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Pre-Formulation Study, Analytical Method Development And Validation Of Rivaroxaban

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Abstract
The main objective was to develop and validate the UV-spectrophotometric method for the estimation of rivaroxaban (RIV). Method was validated for different parameters like linearity, precision, and ruggedness study. Calibration curve was constructed for RIV standards by plotting the concentrations versus peak area ratios. The graph proved that the method was linear in investigation concentration range with correlation coefficient R^2 value of 0.9863 The intraday and interday precision (RSD %) was found to be 1.49 and 1.11% respectively. The robustness were changed relatively between 99.8 – 102.4% within these small changes on mobile phase component and column temperature. The results were evaluated statistically, and there were no significant differences ($p>0.05$) within the results. Applying same procedures by two different operators showed the ruggedness of the developed method. The analysis results having no significant difference indicate that the proposed method is robust. The FTIR spectra of rivaroxaban was performed and analysed. Results of IR confirmed the presence of the main functional group
Key words: *Rivaroxaban, Method development, Validation, Pre-formulation*

INTRODUCTION

Anticoagulants are given to prevent the blood from clotting or prevent to existing clots from getting larger. Clots can block the blood flow to the heart muscle or block the blood flow to the brain. These cause a heart attack or a stroke. Rivaroxaban (RIV), an oral oxazolidinone-based anticoagulant, is a potent, selective direct inhibitor of factor Xa that is used in the prevention of venous thromboembolism in adult patients after total hip replacement or total knee replacement surgery. RIV (Figure 1) is a small molecule (molecular mass: 436 g mol-1) that is almost insoluble in water and exhibits high plasma protein binding (92–95%) in humans, with serum albumin being the main binding component [1-3].

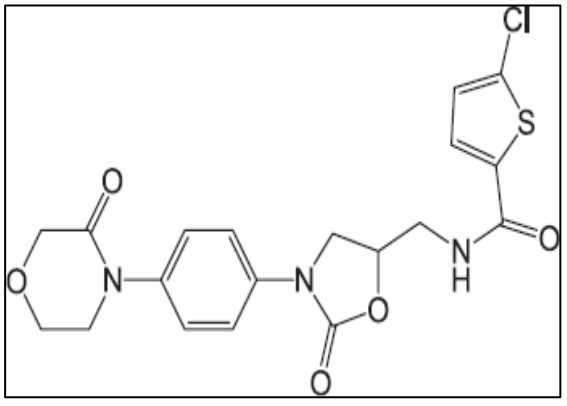

Fig. 1:Chemical structure of rivaroxaban

The potency of factor Xa inhibition occurs primarily as a result of RIV binding with high selectivity to the S1 and S4 pockets of the serine endopeptidase [4]. Inhibition of factor Xa interrupts the intrinsic and extrinsic pathway of the blood coagulation cascade, inhibiting both thrombin formation and development of thrombi. RIV does not inhibit thrombin (activated Factor II), and has no effects on platelets have been demonstrated [5-6]. The RIV was approved for marketing by Health Canada and European Commission in 2008. In the literature, there is a reported HPLC-MS method for the determination of RIV in human plasma for pharmacokinetic studies [7]. However, there is no method reported for the quantification of RIV in pharmaceutical dosage forms. It is well known that quality control is an important task in the pharmaceutical industry. The term quality control refers to the sum of all procedures undertaken to ensure the identity and purity of a particular pharmaceutical [8]. Quality control measurements include stability testing of the drug formulation, dissolution testing and analysis of raw materials and synthesis products. A pharmaceutical company usually has to measure a large number of quality control samples and UV is a unique technique for the analysis of wide variety of samples [9].

In this study, it was aimed to develop an accurate, precise, robust, rapid and selective UV method for determination of RIV in tablet dosage forms. The stability of RIV was evaluated and also a forced degradation procedure was applied under stress conditions like high temperature, acidic-alkali conditions, and irradiation with UV light. The developed method was fully validated according to the ICH [10-12] guidelines and observed that it was capable of determining RIV in the presence of forced degradation products. Therefore, it could be concluded that this method could be proposed for the quality control process of RIV in pharmaceutical industry.

