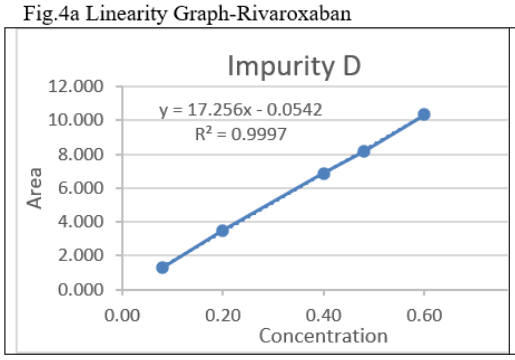

Fig.4c Linearity Graph-Impurity -D

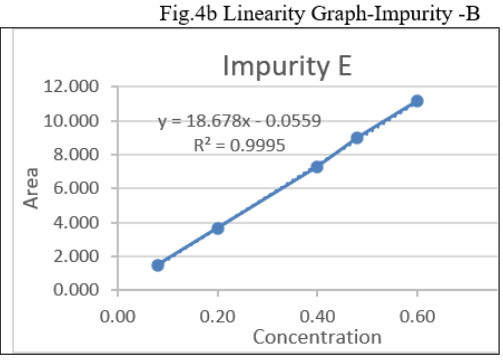

Fig.4d Linearity Graph-Impurity -E

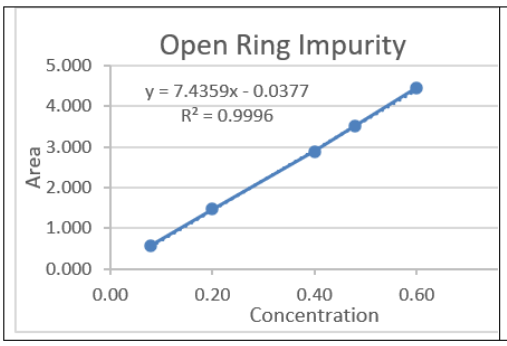

Fig.4e Linearity Graph-Open Ring

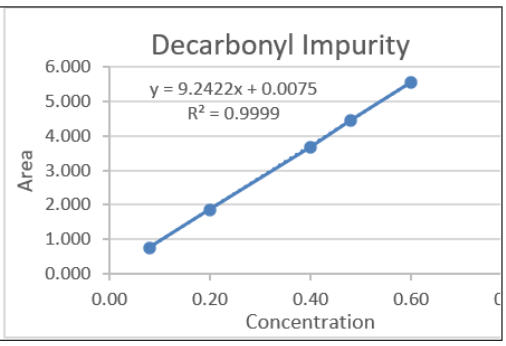

Fig.4f Linearity Graph-Decarbonyl

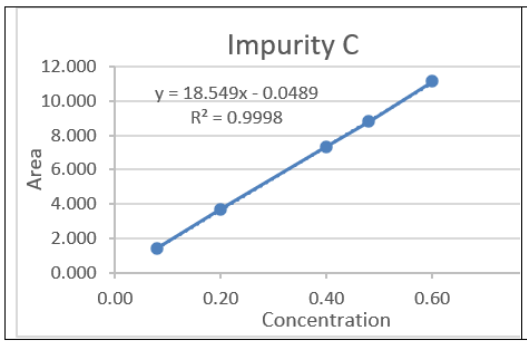

Fig.4e Linearity Graph-Impurity C

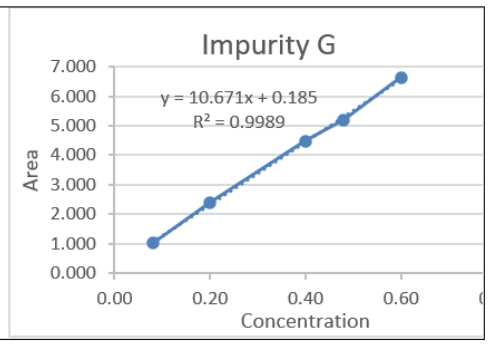

Fig.4f Linearity Graph- Impurity G

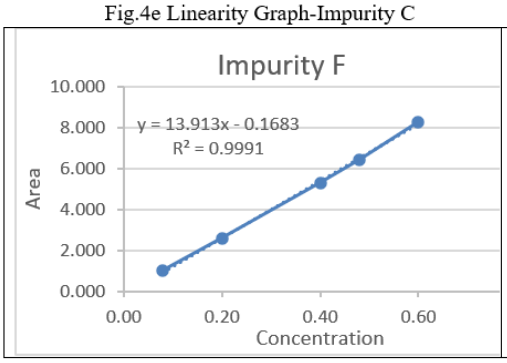

Fig.4e Linearity Graph-Impurity F

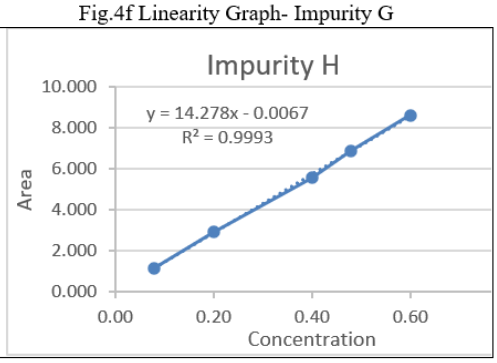

Fig.4f Linearity Graph- Impurity H

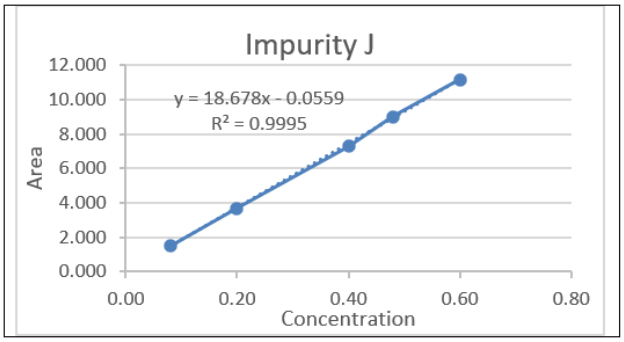

Fig.4g Linearity Graph-Impurity -J

Accuracy

The % recovery and %RSD of Rivaroxaban known and unknown impurities for all level observed within the specified acceptance limit that confirms the method's ability to accurately recover the analyte from the 0.125µg/mL (LOQ) to 5µg/mL (200%) and It is compatible of accurately quantify eluting impurities from the LOQ to 200% range w.r.t. Specification values. Refer to Table 5 for detailed accuracy results.

Table 5: Accuracy Results (in Percentage)
Limits: Accuracy of each level (n=3) must be within 80-120%, %RSD (n=3) ≤ 10%.

% Recovery Level		Riva.	Imp B	Imp D	Imp E	Open Ring	Decar-bonyl	Imp C	Imp G	Imp F	Imp H	Imp J
LOQ Level -1	SPL-1	96.7	104.0	89.5	105.6	97.9	100.8	101.7	92.8	92.8	102.0	101.5
