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NEW SPECTROPHOTOMETRIC METHODS FOR THE QUANTITATIVE ESTIMATION OF RIVAROXABAN IN FORMULATIONS

P.V.V.Satyanarayana*,

Alavala Siva Madhavi

Department of Chemistry, AcharyaNagarjunaUniversity, Nagarjuna Nagar, Guntur, Andhra Pradesh, India

Contact Mail id: sivamadhavi@rocketmail.com

ABSTRACT

Rivaroxaban is used as an oral anticoagulant. The quantitative estimation of Rivaroxaban the analytical methods present are of relatively expensive and hence need for the simple analytical methods. In this proposed research work five such new spectrophotometric methods have been developed and applied for routine determination of Rivaroxaban in pharmaceutical formulations and bulk dosage forms. These methods are based on the color formation of the drug on binding with the different reagents. All these method have different linearity ranges. All these method are simple, Accurate, precise and very effective even at low concentrations and used for the quantitive estimation of Rivaroxaban in commercial formulations

KEYWORDS: Rivaroxaban, Spectrophotometric Methods, INH, Naptha Quinone sulphate, Haemtoxylin, 2,2 Bipyridine , 4-Amino phenazone.

INTRODUCTION

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 Pharmaceutica.[2] It is the first available orally active direct factor Xa inhibitor. Rivaroxaban is well absorbed from the gut and maximum inhibition of factor Xa occurs four hours after a dose. The effects lasts 8–12 hours, but factor Xa activity does not return to normal within 24 hours so once-daily dosing is possible. 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.[1]

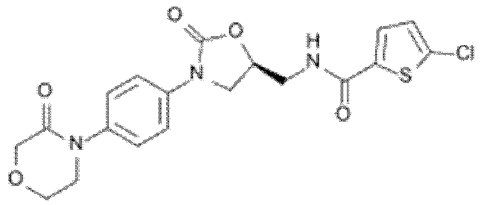

Figure 1: Structure of Rivaroxaban

Rivaroxaban has predictable pharmacokinetics across a wide spectrum of patients (age, gender, weight, race) and has a flat dose response across an eightfold dose range (5–40 mg).[6] Clinical trial data have shown that it allows predictable anticoagulation with no need for dose adjustments and routine coagulation monitoring.[1] However, these trials have excluded patients with liver disease and end-stage liver disease; therefore, the safety of rivaroxaban in these populations is unknown. Rivaroxaban bears a striking structural similarity to the antibiotic linezolid: both drugs share the same oxazolidinone-derived core structure. Accordingly, rivaroxaban was studied for any possible antimicrobial effects and for the possibility of mitochondrial toxicity, which is a known complication of long-term linezolid use. Studies found that neither rivaroxaban nor its metabolites have any antibiotic effect against Gram-positive bacteria. As for mitochondrial toxicity, in vitro studies found the risk to be low, and not likely to be of clinical consequence because rivaroxaban is only meant (and approved) for shortterm
use.[6]

Experimental Procedure:

All chemicals used were of analytical reagent grade and distilled water was used to prepare all solutions. Double beam UV-Visible Spectrophotometer is used for measuring the absorbance's of the color formed during the analysis.

Preparation of reagents:

2,2 Bipyridine : Weigh accurately 200 mg of 2,2 Bipyridine and was dissolved in 100 ml of distilled water with warming.

4-Amino Phenazone solution: Weigh accurately 500mtg of 4-Amino Phenazone and was dissolved in 100 mL of Methanol containing 1 mL of conc. HCl.

Haematoxylin : 0.2 % in methanol.

Chloramine T: 0.4 % in distill water.

Iso Nicotanic hydrazide solution: Weigh accurately 800 mg of Iso Nicotanic hydrazide and is dissolved in 100 mL of MeOH containing 1% of conc. HCl.

Naptha Quinone Sulphate: Weigh accurately 100 mg of Naptha Quinone Sulphate and was dissolved in 100 ml of distilled water.

Fe (III) solution: Accurately 250 mg of anhydrous ferric chloride was weighed and was taken in a100 ml graduated volumetric flask. It was dissolved in little amount of distilled water and the final volume was made up to the mark with distill water.

