RP-HPLC Stability Indicating Method Development and Validation for Estimation of Rivaroxaban in Active Pharmaceuticals Ingredients

Sachin S. Garad[1*], Dhananjay V. Mane[2]

[1*,2]School of Science and Technology, Yashwantrao Chavan Maharashtra Open University, Nashik 422222, Maharashtra, INDIA

***Corresponding Author:** Sachin S. Garad

*School of Science and Technology, Yashwantrao Chavan Maharashtra Open University, Nashik 422 222, Maharashtra, INDIA Email: sachingarad.doc@gmail.com, Mobile No.: 9527129183

Abstract

Rivaroxaban is Anti-hypersensitive drug. In this study RP-HPLC stability indicating method was developed for Rivaroxaban in Active Pharmaceutical Ingredients. Hemochrom Intsil C18 (250mm X 4.6mm X 5µm) HPLC column was used at temperature 35°C.Gradient elution was achieved with 0.01M KH_2PO_4 Buffer and Acetonitrile-Water (20:80) v/v. The flow rate was 1.0 ml min^{-1} wavelength used was 240 nm. Run time was kept 30 min. The development method was validated according to ICH guideline and found Linear over the range 50 µg ml^{-1} to 500 µg ml^{-1}. The method was studied for force degradation i.e. stress studies parameter and method was capable for separation of degradation product and estimation of Rivaroxaban specifically, selectively, accurately and preciously.

Keywords: Rivaroxaban, HPLC, Force Degradation, Validation.

INTRODUCTION

Rivaroxaban is Anti-hypertensive drug used to prevent heart attack, Rivaroxaban Fig. 1, IUPAC name is (S)-5-chloro-N-{2-oxo-3-[4-(3-oxomorpholin-4-yl) phenyl]-1,3 oxazolidin-5-yl-methyl} thiophen-carboxamide.

Rivaroxaban sold under the brand name Xarel-to. Rivaroxaban is an anticoagulant medicati- on (blood thinner) used to treat and prevent blood clots. Specifically it is used to treat deep vein thrombosis and pulmonary emboli and prevent blood clots in atrial fibrillation and following hip or knee surgery. Rivaroxaban also used as veterinary drugs as blood clothing agent in Animals like Dogs and Cats. Rivaroxaban was initially developed by Bayer. In the United States, it is marketed by Janssen Pharmaceuticals (a part of Johnson & Johnson). It was the first available direct factor Xa inhibitor which is taken by mouth. (1)

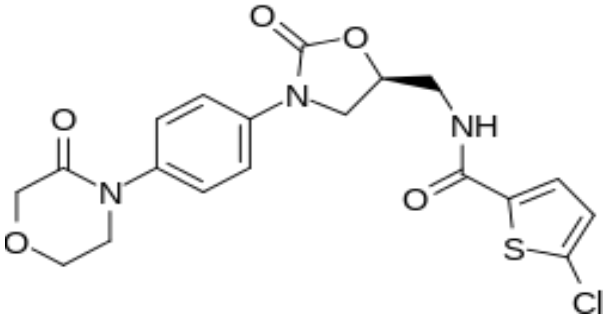


Fig.1 Structure of Rivaroxaban

Various regulatory Agencies including International Conference on Harmonization (ICH), US Food and Drug Administration (FDA), European Directorate for the Quality of Medicines etc. are accentuating on the estimation of drugs and whether method is capable of stability indicating or not. Very few methods available in the literature for the estimation of Rivaroxaban in Active pharmaceuticals ingredients with force degradation data. Few methods were reported for the determination of Rivaroxaban in pharmaceutical dosage forms and Active pharmaceuticals using HPLC (2-7). But detailed stability indicating Assay method and stress study not available yet and it's very important for Drug master file (DMF) submission to carried out this study as per regulatory requirement so present aim is to develop and validate the stability indicating Analytical method for Rivaroxaban estimation.

