International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.5, No.3, pp 1254-1263, July-Sept 2013

Photolytic-Thermal Degradation Study And Method Development Of Rivaroxaban By RP-HPLC

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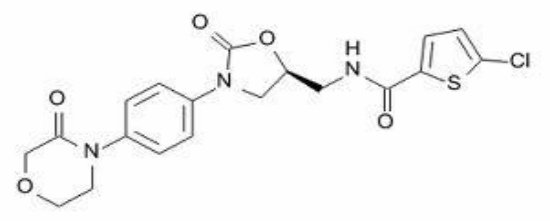
Abstract: A simple, precise and accurate RP- HPLC method has been developed and validated for assay of Rivaroxaban. An isocratic separation was achieved using a phenomenex C18 (250×4.6 mm, 5µm), 100°A particle size columns with a flow rate of 1 ml/min and using a PDA detector to monitor the elute at 250 nm. The mobile phase consisted of Methanol: Acetronitrile (50:50 v/v). The method was validated for specificity, linearity, precision, accuracy and robustness. The method was linear over the concentration range of 20-100 µg/ml (r^2= 0.99995).The method was found to be robust and suitable for assay of Rivaroxaban in a tablet formulation. The Drug Substance was subjected to Thermal and Photolytic Degradation. Degradation products resulting from the stress studies did not interfere with the detection of Rivaroxaban and the assay is thus stability-indicating.
Keywords: Rivaroxaban, Oral anticoagulant, 250 nm, stability indicating method, Oxidation Degradation, Photolytic Degradation.

Introduction [1-7]

Anticoagulants are often called blood thinners. They help prevent blood clots from forming and growing and reduce your risk for heart attack, stroke and blockages in your arteries and veins. Rivaroxaban is an oral anticoagulant invented and manufactured by Bayer; in a number of countries it is marketed as Xarelto.[1] In the United States, it is marketed by Janssen Pharmaceutical.[2] It is the first available orally active direct factor Xa inhibitor. The effects last 8–12 hours, but factor Xa activity does not return to normal within 24 hours so once-daily dosing is possible.[3-4] There is no specific way to reverse the anticoagulant effect of Rivaroxaban in the event of a major bleeding event, unlike warfarin. Rivaroxaban is an oxazolidinone derivative optimized for inhibiting both free Factor Xa and Factor Xa bound in the Prothrombinase complex.[5]It is a highly selective direct Factor Xa inhibitor with oral bioavailability and rapid onset of action. Inhibition of Factor Xa interrupts the intrinsic and extrinsic pathway of the blood coagulation cascade, inhibiting both thrombin formation and development of thrombi. Rivaroxaban does not inhibit thrombin (activated Factor II), and no effects on platelets have been demonstrated.[6]In September 2008, Health Canada and European Commission granted marketing authorization for Rivaroxaban as one 10 mg tablet taken once daily for the prevention of venous thromboembolism (VTE) in patients who have undergone elective total hip replacement or total knee replacement surgery. It was approved by CDSCO on 30 January 2010. In December 2011 Rivaroxaban has been approved by the European Commission for use in two new indications: prevention of stroke and systemic embolism in adult patients with non-valvular atrial fibrillation (AF) with one or more risk factors and treatment of deep vein thrombosis (DVT) and prevention of recurrent DVT and pulmonary embolism (PE) following an acute DVT in adults.

On July 1, 2011, the U.S. Food and Drug Administration (FDA) approved Rivaroxaban for prophylaxis of deep vein thrombosis (DVT), which may lead to pulmonary embolism (PE), in adults undergoing hip and knee replacement surgery.[3] On November 4, 2011, the U.S. FDA approved Rivaroxaban for stroke prophylaxis in patients with non-valvular atrial fibrillation.[4]