HCl solution (1N): Prepared by diluting 86 ml of conc. HCl to 1000 ml with distilled water and standardized.

Buffer pH 7: 390 ml of 0.067 M KH_2PO4 was prepared and is added to 610 ml 0.067 Na_2HPO_4 in Distill water.

NaOH Solution: Weigh accurately 20g of Sodium Hydroxide and was dissolved in 100 ml of distilled water.

Preparation of working standard drug solution:

The standard Rivaroxaban (100 mg) was weighed accurately and transferred to volumetric flask (100 ml). It was dissolved properly and diluted up to the mark with methanol to obtain final concentration of 1000 µg /ml (stock solution I). 10 ml of stock solution I was diluted to 100 ml with Methanol (Stock solution II, 100 µg/ml) and the resulting solution was used as working standard solution.

Methods:

2,2 Bipyridine Method: (M1)

From the standard stock solution II of Rivaroxaban, appropriate concentration(20 to120 ppm) is pipetted out in to a 25 ml volumetric calibrated tube, 0.5 ml FeCl3 solution and 2 ml of 2,2 Bipyridine were added. The tube was heated in water bath up to 30 min. after cooling the tube 1 ml of acid was added and make up to 25 ml with distilled water. Make up to 25 ml volume. The absorbance of the formed color was measured after 5min at 470 nm against a reagent blank.

4-Amino Phenazone Method: (M2)

From the standard stock solution II of Rivaroxaban 0.5 mL of standard drug solution (3-21ppm) were transferred into a series of 10 mL calibrated tubes. Then 3.0 mL of 4-Amino Phenazone solution was added to each tube and kept aside for 15 min. Later the solution in each tube was made up to 10 ml with methanol. The absorbance was measured at 450 nm against the reagent blank.

Haematoxylin Method: (M3)

To series of 25 mL tubes 1 mL of Haematoxyline and 1mL of Chloramine T was added. To this add 10 mL of pH 7.0 buffer solution. Kept a side for 20 min. Then sample solution ranging to 30– 90 µg of drug , kept in a water bath at 70 ^{0}C for 5 min. cool to room temperature and made up to 25 mL with distill water. The absorbance was measured at 740 nm against the reagent blank.

Iso Nicotanic hydrazide Method: (M4)

Aliquot of standard drug solution (5-30ppm) was delivered into a series of 10 ml of calibrated tubes. Then 2.0 mL of Iso Nicotanic hydrazide solution was added to each tube and heated for 10 min at 60 ^{0}C. The solution in each tube was cooled and made up to 10 mL with methanol. The absorbance was measured at 470 nm against the reagent blank.

Naptha Quinone Sulphate Method: (M5)

Aliquot of standard drug so0lution was transferred in to a series of calibrated test tubes containing 0.2ml of NaOH and 0.2 of Naptha Quinone Sulphate reagent solution was added in each tube and the contents were heated at 50^{0}c for a min and cooled for 2min ice water. This operation was performed in the dark. After cooling the contents in the tube were rinsed with 1ml of water. These rinsing were transferred in to 25ml separating funnel containing 10ml of dichloro methane and shaken immediately for 5sec. the whole organic layer from the bulk was collected from the funnel after 2min of mixing 3ml DNPH was added. It was heated for 10min at 50^{0}c by using air condenser and chilled in ice water. Then .5ml of concentrated sulphuric acid was added slowly, mixed the absorbance were measured after 5min at 500nm against the reagent blank.

Assay Procedure for Formulations:

An amount of finely ground tablet powder equivalent to 100 mg of Rivaroxaban (XARELTO - 10mg) was accurately weighed into a 100 ml calibrated flask, 60 ml of water added and shaken for 20 min. Then, the volume was made up to the mark with water, mixed well, and filtered using a Whatman No 42 filter paper. First 10 ml portion of the filtrate was discarded and a suitable aliquot of the subsequent portion (1000 µg mL-1 Rivaroxaban) was diluted appropriately to get suitable concentrations for analysis by proposed methods.