EXPERIMENITAL

Material and Methods:

Rivaroxaban working standard having purity ≥ 99% was purchased from Sigma Aldrich Ltd, Mumbai. Potassium dihydrogen orthophosphate, Potassium hydroxide, Acetonitrile, Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide (3O%) procured from Merck Ltd. Mumbai.

Instruments and Chromatographic Conditions:

The chromatographic analysis was performed using Waters LC system equipped with PDA detector (Waters, USA). HPLC Column used was Hemochrom Intsil C18 (250mm X 4.6mm X 5µm) software used for analysis was Empower 3.

General Procedure:

The chromatographic separation was achieved at column temperature of 35°C. The mobile phase A was 0.01M potassium dihydrogen phosphate buffer and the mobile phase B was Acetonitrile: Water (20:80), and the gradient program of the mobile phase is shown in Table 1. The mobile phase was filtered through 0.45 µm filter, and degassed by sonicater before uses. The flow rate of the mobile phase was 1 mL/min. The injection volume was 10µL, and the detection was performed at 240 nm.

Table No. 1: LC Gradient time program.

Time (Min)	% Mobile Phase A	% Mobile phase B
0.0	70	30
20.0	20	80
20.1	10	90
25.0	10	90
25.1	70	30
30.0	70	30

Preparation of standard solution:

Weigh accurately about 50.0 mg of Rivaroxaban working standard into a 50 mL volumetric flask, add about 30 mL of diluent sonicate to dissolve and make up to the mark with diluent and mix well. Pipette out 5.0 mL of the above standard solution into a 50 mL of volumetric flask and make up to the mark with diluent and mix well.

Preparation of Sample Solution:

Weigh accurately about 50.0 mg of Rivaroxaban sample into a 50 mL volumetric flask, add about 50 mL of diluent, sonicate to dissolve and make up to the mark with diluent and mix well. Pipette out 5.0 mL of the above standard solution into a 50 mL of volumetric flask and make up to the mark with diluent and mix well.

Development Detection Method:

Reverse phase High performance liquid chromatography (RP-HPLC) method is used for estimation of Rivaroxaban and force degradation study please refer Typical HPLC chromatogram for Rivaroxaban in fig.2 In which first developed RP-HPLC method for Assay on HPLC and then validated it as per ICH guideline. In validation force degradation study also studied in specificity parameter with the help of HPLC.

Fig.2: Typical HPLC chromatogram of Rivaroxaban

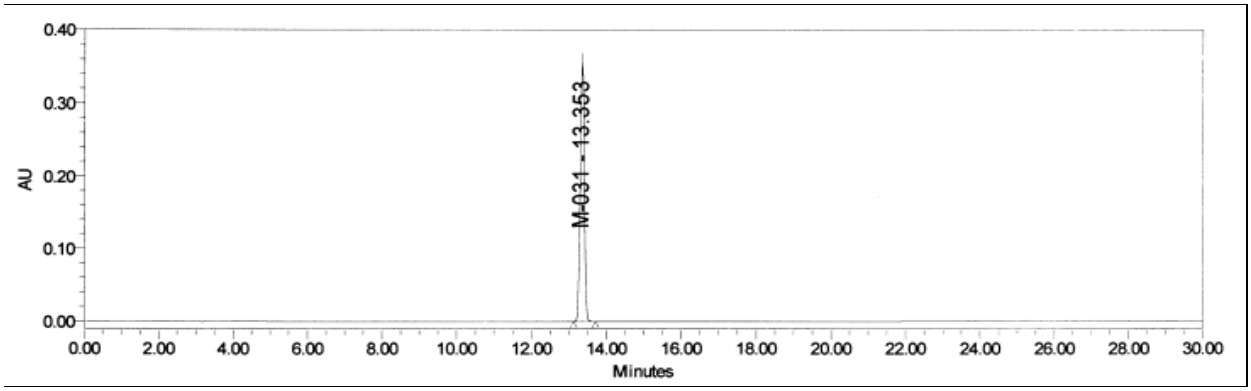
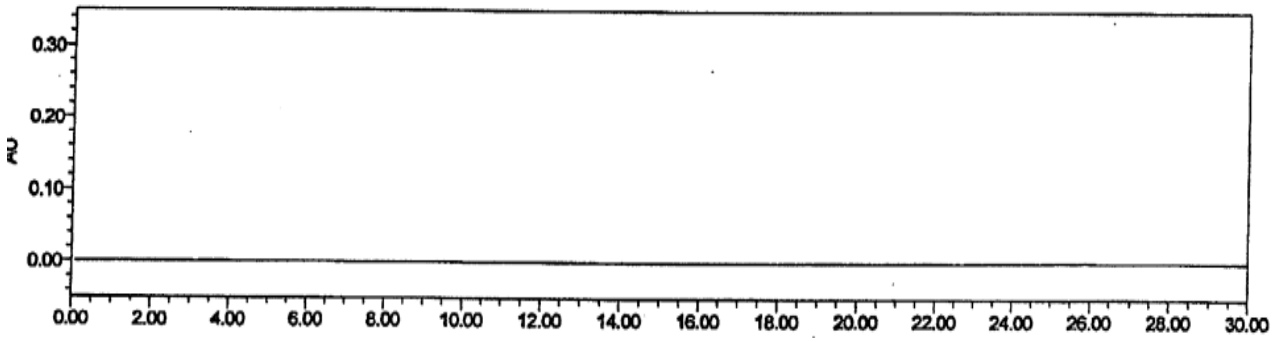