Structure of Rivaroxaban

IUPAC NAME of Rivaroxaban is (S)-5-chloro-N-{[2-oxo-3-[4-(3-oxomorpholin-4-yl) phenyl] oxazolidin-5 yl] methyl}thiophene-2-Carboxamide. It's Molecular Formula is $C_{19}H_{18}ClN_3O_5$ and Molecular Mass is 435.882 g/mol. Literature survey reveals Colorimetric method [8-9], RP-HPLC method [10] and bio analytical method [11] for estimation of Rivaroxaban. The stability of a drug substance or drug product is defined as its capacity to remain within established specifications, i.e. to maintain its identity, strength, quality, and purity until the retest or expiry date. Stability testing of an active substance or finished product provides evidence of how the quality of a drug substance or drug product varies with time under a variety of environmental conditions, for example temperature, humidity, and light. Knowledge from stability studies is used in the development of manufacturing processes, selection of proper packaging and storage conditions, and determination of product shelf-life. There was no reported stability indicating analytical method for analysis of Rivaroxaban in the presence of its degradation products in pharmaceutical dosage forms. The objective of this work was to develop a new, simple, economic, rapid, precise, and accurate stability-indicating HPLC method for quantitative analysis of Rivaroxaban, and to validate the method in accordance with ICH guidelines.

Material And Methods

➢ Instruments And Reagents

A HPLC Instrument with UV-Visible and photodiode array detector LC-2010CHT, Shimadzu, Japan was used for the absorbance measurements. Sartorious CP225D Analytical balance was used for weighing the samples. D120/1H, Trans-o-sonic- Ultra Sonicator was used for Sonication of solution. All the chemicals used were of analytical grade. Pure Rivaroxaban was procured as a gift sample from Mega Fine Pharma, Mumbai, India.

➢ Preparation Of Standard Stock Solution (500 µg/ml)

25 mg of Rivaroxaban was weighed accurately and transferred into a clean, dry 50 mL volumetric flask, dissolved with sufficient volume of diluent (50:50 v/v% Methanol: acetonitrile) and volume was adjusted to 50 mL with diluent to get a concentration of 500 µg/mL.

Selection Of Wavelength

The standard solution of Rivaroxaban was injected under the chromatographic conditions. Detection was carried out at different wavelengths but the best response was achieved at 250 nm with PDA detector. Therefore it was chosen as the analytical wavelength.

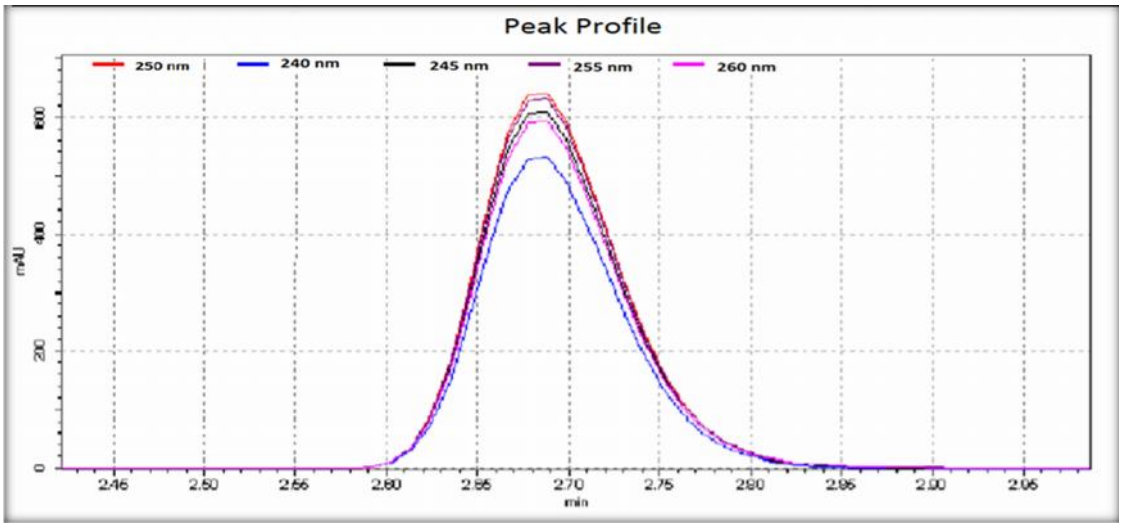


Figure 1- Selection of wavelength by PDA Detector

Selection Of Mobile Phase:

The scanning of Rivaroxaban was done by preparing 50 µg/ml solution of drug separately in combination of various solvent systems (varying the ratio and/or nature of organic modifier), at the end of these studies acetonitrile: Methanol (50: 50 v/v) was selected as the best mobile phase because in that drug was showing good elution (figure 2).

