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DEVELOPMENT AND VALIDATION OF RIVAROXABAN BY USING RP-HPLC METHOD IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Rivaroxaban in bulk & pharmaceutical dosage form. Chromatographic separation of Rivaroxaban was achieved on Waters Alliance-e2695, by using Agilent SB C18 (250mm x 4.6mm, 3.5µm) column and the mobile phase containing ACN and 0.1% Formic acid in the ratio of **70:30% v/v**. The flow rate was 0.8 ml/min; detection was carried out by absorption at **269nm** using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Rivaroxaban were NLT 2000 and should not more than **2** respectively. % Relative standard deviation of peak areas of all measurements always less than **2.0.** The proposed method was validated according to ICH guidelines. The RP-HPLC method was found to be linear over the concentration range from 12.5-75 µg/ml with correlation coefficient of (r^2) 0.99978.the retention time for bulk rivarobaxan was found to be 3.029 min. LOQ of method was 0.5 µg/ml and LOD of method was found to be 0.15 µg/ml. The method was found to be simple, economical, suitable, precise, accurate & robust method which can be applied for the regular analysis of rivarobaxan in bulk as well as pharmaceutical dosage form.

Keywords: RP-HPLC, Rivaroxaban, Retention time, Formic acid

INTRODUCTION:

Rivaroxaban Figure 01 is an oral oxazolidinone-based anticoagulant; it is a potent and selective direct inhibitor of factor Xa for the prevention of venous thromboembolism in adult patients after total hip replacement or total knee replacement surgery. It is also used in treatment of pulmonary Embolism and to prevent blood clots **[1]**. Chemically it is 5-chloro-N-[[(5S)-2-oxo-3- [4-(3- oxomorpholin-4-yl) phenyl]-1, 3-oxazolidin-5-yl] methyl] thiophene- 2-carboxamide, shown in the Figure 01 . It is an empirical formula $C_{19}H_{18}Cl\ N_3O_5S$ and molecular weight of 435.882 g/mol **[2-3]**.

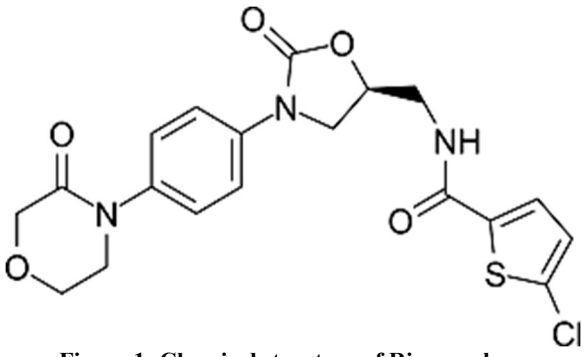

Figure 1: Chemical structure of Rivaroxaban

Literature survey revealed that few analytical methods have been reported for estimation of Rivaroxaban individually or in combination with other drugs. The reported methods are Spectrophotometric **[4]**, RP-HPLC **[5]** and HPTLC **[6]** methods. The present study was aimed to develop a simple, sensitive, rapid and precise RP-HPLC method for estimation of Rivaroxaban in bulk and pharmaceutical dosage forms. The analytical method was validated according to ICH validation parameters.

MATERIALS AND METHODS:

Instrumentation

The analysis was performed by using a chromatographic system.

- HPLC - ALLIANCE Model instrument (Waters e 2695- Empower software2.0versions)
- Weighing balance (Sartouris)
- UV/VIS Spectrophotometer (UV-1700)
- Ultra Sonicator – UCA 701 Model (Unichrome)
- Pump – Isocratic model

Chemicals and Reagents:

- Acetonitrile, Formic acid, HPLC grade Water

Determination of Working Wavelength (λ_{max}):

In estimation of the drug isobestic wavelength was used. Isobestic point is the wavelength

where the molar absorptivity is the same for the substances that are inter convertible. So this wavelength was used in estimation of drug accurately. The wavelength of maximum absorption of the solution of the drug in mixture of Acetonitrile and 0.1% Formic acid (70:30 v/v) were scanned using PDA Detector within the wavelength region of 200–400 nm against Acetonitrile and 0.1% Formic acid (70:30 v/v) as blank.