Materials and Methods Pre-formulation study Organoleptic Properties

The organoleptic studies of drug general appearance like nature, color, odor, taste etc. were performed and observed.

Solubility study

Method: Saturation Shake-Flask (SSF)

Aqueous solubility is an important physicochemical property of drug substance which determines its systemic absorption and in turns its therapeutic efficacy. Solubility of drug was determined in Acetonitrile (ACN), Water, Methanol, DMSO and Dimethyl formamide. The sample was qualitatively tested for its solubility in various solvents. It was determined by shaking 2 mg of drug sample in 5 ml of solvent in a small test tube and observed to disappear the sample completely.

Melting point

Capillary method was adopted for the determination of melting point of Rivaroxaban

Partition coefficient:

The partition coefficient of drug was examined in, n-Octanol: water solvent system. It was determined by taking 5mg of drug in a separating funnel containing, 20ml of n-Octanol and 20 ml water. The separating funnel was shaken for 2 hours in a wrist action shaker for equilibrium. Two phases were separated and the amount of drug in aqueous phase was analysed spectrophotometrically at 248 nm. The partition coefficient of drug was calculated using the following formula-

Partition coefficient, $K = \frac{Amount of drug\ in\ organic\ Phase}{Amount of drug\ in\ aqueous\ phase}$

UV Method Development

Method was validated for different parameters like linearity, precision, and ruggedness study.

Standard stock solution preparation

About 5mg of rivaroxaban was weighed and transferred into 5ml volumetric flask. The volume was made up to 5ml using respective solvent to obtain a solution that has a concentration 1000 µg/ml. 1ml of this stock solution was taken and then diluted up to 10 ml using respective solvent to obtain a solution that has a concentration 100 µg/ml which is standard stock solution**.**

Lambda max

From the above stock solution 0.4ml sample was transferred into a 5 ml volumetric flask and the volume was made up to mark with solvent to prepare a concentration of 8 µg/ml. The sample was scanned by UV-VIS Spectrophotometer in the range of 200- 400 nm, using respective solvent as a blank. The wavelength corresponding to the maximum absorbance (max) was found 248 nm.

Linearity curve

Aliquots of 4, 8, 10, 12, 14, 16, and 18 µg/ml prepared utilizing 100 µg/mL Rivaroxaban working standard solution were accurately transferred into a series of 5 mL calibrated flask and made up to the mark with solvent. The absorbance of the resulting solution was measured 248 nm against ACN: Water blank. Calibration curve was prepared by plotting the absorbance vs concentration of drug. Seven points calibration curve were obtained in a concentration range from 4-18 µg/ml for drug. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was $y = 0.0301x + 0.0201$ with correlation coefficient $R2 = 0.9863$**.**

Precision

Both Inter- day precision and Intra-day precision were carried out as per the statistical requirement to support reproducibility of the method.

Intra Day Assay

The assay procedure was carried out in the same day in the duration of 2 hours to 3 hours at fixed concentration and the results were compared.

Inter Day Assay

The assay procedure was carried out in the after day in the duration of 24 hours at fixed concentration and the results were compared.

Robustness

To determine the robustness, the same procedure was carried out by changing the temperature and the result is compared with the same previous procedure.

Ruggedness

The ruggedness of the method was determined by carrying out the analysis using two different analysts and the respective absorbance was noted. Ruggedness of the methods was assessed by carrying out assay 6 times with different analyst by using same equipment.

Fourier transmission Infra-Red Spectroscopy

The KBr disc was prepared using 1 mg of drug in 100 mg of spectroscopic grade KBr which has been dried using IR lamp. Both KBr and drug was mixed and subjected to hydraulic pressure to form disc. This disc was placed in FT-IR chamber. Infrared spectrum was recorded in the 4000 - 400 cm-1 region.

RESULTS AND DISCUSSION

Table 1: Organoleptic evaluation

S. No.	Drug	Parameter	Inference
1	Rivaroxaban	Colour	White
2		Odour	Odourless
3		Taste	Complies

Table 2: Solubility profile of Rivaroxaban in different solvents.