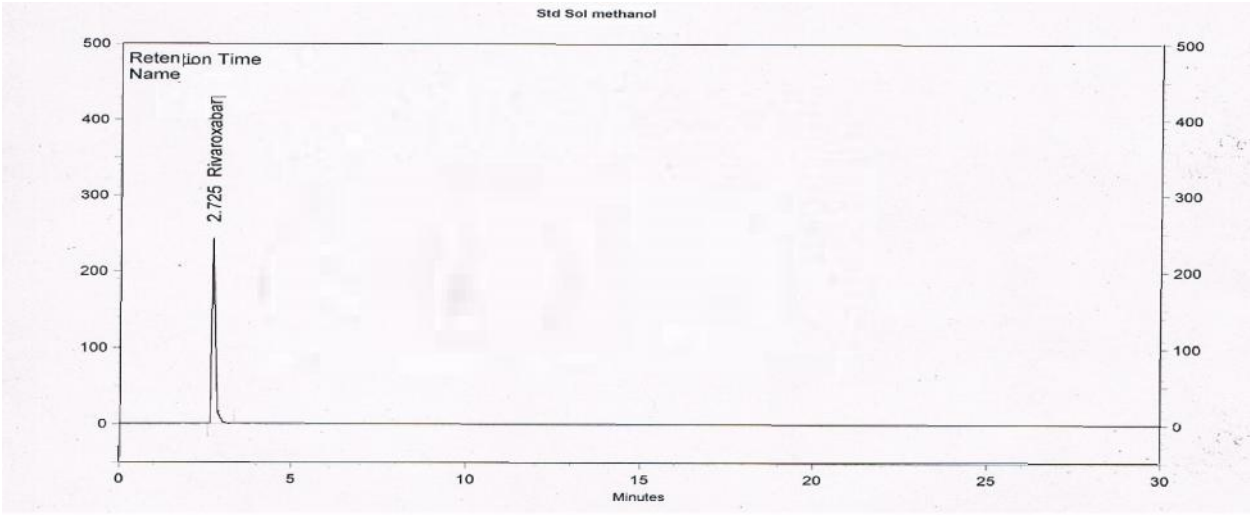


Figure 2- Chromatogram of 50 µg/ml of Rivaroxaban using studies acetonitrile: Methanol (50: 50 v/v) as a mobile phase.