Table 1: Chromatographic Conditions

Column	**Agilent SB C18 (250mm x 4.6mm, 3.5µm)**
Mobile phase	**ACN+ 0.1 %Formic acid, ACN+ 0.1 % orthophsophoric acid.**
diluents	**Mobile phase**
Wavelength	**200-400 nm**
Flow rate	**0.8ml/min**
Injection volume	**20µl**
Mode of separation	**Isocratic**
Temperature	**Ambient ($25^{O}C$)**
Run time	**5-10min**

Table 2: Optimized conditions

Column	**Agilent SB C18 (250mm x 4.6mm, 3.5µm)**
Movable phase	**ACN+ 0.1% Formic acid (70:30 v/v)**
Wavelength	**269 nm**
Flow rate	**0.8ml/min**
Injection volume	**20µl**
Run time	**5min**
Observation	**This method is suitable for validation**

Rivaroxaban was analyzed with Agilent SB C18 (250mm x 4.6mm, 3.5µm) for the chromatographic separation and column was maintained at ambient temperature. The mobile phase was composed of a mixture of acetonitrile and 0.1 % Formic acid in the ratio of 70:30 v/v and it was delivered at a flow rate of 0.8 mL /min and detection was monitored at 269 nm with PDA detector. Mobile phase was used as diluent. Injection volume was 20 µl. The run time was 5 min. The retention time of Rivaroxaban was found to be 3.029 min. Optimized conditions are shown in the **Table 2**.

Preparation of Standard stock solution

Accurately weigh and transfer 5 mg of Rivaroxaban working standard into a 10 ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. **[7]** (Stock solution) further pipettes 1 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent. (50ppm of Rivaroxaban)

Preparation of Sample Solution

Accurately weighed and transfer 29 mg of Rivaroxaban sample into a 10mL clean dry volumetric flask add diluent and sonicate it up to 30 min to dissolve, and centrifuge for 30min to dissolve it completely and make

volume up to the mark with the same solvent. Then it is filtered through 0.45micron injection filter (Stock solution). Further pipette 1 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent (50ppm of Rivaroxaban) **[8]**.

Method Development and Optimization

The optimized HPLC conditions several mobile phases of different compositions were tested to develop an optimization of chromatographic conditions such as tailing factor, good peak shape, and theoretical plates. For the selection of the mobile phase primarily 0.1 % orthophosphoric acid: acetonitrile, acetonitrile: 0.1 % formic acid has been tested for different compositions, flow rates and ratios. The chromatography conditions are shown in the **Table 1 [9]**. Finally, mobile phase consisting a mixture of acetonitrile: 0.1 % formic acid (70:30 % v/v) at a flow rate of 0.8 ml/min was found to be satisfactory and proper system suitability parameter results were obtained. The results of system suitability and chromatogram are shown in the Table no.08 and **Figure 3**.

Method Validation

The method was validated for Specificity, system suitability, linearity, accuracy, precision, limit of detection, limit of quantification and robustness by following procedures.

System Suitability

System suitability is an integral part of the chromatographic system. It is verification of tailing factor, theoretical plate count are calculated and compared with standard specification of system **[10]**.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present **[11]**. The specificity of an analytical method is to determine the effect of excipients and other additives that are generally present in the formulation. The test results obtained were contrasted with the results of standard drug.

Linearity

Linearity is the ability (within specified range) to obtain test results are directly proportional to the concentration of analyte in the sample. Linearity is evaluated by visual inspection of plot of signal as a function of analyte concentration. If there is a linear relationship test results are calculated by regression line by method of least squares **[12]**. The linearity of the method was determined at six concentration levels ranging from 12.5-75 µg/ml for Rivaroxaban. Evaluation of the drug was performed with PDA detector at 269 nm; peak area was recorded for all the peaks. The correlation coefficient value of Rivaroxaban was 0.99978. The results shown that an

excellent correlation exists between peak area and concentration of drug within the concentration range indicated.

Range

The range of analytical procedure is the interval between the upper and lower concentration of analyte in the sample **[13]**.

