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SPECTROSCOPIC INVESTIGATION AND VALIDATION OF ACTIVE PHARMACEUTICAL INGREDIENT IN PURE FORM AND IN MARKETED FORMULATIONS

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ABSTRACT

Rivaroxaban is an anticoagulant medication (blood thinner) used to treat and prevent blood clots. Rivaroxaban inhibits both free and bound Factor Xa in the prothrombinase complex. It is a selective direct factor Xa inhibitor with an onset of action of 2.5 to 4 hours. This paper describes a simple, accurate, specific and validated method for the estimation of Rivaroxaban in pure and in tablet dosage form. A simple study was carried out of all the parameters established as per ICH guidelines to validate an analytical method for estimation. The method showed high sensitivity with reproducibility in results. The wavelength maxima (λ_{max}) was found to be 250 nm. The linearity for this method was found to be in the range of 2 – 10µg/ml. The calibration curve (Fig -2) was drawn by plotting graph between absorbance and concentration. This method showed a correlation coefficient of 0.9997. The regression equation of the curve was Y= 0.02897x+0.05032. Method was successfully validated. In addition, this proposed method was simple, sensitive, and easy to apply and requires relatively inexpensive instruments. The proposed method can be used for routine analysis of Rivaroxaban in bulk as well as in the commercial formulations.

KEYWORDS: Rivaroxaban, UV Spectroscopy, Tablet dosage form, Prothrombinase, Statistical validation, ICH Guidelines.

INTRODUCTION

It is an anticoagulant medication (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. It is taken by mouth. Rivaroxaban was patented in 2007 and approved for medical use in the United States in 2011. In the United States, it will not be available as a generic medication until 2024. In those with non-valvular atrial fibrillation, it appears to be as effective as warfarin in preventing ischemic strokes and embolic events. Rivaroxaban is associated with lower rates of serious and fatal bleeding events than warfarin, though rivaroxaban is associated with higher rates of bleeding in the gastrointestinal tract. Because of the difficulty associated with managing bleeding, rivaroxaban should be discontinued at least 24 hours before surgery, then restarted as soon as adequate hemostasis is established. The most serious adverse effect is bleeding, including severe internal bleeding. Rivaroxaban is associated with lower rates of serious and fatal bleeding events than warfarin but is associated with higher rates of bleeding in the gastrointestinal tract.

Rivaroxaban inhibits both free and bound Factor Xa in the prothrombinase complex. It is a selective direct factor Xa inhibitor with an onset of action of 2.5 to 4 hours. 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. It allows predictable anticoagulation and dose adjustments and routine coagulation monitoring; dietary restrictions are not needed.

In the present study, the method UV-Spectroscopy was based on estimation of Rivaroxaban. Absorption spectrometry is the measurement of the selective absorption by atoms, molecules of electromagnetic radiation having a definite and narrow wavelength range. Absorption spectroscopy encompasses the wavelength regions: UV (200-380nm) Visible (380-780nm). Near –IR (780nm - 2.5µm) and Far -IR (2.5 - 40µm) from the UV spectra 250 nm was selected as λmax for analysis of Rivaroxaban, using Ethanol as solvent. It was observed that Rivaroxaban in Ethanol was stable for 3.2 hrs.

MATERIALS AND METHODS

Rivaroxaban was obtained as a gift sample from Burgeon Pharmaceuticals Pvt.Ltd., Pondicherry. Xarelto 20 mg, Bayer Zydus PharmaPvt Ltd″ Tablets containing 20 mg of

Rivaroxaban was purchased from local Pharmacy. All the solvents and chemicals used were of analytical reagent grade and procured from Chemicals and solvents are from Qualigens India pvt. Ltd., and Lobe Chemist India Ltd.

Instruments

Kerro P5 Series Precision Electronic Balance, Model Bl -3003, T60 UV-Visible spectrophotometer with 1 cm matched quartz cells, Sonicator Sonica Ultrasonic cleaner model 2200 mH.

Method – Simple UV- Spectroscopy

The solubility of Rivaroxaban was determined in a variety of solvent ranging from non polar to polar using essentially a method of Schefter and Higuchi. The drug was found to be freely soluble in DMSO, Ethanol, Methanol and Sparingly soluble in Acetic acid. Considering the economic factor and the drug were stable in Ethanol for 3.2 h. Ethanol was selected as the solvent for method.

Preparation of standard stock solution

10 mg of Rivaroxaban raw material was accurately weighed and transferred into the 100 ml volumetric flask and dissolved in minimum quantity of Ethanol and made up to 100 ml with double Ethanol.