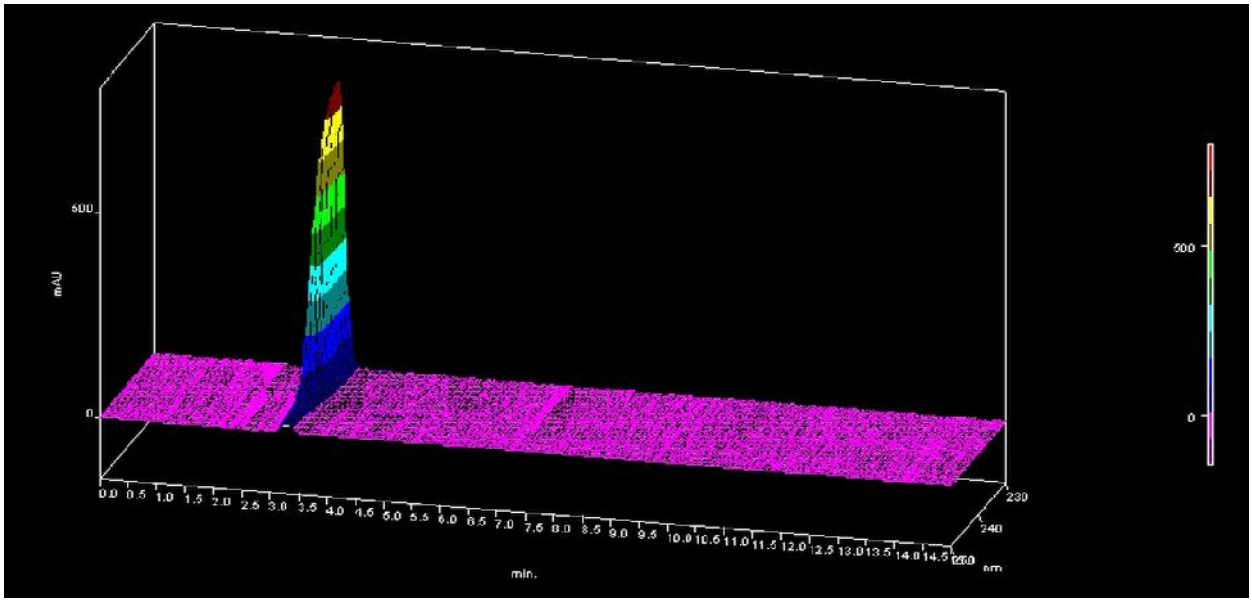


Figure- 3 3D plot of standard solution by PDA detector for HPLC method

Method Validation :[19]

Specificity

Analysis of specificity was carried out by using PDA detector. Standard solution of 50 µg/mL was injected and the peak purity curve was obtained. According to peak purity curve, no impurity was attributed to the analyte peak. Peak purity curves for standard solutions are shown in figure 4.

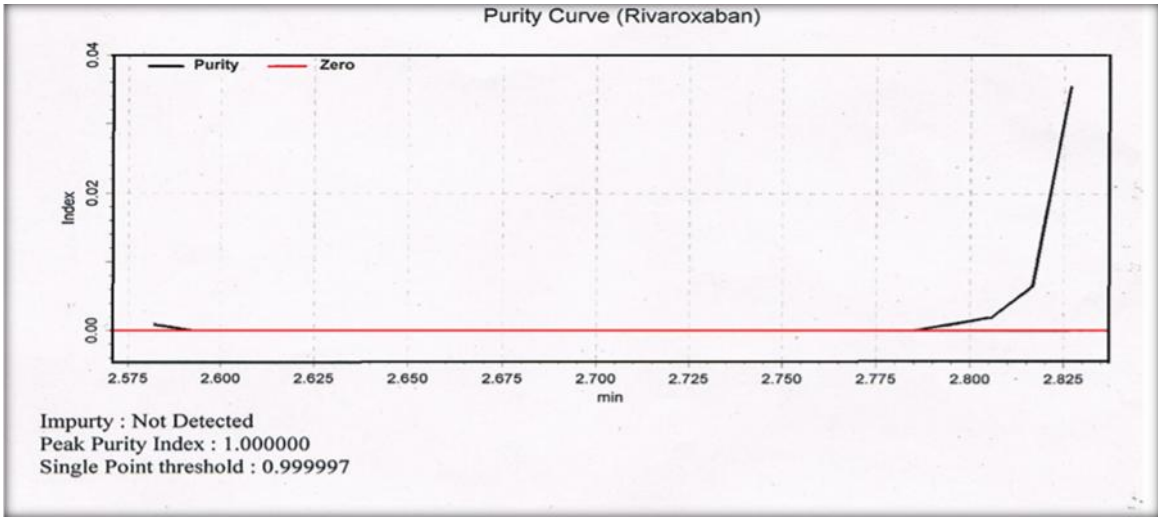


Figure-4 Peak purity curve of standard solution for HPLC method.

The method validation was carried out as per ICH Q2 (R1) guidelines. The following validation parameters; linearity and range, accuracy and precision, limit of detection (LOD), limit of quantification (LOQ) and robustness were studied.

Linearity

The portions of 0.4 mL, 0.8 mL, 1.2 mL, 1.6 mL, and 2.0 mL of 500 µg/mL of standard stock solution of Rivaroxaban were transferred separately to a series of 10 mL of volumetric flasks and volume was adjusted to 10 mL with diluent to obtain the concentrations of 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL and 100 µg/mL respectively. 20 µl of each of these standard solutions of Rivaroxaban were injected under the operating chromatographic conditions into the system. Calibration curve was constructed by plotting (peak areas v/s concentrations) of Rivaroxaban.

Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of the method was determined by calculating recovery of Rivaroxaban by the standard addition method.

Precision

I. Intraday Precision

Solutions of Rivaroxaban containing 2, 6 and 12 µg/mL series were analyzed three times on the same day and % RSD was calculated.

II. Interday Precision

Solutions of Rivaroxan containing 2, 6 and 12 µg/mL series were analyzed on three different days and % RSD was calculated.

Robustness:

The robustness of the method was established by making deliberate minor variations in the flow rate and Temperature.