S. No.	Solvents	Category
1.	Dimethyl sulfoxide	Freely soluble
2.	Dimethyl formamide	Freely soluble
3.	Acetonitrile	Freely Soluble
4.	Water	Soluble
5.	Methanol	Slightly soluble

Table 3: Melting point

S. No.	Drug	Specification	Inference
1	Rivaroxaban	227-230º C	228ºC

Table 4: Partition coefficient

S. No.	Solvent	Partition coefficient
1	n-Octanol: water	1.3

Table 5: Calibration curve

Conc. (µg/ml)	Avg Abs
4	0.149
6	0.171
8	0.27
10	0.322
12	0.399
14	0.437
16	0.518
18	0.54
Mean	0.35075
SD	0.148283271
%RSD	42.28

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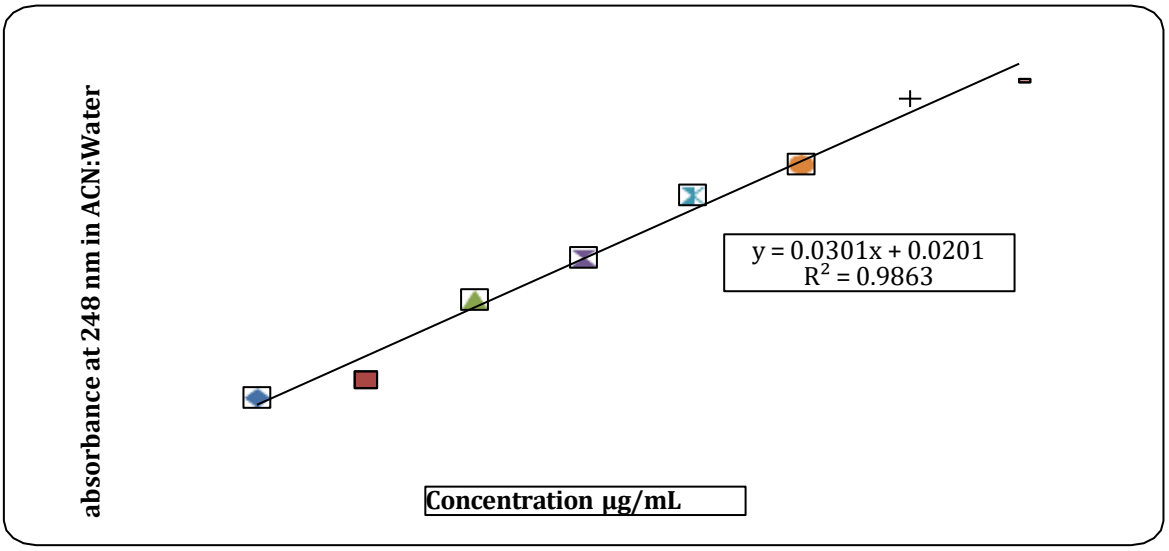