Accuracy

Accuracy of analytical method is 'measure of how close the experimental value to the true value' accuracy of the method was determined by standard addition method **[14]**. A known amount of standard drug is added to the fixed amount of pre-analyzed injection solution. Percent recovery is calculated by comparing the area before and after addition of the standard drug. The standard addition method is performed at 50 %, 100 % and 150 % level. The solutions are analyzed in triplicate at each level as per the proposed method.

The accuracy of the method was determined by calculating recovery of Rivaroxaban by the method of standard addition. Known amount of Rivaroxaban was added to a pre-quantified sample solution and the amount of Rivaroxaban was estimated by measuring the peak area ratios and by fitting these values to the straight-line equation of calibration curve **[15]**. The recovery studies were carried out three times over the specified concentration range of 50 %, 100 % and 150 % levels. The amount of Rivaroxaban was estimated by measuring the peak area ratios by fitting these values to the straight-line equation of calibration curve. From the above determination, percentage recovery and mean of percentage recovery were calculated.

Precision

The closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions **[16]**. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. The intra-day precision study of Rivaroxaban was carried out by estimating the correspondence responses six times on the same day with 50 % concentration and inter-day precision study of Rivaroxaban was carried out by estimating the correspondence responses six times next day with 50% concentration **[17]**.

Limit of detection and Limit of Quantification

Limit of detection (LOD) is defined as the lowest concentration of analyte that gives a detectable response. Limit of Quantification (LOQ) is defined as the lowest concentration of analyte that can be quantified with a specified level of accuracy and precision **[18]**. For this study, six replicates of the analyte at lowest concentration are measured and quantified. The limit of detection (LOD) and limit of quantification (LOQ) of the developed

method were determined by injecting progressively low concentrations of the standard solution using the developed RP-HPLC method **[19]**.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters **[20-21]**. The robustness of the proposed method is estimated by

a) changing flow rate of the mobile phase
b) Composition of the mobile phase.

RESULTS AND DISCUSSION

The RP-HPLC procedure was optimized with a view to develop an accurate assay method for the determination of Rivaroxaban in bulk and pharmaceutical dosage form by using Agilent SB C18 (250mm x 4.6mm, 3.5µm) column with mobile phase of acetonitrile and formic acid in the ratio of 70:30 v/v. The flow rate of mobile phase at 0.8 ml/min and the component was monitored and detected with PDA detector at 269 nm. The eluted drug peak with good shape and well resolved. The results of both conditions of chromatography and optimized chromatographic conditions were shown in Table 01, 02 and Figure 03. The retention time, number of theoretical plates and tailing factor of Rivaroxaban was found to be 3.029 min., 14774 and 1.06 respectively; results are shown in the Table 08, which indicates efficient performance of the column. The method was linear in the range of 12.5-75 µg/ml for Rivaroxaban with correlation coefficient of 0.99978. The regression equation of Rivaroxaban concentration over its peak area ratio was found to be Y=40608.93x, where X is the concentration of Rivaroxaban and Y is the respective peak area. The linearity results were shown in **Table 3** and **Figure 2**.

The mean % recoveries were found to be from 99.5 to 100 % which indicate the method is accurate. The accuracy results were shown in **Table 4**. The percentage RSD for system precision for rivarobaxan was found to be 0.31. The % RSD for intra-day precision and inter-day precision for Rivaroxaban were found to be 0.38 and 0.45, the values were less than 2 % which indicate the method is precise. The results of precision studies were shown in **Table 5 and 6**. The limit of detection (LOD) and limit of quantification (LOQ) for Rivaroxaban were found to be 0.15 µg/ml and 0.50 µg/ml, which indicate the sensitivity of the method. The results of LOD and LOQ were shown in **Table 7** and **Figure 4 and 5**.

The robustness was performed for the flow rate variations from 0.72 ml/min to 0.88 ml/min. The method is robust only in less flow condition ± 2%. The results are shown in the **Table 8** summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ± 0.08 ml/min. The method is robust only in less flow condition. The typical variations studied under this parameter were mobile phase flow rate. Overall % RSD was found to be less than 2% for all the variations which indicates that the proposed method is robust.