Fig. 3: Blank HPLC Graph



RESULTS AND DISCUSSION

Forced degradation studies provide the approach toward analyse the stability of drug samples in pharmaceutical industries. Outcome of this study was, we are known that wetter our method is capable or not for determining degradation product, Determination of innate stability of the drug molecule, Acceptance criteria for force degradation as per ICH and regulatory guideline The net degradation should be between 1-20%, at least 1% degradation should be achieved, if not justified. Purity angle should be less than purity threshold for Rivaroxaban. The peaks should not have any flag in purity results table (For waters empower software).

Prepared standard solution, sample and blank as per methodology. Performed Acid hydrolysis stress study, Base hydrolysis stress study, Peroxide oxidation stress study, Water degradation stress study,

Photolytic stress study (1.2 million lux hours and 200 watts/ square meter), Heat stress study (60°C/7 days) and Humidity stress study (25°C/90% ±5RH/7 days). Prepared samples, blanks and inject into HPLC system (PDA detector). No interference was observed for degradation products, purity angle is less than purity threshold for Rivaroxaban peak and there are no purity flags. This indicates that the method is specific for the analysis. The results are summarized in Table No-3.

Table No. 2: Typical HPLC Sequence for Force degradation study (Stress study):

Sr. No.	Sample Name	No. of Injection
1	Blank	1
2	Standard	6
3	As is sample	1
4	Acid degradation sample	1
5	Base degradation sample	1
6	Oxidation degradation sample	1
7	Water degradation sample	1
8	Photolytic degradation sample	1
9	Bracketing Standard solution-1	1
10	UV degradation sample	1
11	Heat stress study sample	1
12	Humidity stress study sample	1
13	All respective Blank	1 (Each)
14	Bracketing Standard solution-2	1

Table No. 3: Result Obtained for Force degradation study (Stress study):

Sample Name	% Assay	% Degradation	Purity Angle	Purity Threshold	Purity flag
As is sample	100.1	NA	0.051	0.244	No
Base sample (l N NaOH /5mL/R.T/20 min)	90.2	9.9	0.047	0.241	No
Acid sample (l N HCI/ 5 mL/60°C/2 Hrs.)	94.2	5.9	0.048	0.242	No
H_2O_2 sample (3O% H_2O_2 / 5 mL/ 60°C/ l2 Hrs.)	98.5	1.6	0.054	0.244	No
Water sample (Water /5 mL/60°C /2 Hrs.)	99.8	0.3	0.049	0.244	No
As is sample	100.3	NA	0.056	0.249	No
Photolytic sample (1.2 million lux hours)	99.9	0.4	0.062	0.249	No
UV sample (200 watts/square meter)	99.9	0.4	0.069	0.251	No

Analytical method Validation:

Analytical method validation (8) studied for development method for Specificity, Precision, Linearity and Range, Accuracy by recovery, Robustness and solution stability.