Limit Of Detection And Limit Of Quantification

Purpose

LOD and the LOQ of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines.

$$LOD = 3.3 \times \sigma/S$$
$$LOQ = 10 \times \sigma/S$$

Where,
σ = Standard deviation of the response
S = Slope of calibration curve.

Degradation Studies [12-18]

➢ Photolytic Degradation

100mg of bulk drug is put into the Petridish and placed under direct sun light and UV Light for 1 hr.10mg is weigh and make up to 10 ml , using Mobile Phase. From above solution 0.5 ml is withdrawn and make up to 10 ml (50 ppm) using Mobile Phase.Similar Procedure is followed for 2hr, 4 hr,6 hr,1 day and 3 day.

➢ Thermal Degradation

100mg of bulk drug was taken in a cleaned Petridis and was put it into the oven at 60 ^{0} C and 80 ^{0}C for 1 hour.10mg of bulk drug from the Petridis was weigh and Dissolved in 10 ml of Mobile phase 0.5 ml of above

solution is withdrawn and make up to 10 ml with mobile phase (50 ppm).Similar Procedure is followed for 2hr, 4 hr, 6 hr,1 day and 3 day.

All samples were subjected to HPLC analysis. The initial analysis of different stressed samples was performed on HPLC system using a C-18 column and mobile phase composed of acetonitrile: Methanol (50: 50). It was filtered and sonicated before use. The injection volume was 20 µl and the flow rate was set at 1ml/min. The detection was carried out at 250nm.

Table-1 Summary of Thermal Degradation at 60 ^{0}C and 80 ^{0}C.

Sr. No.	TIME	% DEGRADATION	
		60 ^{0}C	80 ^{0}C
1	1 Hr	1.16	2.56
2	2 Hrs	4.50	9.10
3	4 Hrs	12.25	17.21
4	6 Hrs	21.76	28.48
5	1 Day	37.96	45.14
6	3 Day	51.29	59.01

Table-2 Summary of Photolytic Degradation in UV Light and Sunlight.

Sr. No.	TIME	% DEGRADATION	
		UV	SUNLIGHT
1	1 Hr	1.8	1.21
2	2 Hrs	7.05	8.69
3	4 Hrs	11.78	13.98
4	6 Hrs	17.47	18.49
5	1 Day	-	29.96
6	3 Day	-	32.25

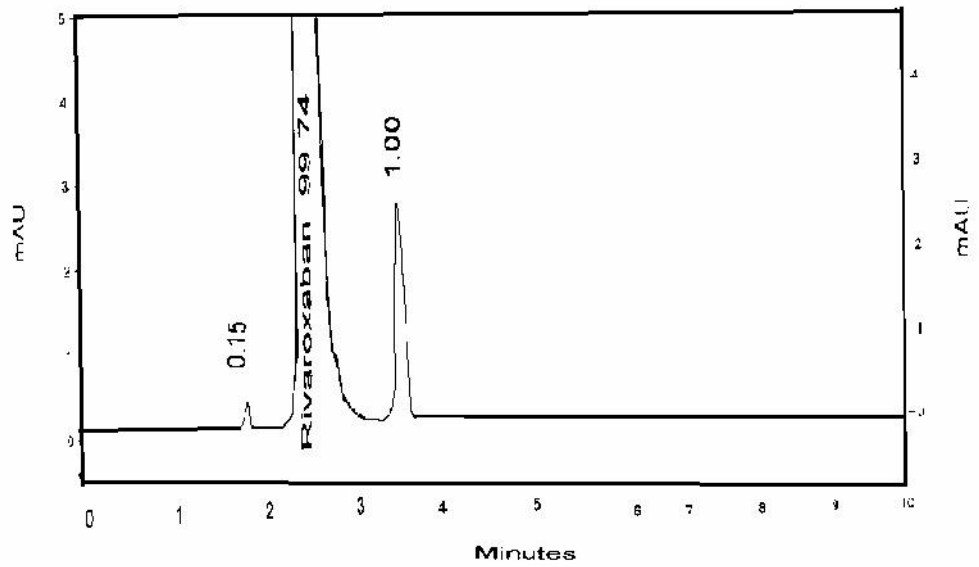


Figure-5 photolytic Degradation of 6 Hr in UV

Table 3 Photolytic Degradation of 6 Hr (UV)