The summary of system suitability parameters and validation parameters were shown in **Table 9 and 11**. Validated method was applied for the determination of Rivaroxaban in commercial tablet formulation that was obtained by injected 3 replicates of the sample solutions. The amount of drug and percentage of assay was found to be 5 mg/tablet and 99.9%. The results are shown ion the **Table 10 and Figure 6, 7**. Typical chromatogram of drug Rivaroxaban was shown in **Figure 3**. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in the formulation did not interfere with the estimation of the drug by the proposed method.

Table 3: Calibration data of Rivaroxaban

S. No.	Rivaroxaban	
	Conc.(µg/ml)	Peak area
1	12.50	522856
2	25.00	1038964
3	37.50	1546354
4	50.00	2087836
5	62.50	2520412
6	75.00	3056381
Regression equation	y=40608.93x+16136.82	
Slope	40608.93	
Intercept	16136.82	
R^2	0.99978	

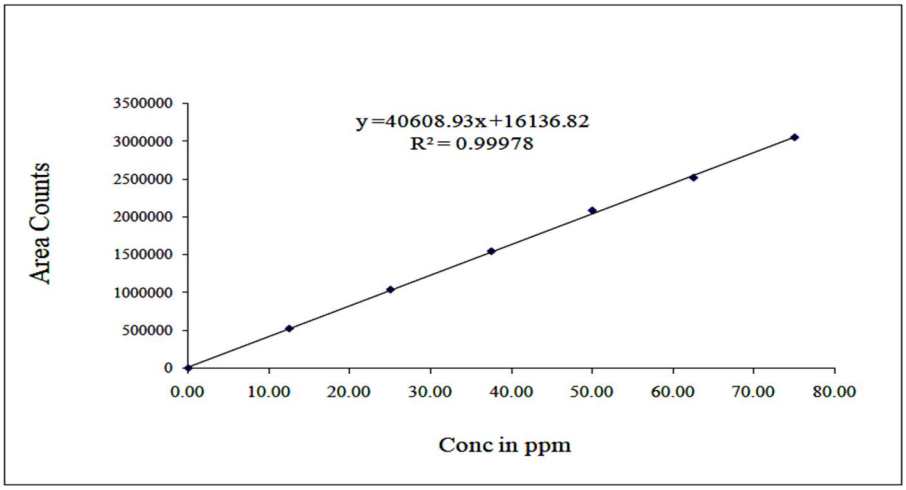

Figure 2: Linearity curve of Rivaroxaban

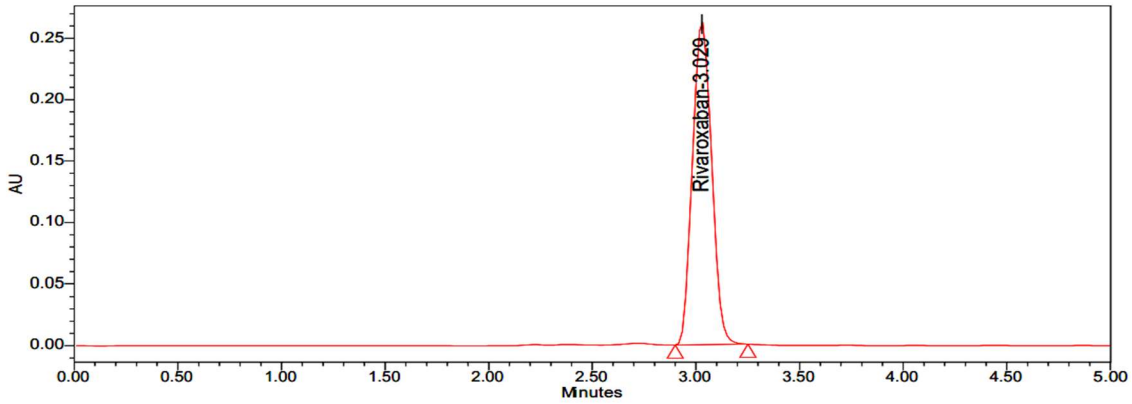

Figure 3: Chromatogram of Rivaroxaban

Table 4: Mean % recoveries

% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean %Recovery
50%	1035624	2.5	2.487	99.5	99.8
	1052558	2.5	2.527	101.1	
	1029967	2.5	2.473	98.9	
100%	2079487	5.0	4.993	99.9	100.0
	2081039	5.0	4.997	99.9	
	2085214	5.0	5.007	100.1	
150%	3094873	7.5	7.431	99.1	99.5
	3096661	7.5	7.435	99.1	
	3128639	7.5	7.512	100.2	