Select ion of λ_{max} and Stability studies

Standard stock solution was further diluted with Ethanol to get 10 µg/ml concentration. The solution was scanned between 200 and 400 nm range using Ethanol as blank. From the UV Spectra 250 nm was selected as λ_{max} for analysis of Rivaroxaban. Stability of the Rivaroxaban in Ethanol was studied by measuring the same solution at this λ_{max} in different time intervals. It was observed that Rivaroxaban in water was stable for more than 3.2 hours.

Calibration Graph and Linearity

In this aliquot of stock solution of Rivaroxaban were transferred in to 10 ml volumetric flask and made up to the mark with Ethanol. The absorbance of different concentration solutions was measured at 250 nm against blank. The samples were found to be linear from 2-10 µg /ml. The calibration curve was plotted using concentration Vs absorbance. The curve obtained was linear in the concentration range of 2-10 µg /ml.

Quantification of formulations

Contents of twenty Tablets of formulation (**Xareltos**) containing 20mg of Rivaroxaban was accurately weighed to find out the average weight. Tablets powder equivalent to 20 mg of Rivaroxaban was transferred in to 50 ml volumetric flask, added Ethanol and made up to the volume. Then the solution was sonicated for 15 minutes. After sonication, the solution was filtered through Whatmann filter paper No.41. From the clear solution, further dilution was made to bring a 10 µg /ml using Ethanol. The prepared solution was measured at 250 nm. The amount of Rivaroxaban was determined by using slope and intercept values from calibration graph.

Recovery studies

To the pre-analyzed formulation, a known quantity of standard solution (2, 10 and 20 µg/ml solution) was added and the contents were mixed well, finally made up to the volume with Ethanol. Absorbance was measured at 250nm. Amount present was calculated from slope and intercept. Then the % recovery was determined.

Statistical validation

The obtained results were treated for statistical validation parameters like Standard Deviation (SD) and Percentage Relative Standard Deviation (% RSD).

RESULTS AND DISCUSSION

The solubility of Rivaroxaban was determined in a variety of solvent ranging from non polar to polar using essentially a method of Schefter and Higuchi. The drug was found to be freely soluble in Ethanol, methanol, DMSO, Ethyl acetate and Benzene Sparingly soluble in Chloroform and 0.1 M NaoH. Rivaroxaban was scanned in the range of 200-400 nm and it shows constant λ_{max} at 250 nm. the optical characteristics such as Beer's law limit (2-10µg/ml), sandell'sensivity (0.021837), correlation coefficient (0.9997), slope(0.02897) and intercept (0.05032), molar absorptivity ($1.02510x10^2$), were calculated for Rivaroxaban in Ethanol.

The limit of detection and limit of quantification were determined from the linearity studies. The limit of detection was found to be 0.6073µg/ml and the limit of quantification was found to be 1.05720µg/ml. It is shown in Table 2. Table 3 shows the result of formulation quantification on XARELTO Tablets repeatability also found to be within the limits 99.06 – 100.60, %RSD value 1.407 and SE value 0.00026.

To evaluate the accuracy of the method, known amount of pure drug (2, 10 and 20 µg/ml solution) was added to the previously analysed solution containing pharmaceutical formulation and the mixture was analysed by the proposed method and the recoveries were calculated. The percentage recovery of Rivaroxaban sample was found with in the limit 99.68%-103.72% Mean of SD 98.19±1.5721 (%RSD 1.7054, SE 0.002681).

SUMMARY AND CONCLUSION

The proposed analytical methods are simple, reliable, rapid, sensitive, reproducible and accurate for the estimation of Rivaroxaban.

The method adopted for our studies are Simple UV-Spectroscopic method.

The drug samples were analyzed by UV spectroscopy using Ethanol as solvent and the average content of drug present in the formulation was found to be 99.51 mg (99.51%).

The above method does not suffer from any interference due to common excipients. Therefore, it was shown that the proposed methods could be successfully applied to estimate commercial pharmaceutical products containing Rivaroxaban. Thus the above studies and findings will enable the quantification of the drug for future investigation in the field of analytical chemistry.

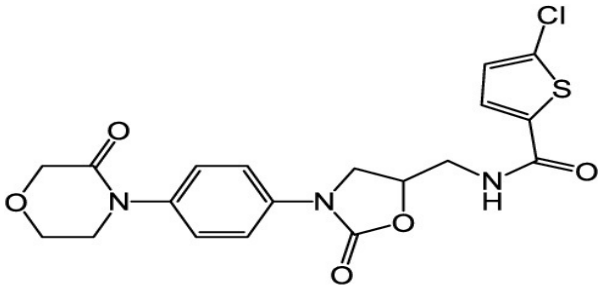

Fig. 1: Structure of rivaroxaban.