Above method is validated as per ICH guideline Q2 R1 (9).

1.0 Specificity & System Suitability:

Ability to assess unequivocally the analyte in the presence of components which may be expected to be present, such as impurities, degradation products, and matrix components. It is a measure of the degree of interference from such things as other active ingredients, excipients, impurities, and degradation products, ensuring that a peak response is due to an analyte only. No any elution observed at RT of principle peak (Shown in Figure 2) so specificity parameter passed. There are 7 potential impurities which are intentionally spiked to show no interference of these impurity at Rivaroxaban peak as shown in fig 4 and Name of these potential impurities are shown in Table 4A.

Fig no 4: Rivaroxaban Typical chromatogram spiked with potential impurities

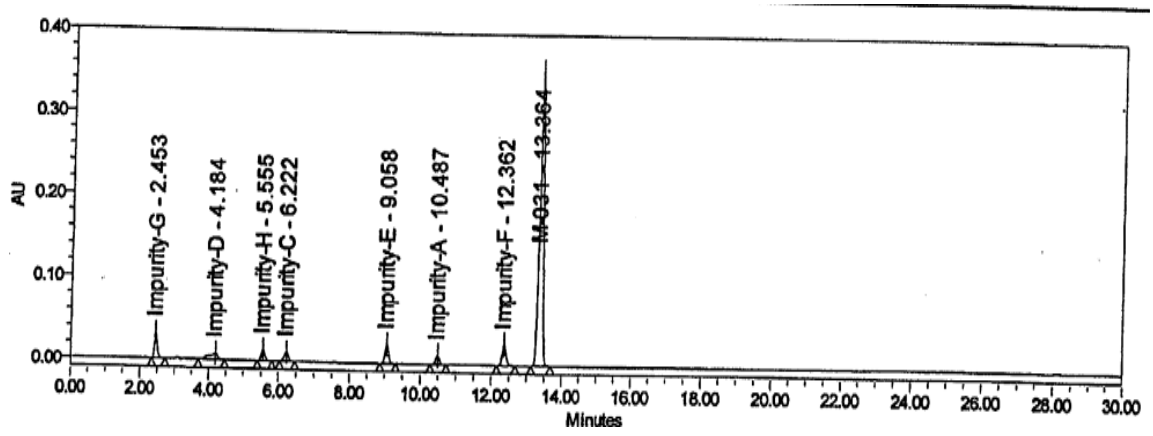


Table No. 4: Result Obtained for Force degradation study (Stress study):

Sr. No	Parameter	Acceptance Criteria	Observation's
1	Tailing Factor	Not more than 0.5	0.1
2	Theoretical Plates	Not less than 5000	72403
3	% RSD	Not more than 2	0.1