Fig. 2: Calibration curve of Rivaroxaban

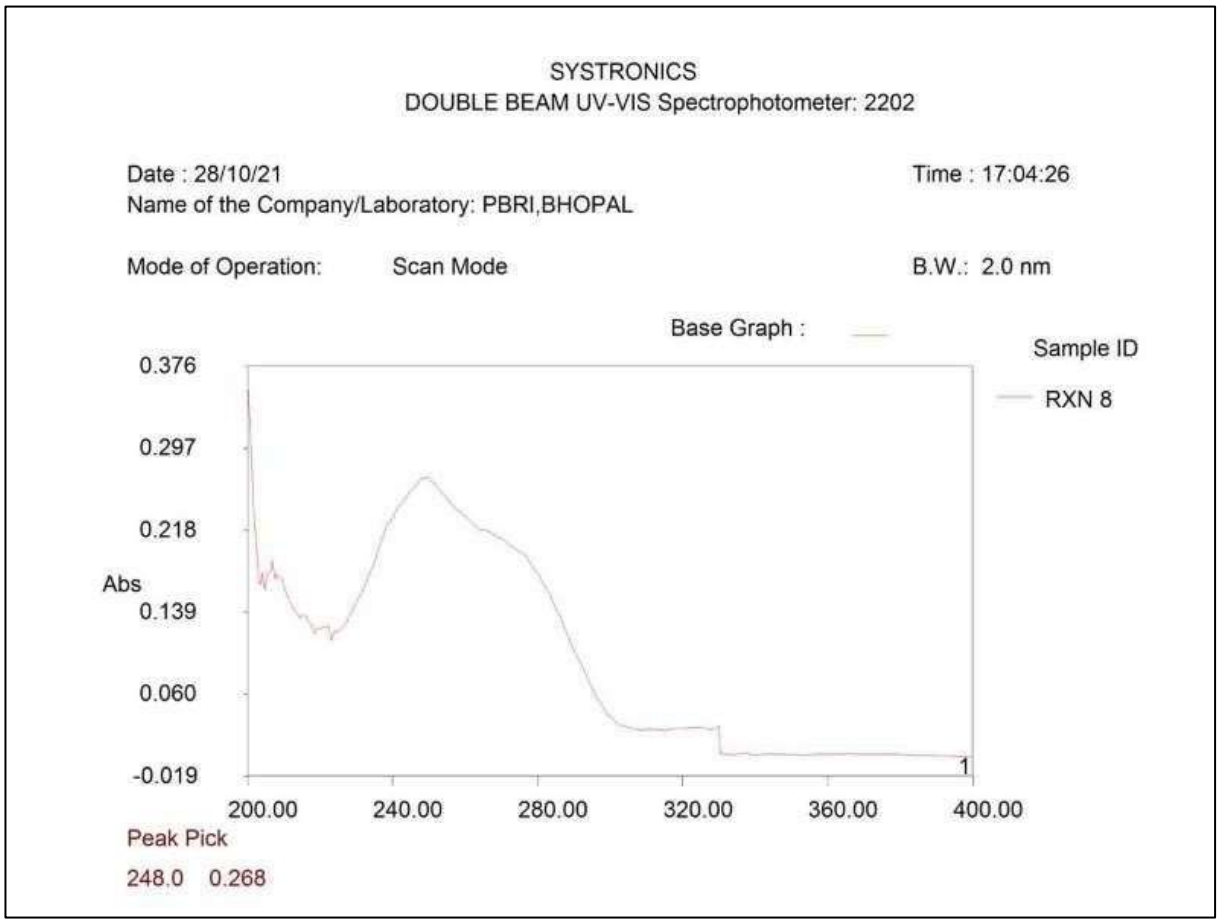

Fig. 3: Lambda max of Rivaroxaban

Concentration (µg/ml)	Absorbance	Statistical analysis	
8	0.269	Mean	0.2705
8	0.271	SD	0.002664583
8	0.267	% RSD	0.74
8	0.273		
8	0.269		
8	0.274		

- **% RSD should be less than 1**

Table 7: Intraday Precision.

Concentration (µg/ml)	Absorbance1	Absorbance2	Absorbance3	AVG % RSD
8	0.269	0.268	0.259	1.49
8	0.271	0.273	0.268	
8	0.267	0.270	0.268	
8	0.273	0.266	0.276	
8	0.269	0.261	0.273	
8	0.274	0.271	0.263	
mean	0.2705	0.268166667	0.267833333	
SD	0.002664583	0.004262237	0.006242329	
% RSD	0.74	1.49	2.24	

Table 8: Inter-day Precision

Concentration (µg/ml)	Absorbance (Day 1)	Absorbance (Day-2)	Absorbance (Day-3)	AVG % RSD
8	0.269	0.273	0.267	1.11
8	0.271	0.263	0.271	
8	0.267	0.269	0.269	
8	0.273	0.273	0.271	
8	0.269	0.267	0.271	
8	0.274	0.265	0.278	
mean	0.2705	0.268333333	0.271166667	
SD	0.002664583	0.004131182	0.003710346	
% RSD	0.74	1.49	1.10	