Method Validation:

Selection of analytical concentration ranges: (linearity test)

Linearity test was evaluated by measuring the absorbance values of standard solutions. The standard stock solution of Rivaroxaban, appropriate aliquots were pipetted out in to a six or seven series of volumetric flasks and add the solutions required in required for each individual method. After color formation absorbance of each concentration was measured at wavelength found for the proposed method. Results were shown in Table: 1 and Standard graphs of linearity for proposed methods were shown below.

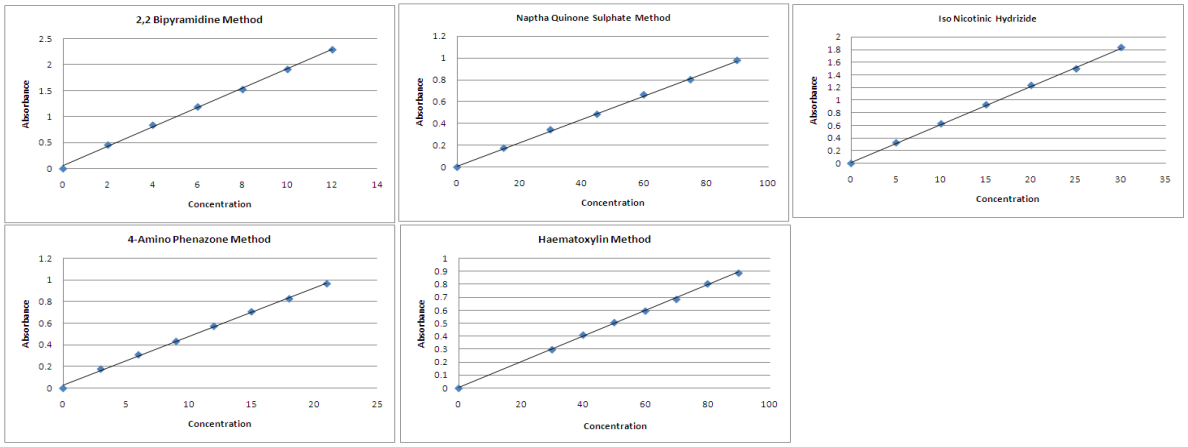

Figure 1: Calibration curves for the proposed methods.

S.NO	Parameter	M1	M2	M3	M4	M5
1	Wavelength Max	470nm	450nm	540nm	480nm	500nm
2	Concentration Range	2-12ppm	3-21ppm	30-90ppm	5-30ppm	15-90ppm
3	Correlation coefficient	0.9992	0.9991	0.9996	0.9997	0.9996
4	Slope	0.187	0.045	0.01	0.06	0.01
5	Intercept	0.051	0.027	0.005	0.016	0.009
6	RSD of Precision	0.47	0.88	0.94	0.52	1.02
7	Average recovery	100.04	99.61	100.29	99.94	100.18
8	Stability period	130min	210min	210min	120min	170min
9	LOD	0.03ppm	0.15ppm	9 ppm	0.15 ppm	1.5 ppm
10	LOQ	0.1ppm	0.5ppjm	10 ppm	0.5ppm	5 ppm
8	% Assay of Formulation	98.17	98.58	98.53	99.3	99.25

TABLE.1 RESULTS

Precision:

To evaluate the accuracy and precision of the methods, pure drug solution (Within the working limits) was analyzed and being repeated six times. The relative error (%) and relative standard deviation (%) were less than 2.0 and indicate the high accuracy and precision for the proposed methods (Table 2).

SNO	M1	M2	M3	M4	M5
Concentration	6PPM	12PPM	60ppm	20PPM	60PPM
1	1.189	0.571	0.596	1.231	0.661
2	1.196	0.579	0.591	1.226	0.669
3	1.188	0.577	0.605	1.235	0.665
4	1.199	0.572	0.601	1.239	0.656
5	1.196	0.566	0.593	1.221	0.654
6	1.185	0.568	0.592	1.232	0.652
RSD	0.47	0.88	0.94	0.52	1.02

TABLE.2 PRECISION RESULT

Recovery Studies:

To ensure the accuracy and reproducibility of the results obtained, known amounts of pure drug was added to the previously analyzed formulated samples and these samples were reanalyzed by the proposed method and also performed recovery experiments. The Percentage recoveries thus obtained were given in Table 3.