Table No. 4 A: List of potential impurities in Rivaroxaban

Sr.No.	Name of Imp.	Structure	Name in Inhouse

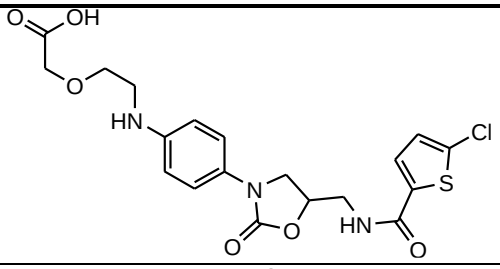
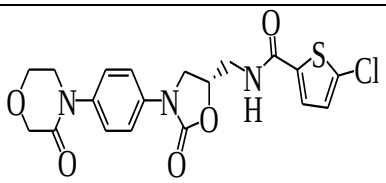
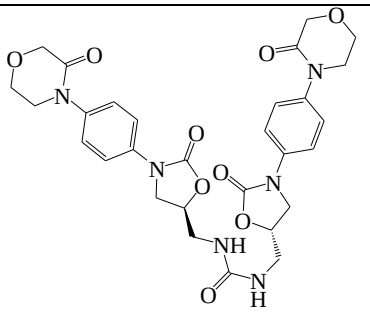
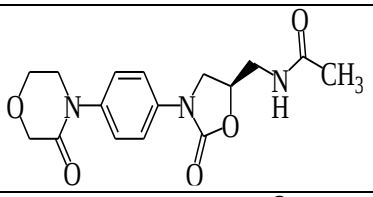
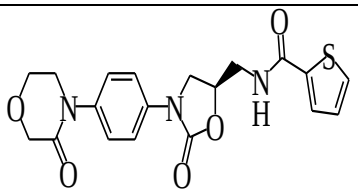
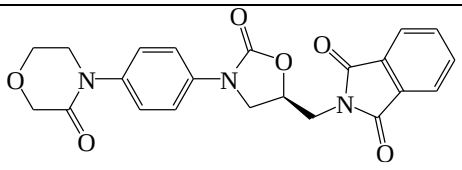
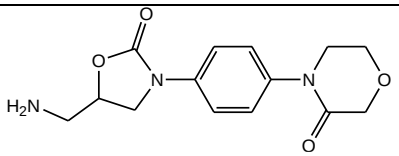
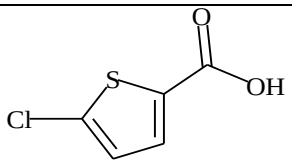
1	{2-[4-(5-{[(5-Chloro-thiophene-2-carbonyl)-amino]-methyl}-2-oxo-Oxazolidin-3-yl)-phenylamino]-ethoxy}-acetic acid		Impurity A
2	5-Chloro-thiophene-2-carboxylic acid {2-oxo-3-[4-(3-oxo-morpholin-4-yl)-phenyl]-oxazolidin-5-ylmethyl}-amide		Impurity B (Chiral)
3	1,3-Bis-{2-oxo-3-[4-(3-oxo-morpholin-4-yl)-phenyl]-oxazolidin-5-ylmethyl}-urea		Impurity C
4	N-{2-Oxo-3-[4-(3-oxo-morpholin-4-yl)-phenyl]-oxazolidin-5-ylmethyl}-acetamide		Impurity D
5	Thiophene-2-carboxylic acid {2-oxo-3-[4-(3-oxo-morpholin-4-yl)-phenyl]-oxazolidin 5-ylmethyl}-amide		Impurity E
6	2-{2-Oxo-3-[4-(3-oxo-morpholin-4-yl)-phenyl]-oxazolidin-5-ylmethyl}-isoindole-1,3-dione		Impurity F
7	4-[4-(5-Aminomethyl-2-oxo-oxazolidin-3-yl)-phenyl]-morpholin-3-one		Impurity G
8	5-Chlorothiophene-2-carboxylic acid		Impurity H

Fig. No. 5 : % RSD for standard Solution:

	SampleName	Name	Vial	Injection	RT	Area	USP Tailing	USP Plate Count	Int Type	Result Id	Result #
1	Standard Solution	M-031	42	1	13.353	2651139	1.0	72403	BB	3346	1
2	Standard Solution	M-031	42	2	13.370	2652443	1.0	72654	BB	3350	1
3	Standard Solution	M-031	42	3	13.351	2653942	1.0	72718	BB	3365	1
4	Standard Solution	M-031	42	4	13.363	2655622	1.0	72785	BB	3349	1
5	Standard Solution	M-031	42	5	13.355	2653710	1.0	72216	BB	3364	1
6	Standard Solution	M-031	42	6	13.363	2655030	1.0	72514	BB	3348	1
Mean						2653648					
% RSD						0.1					