	SPL-2	99.4	105.4	97.1	97.9	95.0	98.4	99.6	105.7	88.2	112.5	99.0
	SPL-3	102.7	103.0	95.7	102.1	101.3	109.9	100.4	93.3	88.7	113.1	95.9
	Mean	99.6	104.1	94.1	101.9	98.1	103.0	100.6	97.3	89.9	109.2	98.8
	%RSD	3.0	1.2	4.3	3.8	3.2	5.9	1.0	7.5	2.8	5.7	2.8
100 % Level -2	SPL-1	98.2	104.4	97.8	101.0	97.4	101.9	101.7	90.9	97.7	103.1	100.0
	SPL-2	98.6	104.7	97.8	98.6	93.1	101.2	101.4	92.3	99.4	97.3	99.8
	SPL-3	99.8	105.4	97.1	99.6	93.7	104.4	102.6	90.3	99.1	99.5	100.1
	Mean	98.9	104.8	97.6	99.7	94.8	102.5	101.9	91.1	98.7	100.0	100.0
	%RSD	0.8	0.5	0.4	1.2	2.5	1.6	0.6	1.1	0.9	2.9	0.2
200% Level -3	SPL-1	99.0	101.8	97.6	100.7	102.6	101.6	101.7	93.0	101.0	102.0	100.3
	SPL-2	99.3	104.1	97.6	101.0	104.2	103.0	101.8	95.0	99.1	100.7	101.2
	SPL-3	100.0	103.1	97.9	100.0	102.8	102.6	101.7	92.4	102.7	102.5	100.1
	Mean	99.4	103.0	97.7	100.6	103.2	102.4	101.7	93.5	100.9	101.7	100.5
	%RSD	0.5	1.1	0.2	0.5	0.9	0.7	0.1	1.5	1.8	0.9	0.6
Overall Mean % Accuracy (n=12)		99.3	104.0	96.5	100.7	98.7	102.6	101.4	94.0	96.5	103.6	99.8
Overall % RSD (n=12)		1.6	1.2	2.8	2.2	4.3	3.1	0.9	4.9	5.5	5.3	1.6

Precision

acceptance limits, confirming that the method is precise and rugged. The Precision and Intermediate precision was performed on individual prepared 100% level six spiked sample solution and calculated % RSD. For detailed results, see Table 6.