Table 5: System precision table of Rivaroxaban

S. No	Concentration Rivaroxaban (µg/ml)	Area of Rivaroxaban
1.	50	2087836
2.	50	2079606
3.	50	2082298
4.	50	2075415
5.	50	2092298
6.	50	2077415
Mean	2082478	
S.D	6464.380	
%RSD	0.31	

Table 6: Intermediate precision (Day_ Day Precision):

Injection	Area	
	Day-1	Day-2
1	2092889	2079684
2	2088794	2095211
3	2071815	2081279
4	2079611	2097220
5	2088397	2073652
6	2079058	2090195
Average	2083427	2086207
Standard Deviation	7893.334	9409.836
%RSD	0.38	0.45

Table 7: Sensitivity parameters (LOD & LOQ) by RP-HPLC

Name of drug	LOD (µg/ml)	S/N	LOQ (µg/ml)	S/N
Rivaroxaban	0.15	3	0.50	10

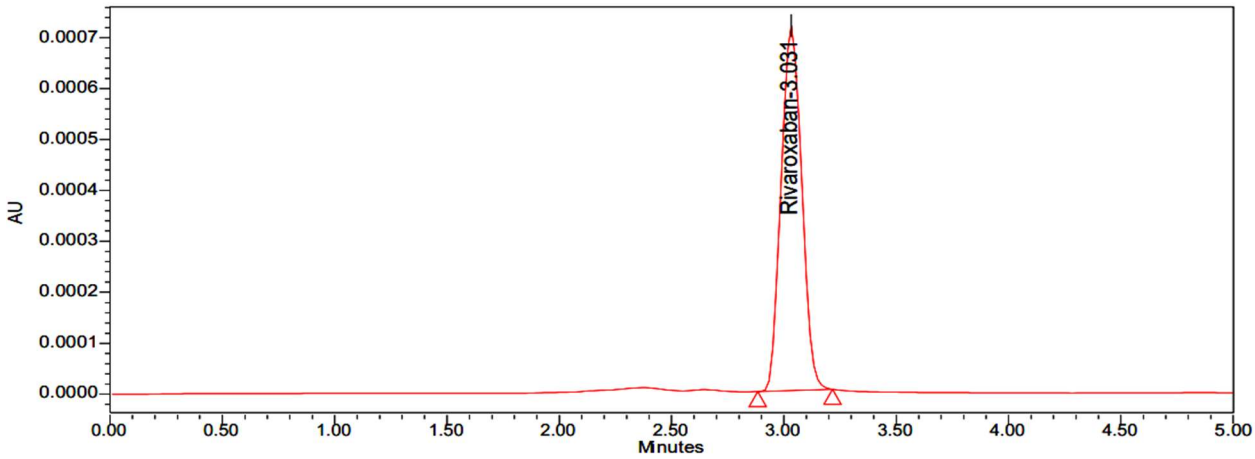

Figure 4: Chromatogram for LOD

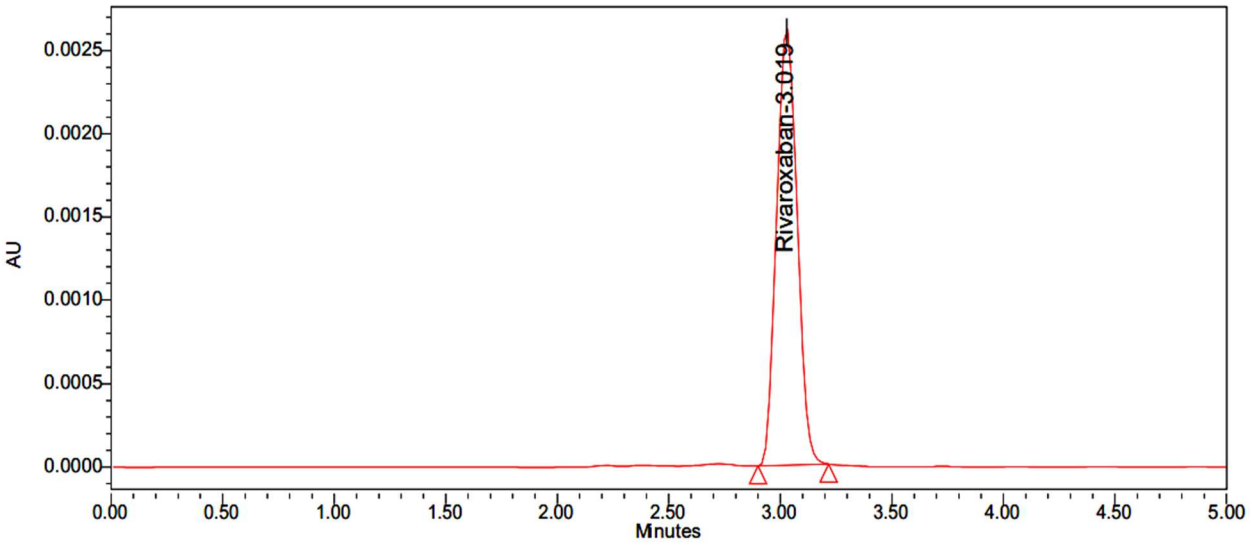

Figure 5: Chromatogram for LOQ

Table 7: Robustness results

Parameter	Rivaroxaban					
	Condition	Retention time(min)	Peak area	Tailing	Plate count	% RSD
Flow rate Change (mL/min)	Less flow(0.72ml)	3.349	1961452	1.12	14855	0.35
	Actual(0.8ml)	3.029	2087836	1.06	14774	0.31
	More flow(0.88ml)	2.778	2245947	1.02	14690	0.61
Organic Phase change	Less Org (63:37)	3.520	1857416	1.15	14882	0.20
	Actual (70:30)	3.027	2079606	1.09	14766	0.31
	More Org (77:23)	2.838	2331411	1.04	14631	0.41

Table 9: System Suitability parameters

S. No	Parameter	Rivaroxaban
1	Retention time	3.029
2	Plate count	14774
3	Tailing factor	1.06
4	%RSD	0.31

Table 10: Assay of Rivaroxaban

Brand	Drug	Area	Average sample area	Std. wt. (mg)	Sample wt. (ml)	Label amount (mg)	Std purity	Amount found (µg/ml)	% assay
Xarelto	Rivaroxaban	2089415 2072663	2081039	5	29	20	99.9	4.997	99.9