Table 9: Result of ruggedness

Analyst-1			Analyst-2	
Concentration (µg/ml)	Absorbance		Concentration (µg/ml)	Absorbance
8	0.269		8	0.268
8	0.271		8	0.271
8	0.267		8	0.263
8	0.273		8	0.269
8	0.269		8	0.271
8	0.274		8	0.273
mean	0.2705		mean	0.269166667
SD	0.002664583		SD	0.003488075
% RSD	0.74		% RSD	1.11

Table 10: Results showing robustness

Temperature 25		Temp 30	
Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
8	0.269	8	0.273
8	0.271	8	0.271
8	0.267	8	0.261

8	0.273	8	0.268
8	0.269	8	0.273
8	0.274	8	0.273
mean	0.2705	mean	0.269833333
SD	0.002664583	SD	0.004750439
% RSD	0.74	% RSD	1.48

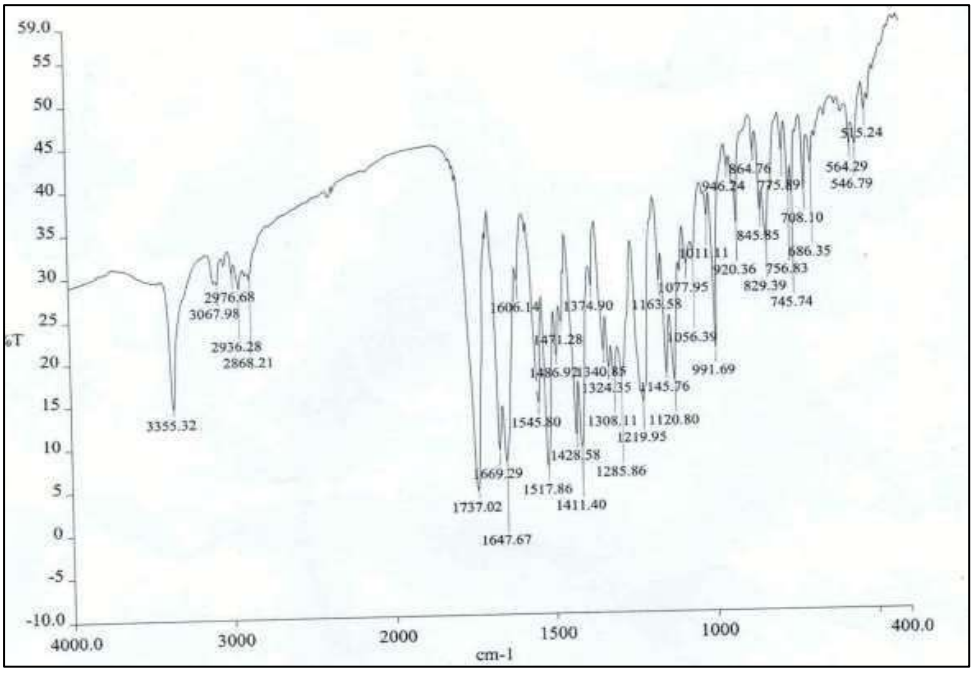

Fig. 4: FTIR of Rivaroxaban

Method validation

The proposed method was validated as to selective, linearity range, sensitivity, precision, accuracy, robustness and ruggedness according to the ICH guideline (ICH, 2005).

Selectivity

Selectivity of a method refers to the extent to which it can determine particular analyte(s) in a complex mixture without interference from other components in the mixture. Selectivity of the method was evaluated by preparing the analytical placebo sample, standard solution and sample of commercial pharmaceutical formulation. The placebo chromatograms did not show any other peaks, thus, it was confirmed the selectivity of the method. The peak purities confirmed that the peaks on the standard solutions, tablet solutions and forced degradation solution are not interfered coming from matrix components. The retention times of peaks and peak areas were identical for the standard solutions and the tablet solutions and the method was capable of separating RIV and IS from forced degradation products.