Table 6: Precision Results,
Limits: %Recovery for impurities should be 80-120%, and %RSD (n=6) ≤ 10%.

Analyte	Spiked Concentration (µg/mL)	Method Precision (n=6)		Intermediate Precision (n=6)		Overall mean	Overall % RSD (n=12)
		Mean	% RSD	Mean	% RSD		
Imp B	0.4	101.0	1.5	97.5	1.0	99.3	2.2
Imp D	0.4	99.0	1.2	98.8	0.7	98.9	1.0
Imp E	0.4	101.1	0.5	100.2	1.4	100.6	1.1
Open Ring	0.4	97.8	1.1	101.0	1.1	99.4	2.0
Decarbonyl	0.4	101.4	2.1	100.0	1.7	100.7	1.9
Imp C	0.4	101.4	0.4	99.5	1.1	100.5	1.3
Imp G	0.4	97.0	1.1	98.6	1.3	97.8	1.4
Imp F	0.4	99.1	1.9	100.8	0.9	100.0	1.7
Imp H	0.4	101.3	0.5	100.5	0.8	100.9	0.8
Imp J	0.4	102.1	1.0	103.6	0.9	102.8	1.2

Filter Study, Robustness and Solution stability study:

The % recovery of all impurities in spiked sample solution for the filter study, robustness study, and solution stability assessment were within the specified range, indicating that the Nylon and PVDF both filter is suitable for use, method is robust for small changes in optimal method parameters do not impact on results, and the Rivaroxaban test solution was found to be stable for up to 72 hours at ambient temperature. For detailed results, see Table 7 & 8.

Table 7: Filter Study, and Solution Stability - Accuracy Results, Limits: 80-120% recovery.

Analyte	Filter paper Study			Solution Stability	
	Centrifuge	0.45µ Nylone	0.45µ PVDF	Initial	72 hours
Imp B	99.0	98.2	97.3	102.3	101.6
Imp D	99.4	98.3	100.0	97.7	96.8
Imp E	98.3	97.1	98.0	101.2	100.8
Open Ring Imp	101.3	97.5	98.0	98.2	97.0
Decarbonyl Imp	99.1	96.4	97.3	101.9	100.8
Imp C	99.9	101.0	99.3	100.8	98.0
Imp G	101.6	99.1	99.0	97.5	97.4
Imp F	100.2	98.7	97.5	96.5	95.7
Imp H	101.7	100.2	101.0	101.6	99.8
Imp J	101.7	100.6	98.6	100.3	99.4

Table 8: Robustness Results- Accuracy Results, Limits: 80-120% recovery.

Analyte	Robustness Study					
	Column Temp. 38°C	Column Temp. 42°C	Flow 0.9 mL	Flow 1.1 mL	Wave length 245nm	Wave length 255nm
Imp B	97.1	103.1	101.2	98.7	99.2	97.2
Imp D	95.7	94.4	94.8	93.6	94.9	94.7
Imp E	96.7	101.6	101.9	95.7	100.9	98.9
Open Ring Imp	98.2	95.9	97.3	96.7	99.5	97.5
Decarbonyl Imp	98.4	100.9	97.3	99.6	97.3	103.9
Imp C	98.6	100.9	99.6	102.7	101.3	100.4
Imp G	101.0	100.4	98.8	104.1	97.9	97.9
Imp F	96.6	105.0	98.0	99.1	98.1	103.3
Imp H	97.6	100.7	103.5	97.5	99.5	96.3
Imp J	98.0	97.1	96.3	101.8	95.1	101.6

Force degradation study:

Samples intentionally treated at different stress conditions were evaluated using the established chromatographic method. Significant degradation was observed under alkaline conditions, while other stress conditions showed minimal degradation. The % degradation values were calculated based on standards, and the relative abundance of each sample concentration was calculated against the % degradation and % Rivaroxaban value. The peak purity was within acceptable limits across all stress samples. This study confirms the method is stability-indicating, ability to monitor and quantify degradant product in test sample while shelf-life studies. See Table 9 for detailed results.

Table 9: Forced Degradation Results

Limits: Mass balance should be within 95-105%.