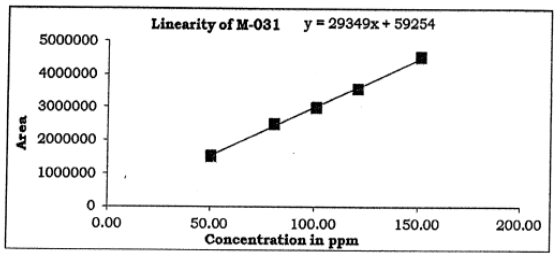
2.0 Linearity and Range:

The detector response was found to be linear Ranges from 50.43 ppm to 151.30 ppm with a correlation coefficient 0.999 for Rivaroxaban the results are summarized in table no.5 and Fig no. 6.

Table no. 5 Linearity result of Rivaroxaban Assay method:

Level	Conc.in ppm	Average Area	No. of Injections	50% level area	150% level area
1-50%	50.43	1525433	1	1522010	4514404
2- 80%	80.69	2491164	2	1532540	4514931
3 -100%	100.87	2987193	3	1526779	4531391
4 -120%	121.04	3563852	4	1519110	4516364
5 -150 %	151.30	4530359	5	1531358	4553213
Correlation Coefficient		**0.99916**	6	1520802	4551853
Intercepts		**59254.34**	**Average**	**1525433**	**4530359**
Slope		**29349.40**	**% RSD**	**0.4**	**0.4**

Fig no. 6 Linearity graph for Rivaroxaban:



3.0 Precision:

3.1 Method Precision:

The precision was evaluated by preparing six samples as per the test method. The % RSD was found to be within acceptance criteria. The assay %RSD results were found to be within acceptance criteria as shown in table no. 6

Table no.6 Method precision results:

Sample No.	% Assay
1	100.1
2	100.1
3	100.1
4	102.0
5	99.5
6	99.4
Average	**100.2**
% RSD	**0.9**

3.2 Intermediate Precision:

Intermediate Precision means repeatability measurement by different days, different column and Different Instruments etc. In this study cumulative RSD is taken into consideration as shown in table no.7.

Table no.7 Intermediate precision results:

	Sample No.	% Assay
Intermediate Precision	1	99.9
	2	99.7
	3	100.1
	4	99.9
	5	100.0
	6	99.3
	Average	**99.8**
	% RSD	**0.3**
	Sample No.	**% Assay**
	1	100.1
Method Precision	2	100.1
	3	100.1
	4	102.0
	5	99.5
	6	99.4
	Average	**100.2**
	% RSD	**0.9**
Cumulative Average		**100.0**
Cumulative %RSD		**0.7**

4.0 Accuracy:

Accuracy study was performed by preparing the test preparation at 50% to 150 % of target test concentration. Analysed six test preparations for 50%, 150% and triplicates at 100% concentration as per the test method. The % individual recoveries of all the samples at each preparation were found to be within the limits as shown in table no.8.

Table no.8 Accuracy Result:

Sample Name	Amount added (mg)	Amount Recovered (mg)	% Recovery	Average % Recovery	%RSD
50 % Prep-1	25.56	25.61	100.2	100.1	0.3
50% Prep-2	25.39	25.46	100.3		
50% Prep-3	25.61	25.74	100.5		
50 % Prep-4	25.59	25.52	99.7		
50% Prep-5	25.68	25.62	99.8		
50% Prep-6	25.62	25.63	100.0		
100% Prep-1	50.52	50.87	100.7	99.7	0.9
100 % Prep-2	50.75	50.20	98.9		
100 % Prep-3	50.66	50.48	99.6		
150 % Prep-1	75.65	74.97	99.1	98.9	0.3
150 % Prep-2	75.74	74.69	98.6		
150% Prep-3	75.71	74.84	98.9		
150% Prep-4	75.54	74.81	99.0		
150 % Prep-5	75.64	74.51	98.5		
150% Prep-6	75.73	75.11	99.2		