Sr No.	Retention Time (min.)	Resolution	% Degradation
1.	1.867	-	
2.	2.645	4.97	17.47 %
3.	*3.787*	*7.35*	

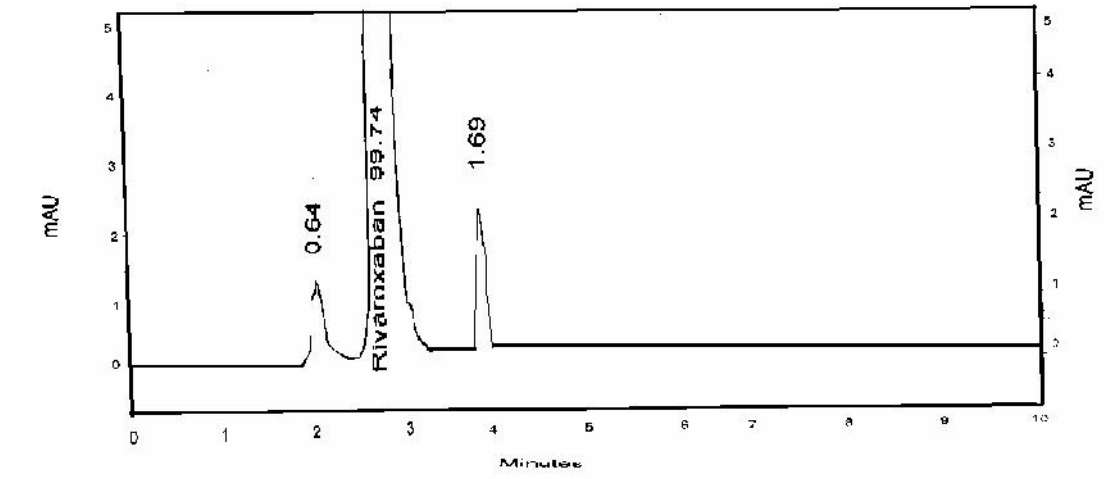


Figure 6- photolytic Degradation of 6 Hr in SL

Table 4 Photolytic Degradation of 6 Hr (SL)

Sr No.	Retention Time (min.)	Resolution	% Degradation
1.	1.867	-	
2.	2.645	5.33	18.49 %
3.	3.797	7.16	

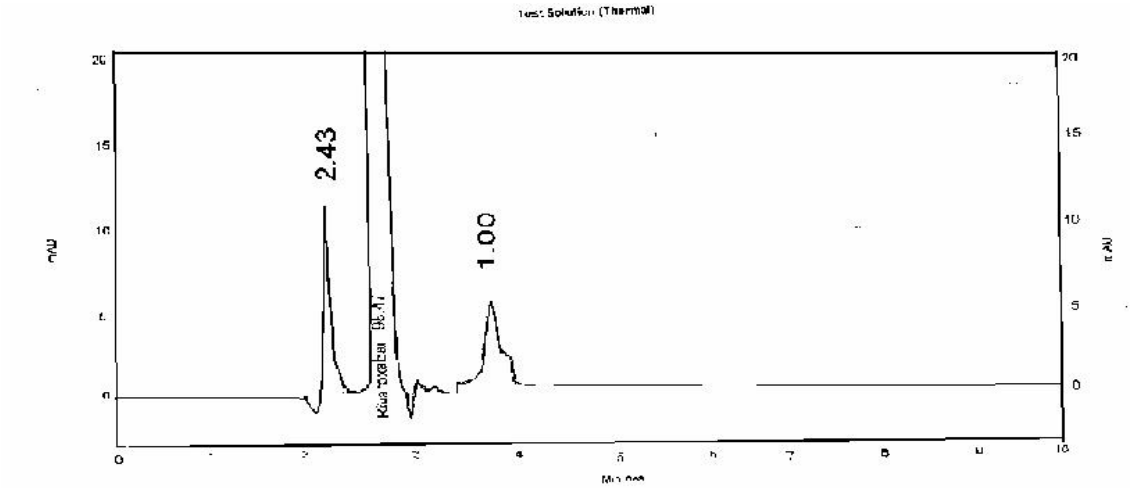


Figure 7 Thermal Degradation of 3 Day at 60 ^{0}C

Table 5 Thermal Degradation of 3 Day (60 ^{0}C)