Sample Stress	% Total Impurity	% Rivaroxaban	Mass Balance	Peak Purity
As such	0.19	100.0	NA	Pass
Photo_Degradation (Exposed to 1.2million lux Hrs. and 200W Hrs./m2 light in photolytic chamber)	0.16	99.8	100.0	Pass
Thermal_Degradation (Heated at 105°C in vacuum oven up to 24hr)	0.18	99.8	100.0	Pass
Acid_Degradation (1N HCl/5ml -24 hrs at room temperature)	3.15	98.5	101.7	Pass
Base_Degradation (1N NaOH/2.5ml -24hrs at room Temperature)	39.99	58.3	98.3	Pass
Oxidation_Degradation (2mL/30%H2O2/Room teperature/24Hours)	0.22	99.8	100.0	Pass

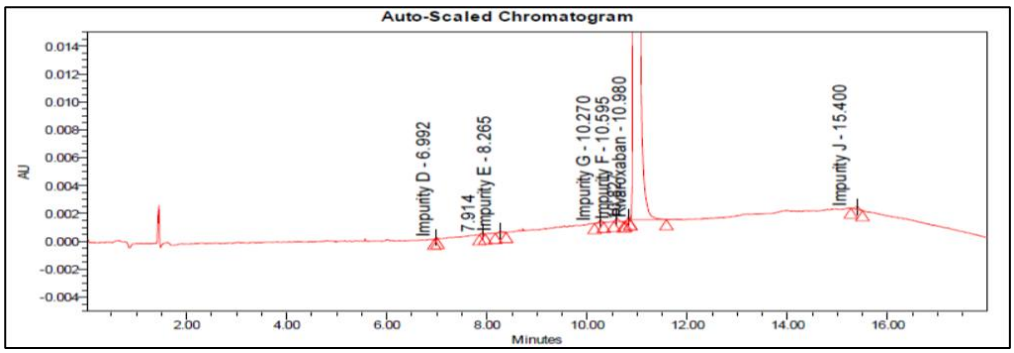

Fig 5a: Observed Chromatogram-Photo degradation

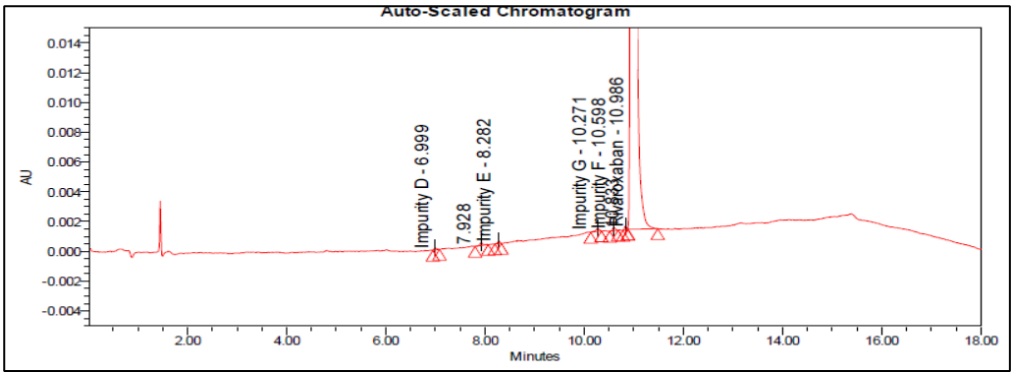

Fig 5b: Observed Chromatogram-Thermal degradation

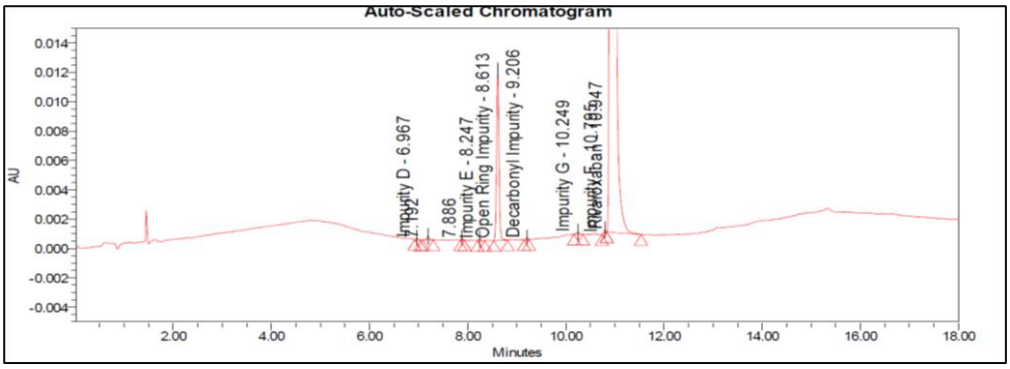

Fig 5c: Observed Chromatogram-Acid degradation

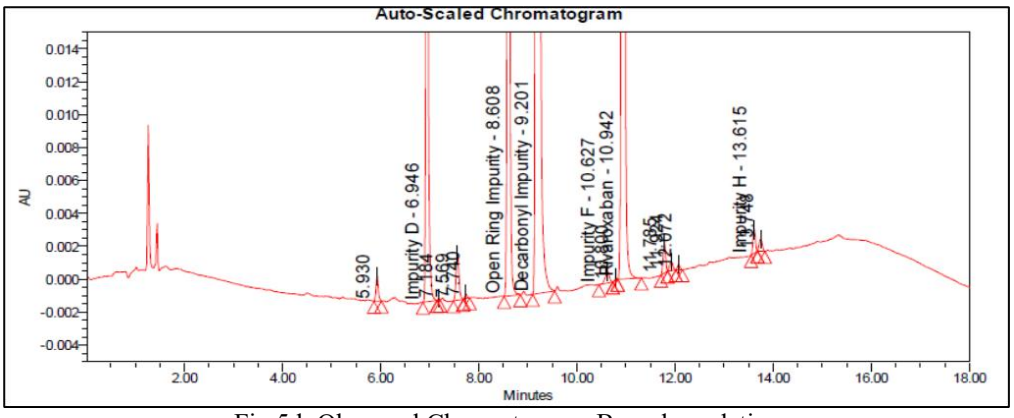

Fig 5d: Observed Chromatogram-Base degradation

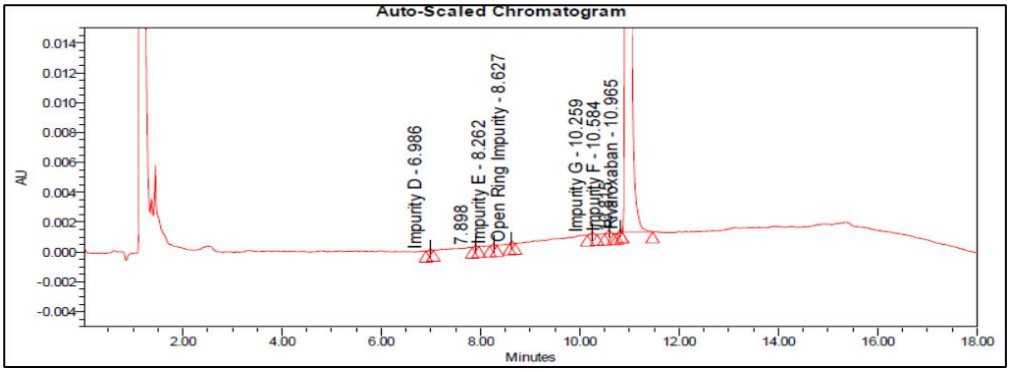

Fig 5e: Observed Chromatogram-Oxidation degradation

V. Conclusion:

A comprehensive RPHPLC method was successfully developed for the separation with good resolution and quantify the Rivaroxaban-related impurities on a C18 YMC Pack column with diluted orthophosperic acid solution. This stability-indicating reverse-phase chromatographic method is fast eluting simple and sensitive, providing reliable detection and quantification of Rivaroxaban impurities. The method is also linear, accurate, and robust, making it suitable for use the quantification of Rivaroxaban-related compounds in pharmaceutical products and APIs. The degradation studies further validate the specificity of this method for its intended use.

The new establish validated chromatographic method is highly recommend, permitting its effective application in formulation of new pharmaceutical products, as well as in the quality evaluation and stability testing of Rivaroxaban in commercial products, Its application plays a crucial role in ensuring the safety and quality of drug formulations.

Acknowledgments

The author expresses gratitude to Prof. Dr. Gayatri Barabde, Research guide, the Institute of Science, Dr. HBSU Mumbai, for her valuable guidance and Chemclues Life Science Laboratories for providing the necessary instrumentation and resources.

Abbreviations

- RP-HPLC: Reverse-Phase High-Performance Liquid Chromatography
- HPLC: High-Performance Liquid Chromatography
- UPLC: Ultra-Performance Liquid Chromatography
- UV: Ultraviolet
- HPTLC: High-Performance Thin-Layer Chromatography
- LCMS: Liquid Chromatography-Mass Spectrometry
- RSD: Relative Standard Deviation
- ICH: International Council for Harmonisation
- NMT: Not More Than
- NLT: Not Less Than

Declarations of interest statement

Conflict of interest

No conflict of interest to declare.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors

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