5.0 Solution Stability:

Established the solution stability of standard and test preparation's on bench top

and in refrigerator. Standard solution was prepared as per test method and injected at initial 1st day and 2nd day. The % RSD of standard solution was found to be within the limits. The result are summarized in table no 9:

Table no.9: Results of standard solution stability % RSD:

Time Interval	% RSD	
	Bench Top	Refrigerator
Initial	NA	NA
Day-1	0.9	0.8
Day-2	0.9	0.7

Table no.10: Results of standard solution stability:

Time Interval	% RSD	
	% Assay	% Difference
Initial Prepration-1	98.8	NA
Bench Top		
Initial Prepration-1	100.1	NA
Day-1 Prepration-1	101.5	-1.7
Day-1 Prepration-2	100.2	-0.1
Day-2 Prepration-1	100.1	-0.3
Day-2 Prepration-2	100.3	-0.2
Refrigerator		
Initial Prepration-1	100.1	NA
Initial Prepration-2	100.1	NA
Day-1 prepration-1	100.2	-0.1
Day-1 prepration-1	100.4	-0.3
Day-2 prepration-2	100.1	0.0
Day-2 prepration-2	100.1	0.0

As per ICH guideline % RSD of fresh standard and Stability standard is within the limit i.e. not more than 2.0 and difference between results from initial values is also within the limit i.e. not more than 2.0 so solution is stable up to two days as shown in table 9 and table no 10.

Robustness:

The method also checked for robustness parameter like flow variation 1ml ± 0.2ml, Column temperature 35°C±5°C, Buffer pH ± 5.8±2, Wavelength variation 240nm±5nm the method found robust.

Conclusion:

The stability indicating method (SIM) for assay of Rivaroxaban has been developed and validated. The method is found to be precise, Specific, Linear, Accurate, Rugged, and Robust and can be used for routine analysis.

Acknowledgement

The authors wish to acknowledge the Hemochrom Pvt. Ltd, Mumbai for availability of Research faculty and acknowledge Sarthi, Pune to funding research.

Reference:

1. https://en.wikipedia.org/wiki/Rivaroxaban
2. Shivshankar V, Gandhimathi M, Ravi TK. Development of validated RP-HPLC method for estimation of rivaroxaban in pharmaceutical formulation. Int J Pharm Anal Res 2015;8:406-10.

3. Souri E, Mottaghi S, Zargarpoor M, Ahmadkhaniha R, Jalalizadeh H. Development of a stability indicating HPLC method and a dissolution test for rivaroxaban dosage forms. Acta Chromatogr 2016;28:347-61.
4. Mehta AR, Maheshwari DG. Development and validation of first UV spectrophoto- metric method and RP-HPLC method for simultaneous estimation of rivaroxaban and ticagrelor in synthetic mixture. J Glob Trends Pharm Sci 2018;9:5275-97
5. RP-HPLC method development and validation for the analysis of riva-roxaban in pharmaceutical dosage forms PVV Satyanarayana, AS Madhavi - Int. J. Sci. Innovations Discoveries, 2012
6. M. Çelebier, T. Reçber, E. Koçak, and S. Altinöz, Braz. J. Pharm. Sci., 49, 359 – 366 (2013).
7. K. Chandra, P. Satya, A. Dhana, et al., Res Desk, 1, 24 (2012).
8. Sachin S. Garad, Dhananjay V. Mane "A New Simple Validated RP-HPLC-PDA Analytical Method for Estimation of Aceclofenec in Active Pharmaceutical Ingredients and Bulk Drug Form Used as Anti-Anti Inflammatory Drug", Journal of Science and Technology, Vol. 07, Special Issue 03, May 2022.
9. VALIDATION OF ANALYTICAL PROCEDURES: TEXT AND METHOD- OLOGY Q2(R1) Current Step 4 version Parent Guideline dated 27 October 1994 (Complementary Guideline on Methodo- logy dated 6 November 1996 incorporated in November 2005)