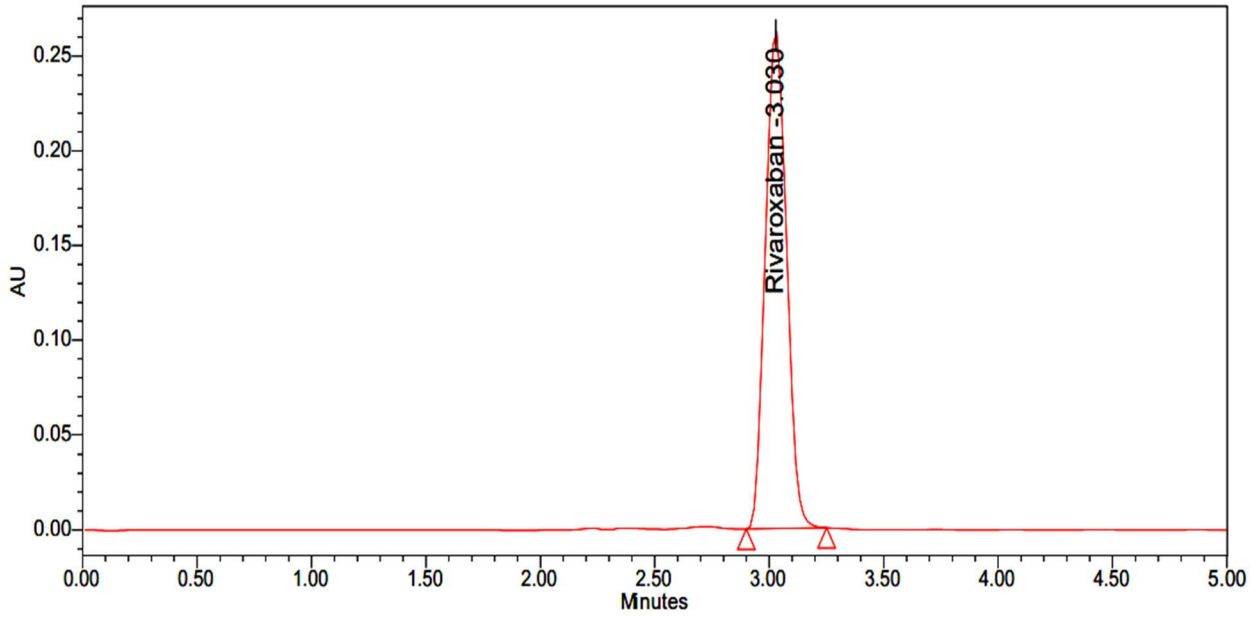

Figure 6: Chromatogram of Assay-1

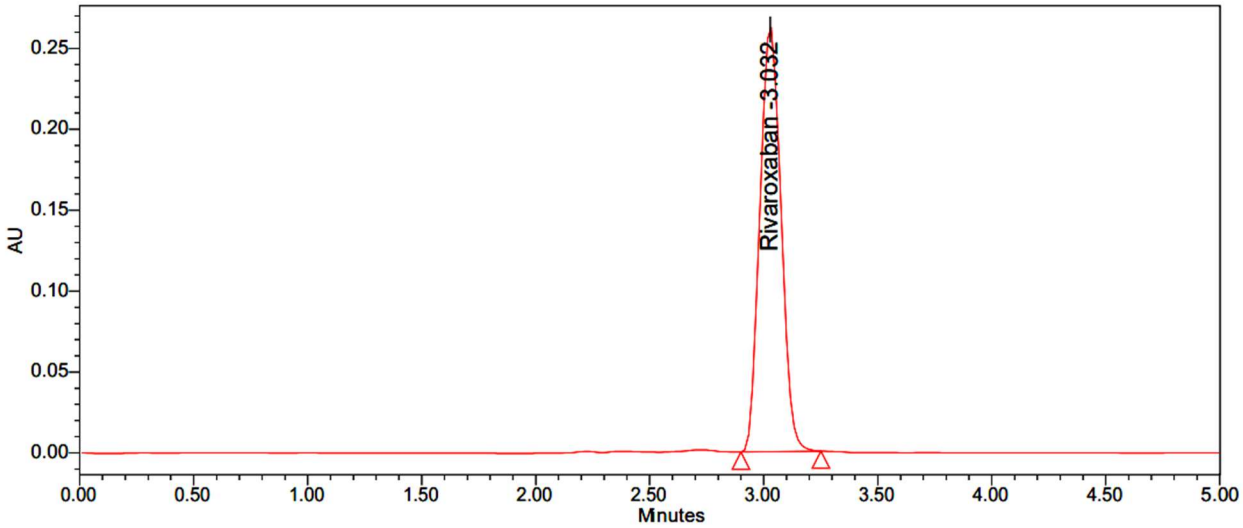

Figure 7: Chromatogram of Assay-1

Table 11: System suitability and validation parameters

S. No.	Parameter	Results
1	Linearity range (µg/ml)	12.5-75
2	Correlation coefficient (r2)	0.99978
3	Retention times (min)	3.029
4	Theoretical plates (N)	14774
5	Tailing factor	1.06
6	Mean % recovery (%)	99.8
6	Repeatability (% RSD)	0.42
7	Reproducibility (% RSD)	0.2
8	Precision a) system	0.31%
	Precision a) Intra day b) Inter day	a) 0.38 b)0.45
9	LOD (µg/ml)	0.15
10	LOQ (µg/ml)	0.5
11	Robustness (% RSD) (0.72ml/min &0.88 ml/min)	0.35
		0.611
12	Assay (%)	99.9

CONCLUSION:

Proposed Study describes new HPLC method for the estimation of Rivaroxaban bulk and in its Pharmaceutical dosage form. The method was validated and found to be simple, sensitive, accurate, precise and robust. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of estimation of Rivaroxaban in regular quality control testing laboratories.

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