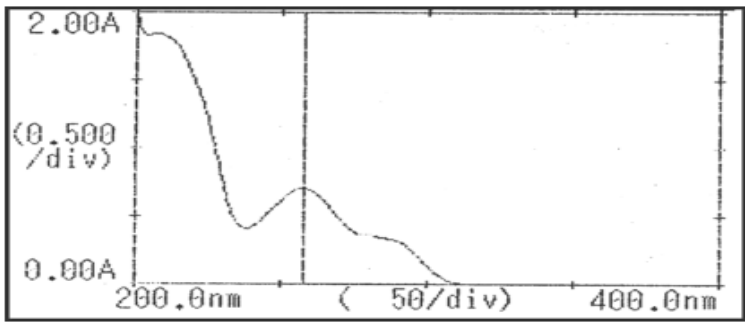

Figure II: UV-Spectroscopic spectrum.

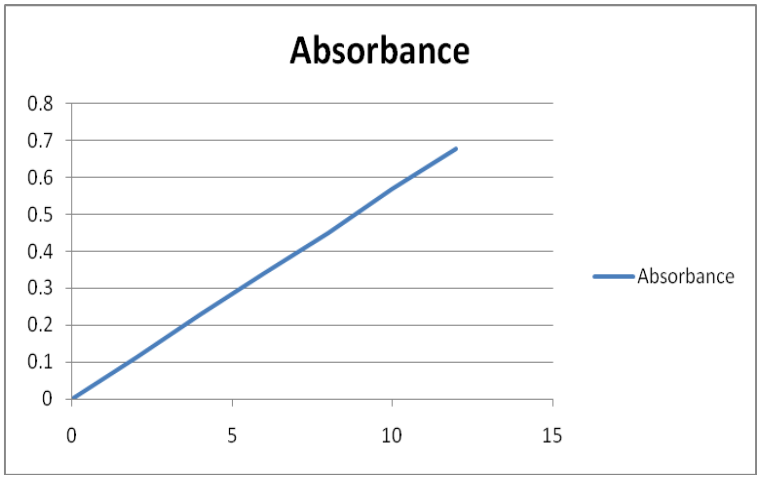

Figure III: Linearity graph.

Table 1: Optical characteristics of rivaroxaban.

Parameters	Method Values
λ_{max}(nm)	250
Beer's law limit(µg/ml)	2-10
Sandell's sensitivity (µg/cm^2/0.001 AU)	0.022417
Molar absorbtivity(L mol^{-1} cm^{-1})	1.02510x10^2
Correlation Co-efficient (r)	0.9997
Regression equation (Y=mx+c)	Y=0.02897x+0.05032
Slope(m)	0.02897
Intercept(c)	0.05032
LOD(µg/ml)	0.6073
LOQ(µg/ml)	1.05720
Standard error of mean of regression line	0.00026

Table 2: Quantification of Formulation- Itoprid by UV method.

S. No.	Labelled Amount (mg/Tab)	Amount found (mg/Tab)	% Obtained	Average %	S. D	%RSD	S. E
1	20	20.06	100.12	99.79	1.57216	1.407	0.00026
2	20	29.71	99.42				
3	20	29.63	99.26				
4	20	29.93	99.86				
5	20	20.01	100.02				
6	20	20.05	100.10				

SD is standard deviation, % RSD percentage relative standard deviation

*Average of six determinations

Table 3: Recovery Studies for Formulation- Itoprid by UV method.

S. No	Amount present (µg/ml)	Amount added (µg/ml)	Amount estimated (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean of ± S.D	%RSD	S.E
1	2.04 1.83 1.78 1.96 2.01 2.03	2 2 2 2 2 2	4.01 3.83 3.76 4.01 3.98 4.00	1.97 2.00 1.98 2.05 1.97 1.97	98.5 100.00 99.00 102.5 98.50 98.50	99.50 ± 1.9216	1.9351	0.7903
2	10.04 9.83 9.78 9.96 10.01 10.03	5 5 5 5 5 5	9.91 9.68 9.77 9.99 10.01 9.89	9.87 9.85 9.99 10.03 10.0 9.86	97.4 97.00 99.80 100.6 100 97.20	98.66 ± 1.8960	1.9255	0.7861
3	20.04 20.83 20.78 20.96 20.01 20.03	20 20 20 20 20 20	99.27 100.1 100.8 100.15 99.31 99.37	19.23 19.27 20.02 19.19 19.30 19.34	98.46 98.54 100.04 98.38 98.6 98.68	98.78 ± 1.0190	0.97374	0.397

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