Sr No.	Retention Time (min.)	Resolution	% Degradation
1.	2.251	-	
2.	2.667	3.25	51.29 %
3.	3.819	7.64	

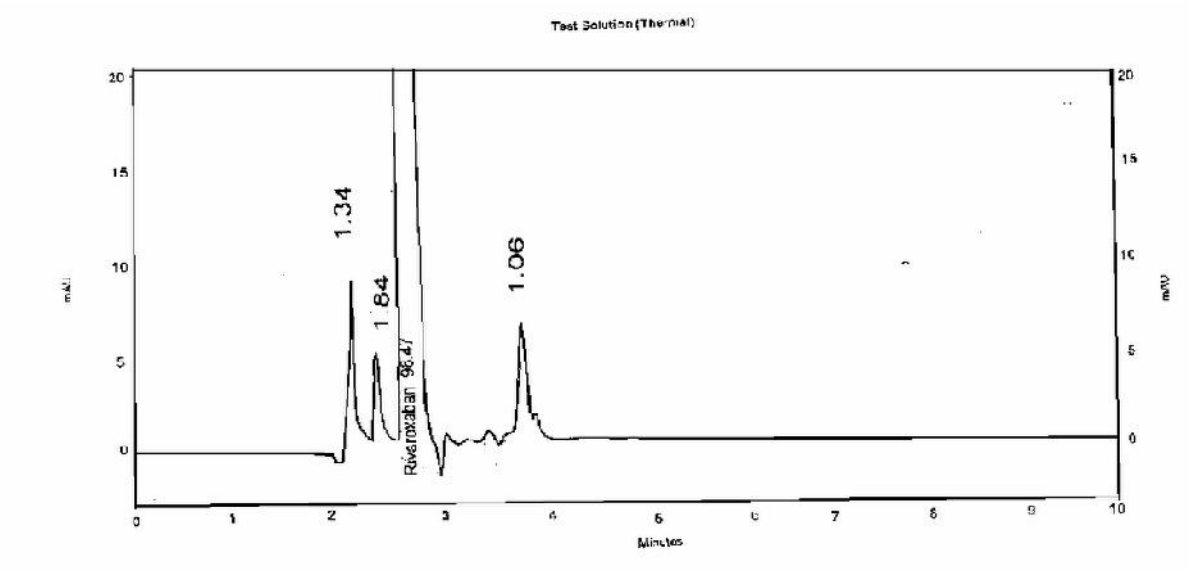


Figure 8 Thermal Degradation of 3 Day at 80 ^{0}C

Table 6 Thermal Degradation of 3 Day (80 ^{0}C)

Sr No.	RetentionTime (min.)	Resolution	% Degradation
1.	2.165	-	
2.	2.496	3.07	59.01 %
3.	2.667	1.98	
4.	3.819	7.31	

Result And Discussion:

Table-7 Regression Analysis Data and Summary of Validation Parameters for HPLC Method

Sr. No.	Validation Parameter	Result
1	UV detection wavelength (nm)	250 nm
2	Linearity range (µg/mL)	20-100
3	Standard Regression equation	y = 79,902.16000x + 8,662.40000
4	Correlation coefficient (R2)	R^2 = 0.99995
5	Precision (%RSD) Intraday (n= 9)	0.143 (Acceptance limit: <1)
6	% Recovery (Accuracy, n = 9)	100.85 %
7	LOD (µg/mL)	0.1277
8	LOQ (µg/mL)	0.38726
9	Robustness Flow rate change Temperature change	 0.22 0.12
10	Assay (% Label claim)	99.68

Conclusion:

A simple, rapid, accurate and precise stability-indicating HPLC analytical method has been developed and validated for the routine quantitative analysis of Rivaroxaban in API. Rivaroxaban undergo degradation in photolytic and Thermal stressed condition to give degradation products. The degradation peak is clearly separated from the drug peak and hence the method is stability Indicating and can be applied to the analysis of routine quality control samples and samples obtained from stability studies.

Acknowledgement

The authors are thankful to the Shree Dhanvantary Pharmaceutical Research Center, Kim for providing faculties to carry out research work.

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