Method	Recovery	Concentration In ppm	Amount found in ppm	% of recovery	Average Recovery
M1	50%	2	1.97	98.5	100.4
	100%	4	4.02	100.5	
	150%	8	8.09	101.125	
M2	50%	6	5.92	98.67	99.61

	100%	12	11.88	99	
	150%	18	18.21	101.17	
M3	50%	30	29.91	99.73	100.29
	100%	60	60.32	100.53	
	150%	90	90.55	100.61	
M4	50%	10	9.96	99.6	99.94
	100%	20	19.83	99.15	
	150%	30	30.32	101.06	
M5	50%	15	14.86	99.1	100.18
	100%	30	30.15	100.5	
	150%	45	40.38	100.95	

TABLE.3 RECOVERY RESULTS

Application to Analysis of Commercial Sample:

In order to check the validity of the proposed methods, Rivaroxaban was determined in commercial formulation. From the results of the determination it is clear that there is close agreement between the results obtained by the proposed methods and the label claim. These results indicating that there was no significant difference between the proposed methods and the reference methods in respect to accuracy and precision.

S.NO	Method	Formulation	Amount prepared	Amount found	% Assay
1	M1	Xarelto (10mg)	6ppm	5.89	98.17
2	M2	Xarelto (10mg)	12ppm	11.83	98.58
3	M3	Xarelto (10mg)	60ppm	59.12	98.53
4	M4	Xarelto (10mg)	20ppm	19.86	99.3
5	M5	Xarelto (10mg)	60ppm	59.55	99.25

TABLE.4 FORMULATION ANALYSIS RESULTS

DISCUSSION

The results obtained method M1 were due to redox reaction followed by complex formation between the anti-oxidant and ferric chloride and 2, 2-bipyridine to form an orange colored solution that exhibited maximum absorption at 470nm against the corresponding reagent blank.

In method M2, 4-Amino phenazone react with the keto group of the drug, results forms a schiffs base. The formed schiffs base show absorbance at 450nm.

In method M3, Haematoxylin and Chloramine T react with each other in basic media and form a compounds haematin. The lone pair electrons on the hetero sulphur group of the drug forms charge transfer spectra when react with haematin. Results the solution attain color. Absorbance of the formed color was measured at 540nm.

In method M4, the keto group of the drug reacts with Iso Nicotinic hydrazode to gives a colored Hydarzone. The formed color chromogen shows absorbance at 480nm.

The presence of imino group of the drug wills responcible for the development of the colored complex in Naptha Quinone Sulphate Method. The imino gropu of the drug undergo nucleophilic substitution with Naptha Quinone Sulphate. Results form a colored complex. The absorbance of the formed color complex was measured at 500nm.

The linearity ranges of Rivaroxaban are found to be 2-12, 3-21ppm, 30-90, 5-30ppm, 15-90 ppm for M1 to M5 respectively. A linear correlation was found between absorbance and concentration of Rivaroxaban. The graphs showed negligible intercept and are described by the equation: Y = a + bX (where Y = absorbance of 1-cm layer of solution; a = intercept; b = slope and X = concentration in ug mL-1 max) .Regression analysis of the Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient(r) for each system according to ICH guide

The accuracy of the proposed methods was further ascertained by performing Accuracy studies. The Relative standard deviations of results for the proposed were very low and the values are within the range below 2. It indicates that the high accuracy and precision for the proposed methods. The Recovery results were very close to the actual range and it revealed that co-formulated substances did not interfere in the determination.

CONCLUSIONS

Five useful micro methods for the determination of Rivaroxaban have been developed and validated. The methods are simple and rapid taking not more than 20-25 min for the assay. These spectrophotometric methods are more sensitive than the existing UV and HPLC methods, and are free from such experimental variables as heating or extraction step. The methods rely on the use of simple and cheap chemicals and techniques but provide sensitivity

comparable to that achieved by sophisticated and expensive technique like HPLC. Thus, they can be used as alternatives for rapid and routine determination of bulk sample and tablets.

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