Linearity

The linearity of the proposed method was established by least squares linear regression analysis of the calibration curve. The regression equation for RXN was obtained by plotting absorbance versus concentration of RXN in the range of 4-18 µg mL-1. The regression equation was y = 0.0301x + 0.0201. The regression coefficient (R2 = 0.9863) was calculated.

Seven points calibration curve were obtained in a concentration range from 4-18 µg/ml for drug. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was y = 0.0301x + 0.0201 with correlation coefficient R2 = 0.9863**.**

Precision and Accuracy

The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). The intermediate precision was assessed by comparing the results obtained on three different days. The experimental values obtained for repeatability (intra-day precision) and intermediate precision (inter-day precision) are found to be 0.269±0.003 and 0.269 ± 0.002and % RSD were recorded 1.49 and 1.11

respectively. [13-14].

Recovery

In order to know whether the excipients in the pharmaceutical formulations interfere with the analysis, the recovery tests were performed by standard addition technique. Three concentration levels were selected and known amounts of RIV standard solutions were added into the tablet solutions. The final concentrations were within the linear range. These solutions were prepared three times and analyzed through the developed method. Comparison of the intercepts of calibration curve (0.0952 ± 0.007 (mean ± SE)) with standard additions technique (0.0936 ± 0.0201 (mean ± SE)) indicates that they were identical and there was no interference coming from matrix components.

Robustness

The robustness of the proposed method was assessed with changes in the analytical temperature. Robustness was carried out at concentration 8 µg/ml at temperature 25℃ and 30℃. The results are expressed as standard deviation and relative standard deviation and was recorded 0.2705±0.002 and 0.264±0.020 whereas % RSD was found 0.74 and 1.48 respectively. The results are compiled in Table

Reproducibility

Ruggedness of the proposed method was evaluated by comparison of the absorbance of RXN that have been measured by two different analysts in the same laboratory. Ruggedness was carried out at 8 µg/ml concentration. The results are expressed as standard deviation and relative standard deviation and it was found 0.2705±0.002 for analyst-1 and 0.268±0.010 for analyst-2 whereas the value of % RSD was recorded 0.75 and 1.11 respectively. The analysis results having no significant difference indicate that the proposed method is robust. The results are presented in Table

Fourier transmission Infra-Red Spectroscopy

IR spectrum of rivaroxaban was interpreted and the functional groups were identified. The first peak was found at 3355.32 cm^{-1}which confirmed the presence of amide group. Aromatic alkane (C-H) stretching peak and in ring peak were identified at 3067.98 cm^{-1} and 1606.14 cm^{-1} respectively. Alkane (C-H) stretching peak was found at 29366.28 cm^{-1}. Free amide (C=O) stretching peak was found at 1737.02 cm^{-1}. Associated amide (C=O) stretching peak was found at 1606.14 cm^{-1}. Aromatic ester (C-O) stretching peak was identified at 1285.86 cm^{-1}. Aliphatic amines (C-N) stretching peak was found at 1145.76 cm^{-1}. Aliphatic ether (C-O) stretching peak was found at 1077.95 cm^{-1}. Aromatic sulfur (C-S) stretching peak was found at 991.69 $cm^{-1}$and halo compound (C-Cl) stretching peak was found at 845.85 cm^{-1}.The FTIR spectra of rivaroxaban confirmed the presence of the main functional group such as amide, alkane, ester, sulfur etc.[Fig. 4]

CONCLUSION

In the present study, an attempt was made to develop a simple, accurate, selective and sensitive spectroscopic method of RIV in pharmaceutical analysis. This method is the only reported method up to date for the determination of RIV in pharmaceutical dosage forms. The method was validated for selectivity, accuracy, linearity, precision (inter-day and intra-day), , robustness and ruggedness in accordance with ICH guidelines. The results from stress testing, including separation of the degradation product and quantification of RIV after exposure to stress conditions show the method is stability-indicating and capable of determining RIV in presence of its degradation products, which indicates the selectivity of the method. The FTIR spectra of rivaroxaban indicated the presence of the main functional group.

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