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Development and validation of UV spectrophotometric method for the determination of rivaroxaban

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ABSTRACT

A new, less time consuming and cost effective UV spectrophotometric method was developed for the quantification of rivaroxaban in bulk. Rivaroxaban was estimated at 270 nm in dimethyl sulphoxide. The linearity range was found to be 2–20µg mL^{-1} (regression equation: Absorbance = 0.1086 × Concentration of drug in µg mL^{-1} + 0.0154; R^2 = 0.9991). The apparent molar absorptivity and sandell's sensitivity were found to be 4.825 × 10^4 L mol^{-1} cm^{-1} and 2.262 × 10^{-3} µg cm^{-2}, respectively. The method was tested and validated for various parameters according to ICH guidelines. The results demonstrated that the procedure is sensitive, selective, precise, accurate, robust, rugged and useful for the determination of rivaroxaban in bulk samples.

Key words: Anticoagulant, dimethyl sulphoxide, linearity, UV spectrophotometry, analysis.

INTRODUCTION

Rivaroxaban (RXN) [1-5] is an orally active anticoagulant, direct factor Xa inhibitor approved for the prevention of venous thromboembolic events in patients who have undergone total hip or total knee replacement surgery. RXN blocks the amplification of the intrinsic and extrinsic pathway of coagulation cascade by binding directly to the catalytic pocket of factor Xa and thereby preventing the formation of thrombus. The half-life of RXN is 5–9 hrs in young subjects and 11–13 hrs in older subjects. RXN is not recommended for use in those under 18 years old and those with severe renal impairment. Chemically, RXN is known as (S)-5-Chlor-N-{2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-ylmethyl}thiophen-2-carbamid (Figure 1).

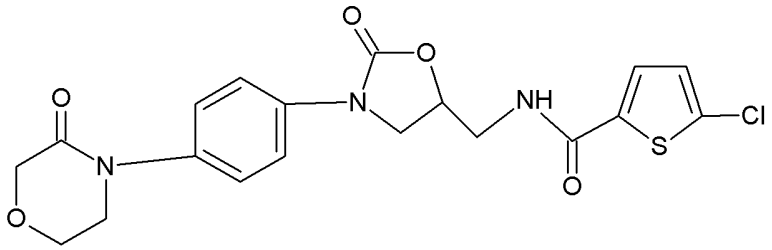

Figure 1: Chemical structure of rivaroxaban

Literature survey revealed that only few methods are available for the analysis of RXN. These methods include visible spectrophotometry [6], reverse phase high performance liquid chromatography (RP-HPLC) [7,8], high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) [9], anti-factor Xa chromogenic assay [10-14] and prothrombin time assay [15]. The reported visible spectrophotometric methods suffer from the disadvantage of heating, preparation of buffer, long reaction time, insufficiently sensitive, less precise and accurate. RP-HPLC, HPLC-MS/MS methods are deficient in simplicity, time consuming, cumbersome, costly and require an expertise operational personal. Anti-factor Xa chromogenic assay and prothrombin time assay methods are applicable only for the plasma samples.

UV spectrophotometry is still the technique of choice because of its simplicity, sensitivity, economical, rapid and easily manageable. Therefore, the aim of this work was directed to develop a rapid, simple, reliable, selective, sensitive and inexpensive UV spectrophotometric method for the determination of RXN. Dimethyl sulphoxide (DMSO) was used as solvent and the absorbance at the wavelength of 270 nm was employed to the quantification of the drug. The linearity of response, sensitivity, accuracy, precision and robustness of the described method for the assay of rivaroxaban were checked.

MATERIALS AND METHODS

2.1. Instrumentation:

All spectrophotometric measurements were carried out using an Elico model SL 159 digital spectrophotometer (Hyderabad, India). The cells used for absorbance measurements were 1-cm matched quartz cells. Samples were weighed by using Essae-Teraoka electronic weighing balance (Goa, India) PG1000 model.

2.2. Standard solutions

A stock standard solution containing 1 mg mL^{-1} of RXN was prepared in DMSO. Working standard solution equivalent to 100 µg mL^{-1} of RXN was prepared by appropriate dilution of stock solution with the same solvent.

2.3. General procedure for the analysis of rivaroxaban

Aliquots of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mL of 100 µg mL^{-1} RXN working standard solution were accurately transferred into a series of 5 mL calibrated flask and made up to the mark with DMSO. The absorbance of the resulting solution was measured at 270 nm against DMSO blank. Calibration curve was prepared by plotting the absorbance *vs* concentration of RXN. The concentration of the unknown sample was read from the calibration curve or computed from the regression equation derived using the Beer's law data.

2.4. Procedure for the analysis of placebo blank

Placebo blank is a mixture of usually added excipients in formulations. Talc (10 mg), starch (10 mg), methyl cellulose (10 mg), sodium citrate (5 mg), magnesium stearate (10 mg) and sodium alginate (10 mg) were accurately weighed and mixed well. The mixture was transferred into a 50 mL calibrated flask and 30 mL DMSO was added to the flask and the content was shaken thoroughly for 15-20 min. The volume was finally diluted to the mark with the same solvent, mixed well and filtered using a Whatman No. 1 filter paper. One mL of the filtrate (placebo blank solution) was diluted to 5 mL with DMSO and the absorbance of the resulting solution was measured at 270 nm.

2.5. Procedure for the analysis of rivaroxaban in synthetic mixture

To the placebo blank of the composition described above, pure RXN was added at three different concentration levels and homogenized. The mixture was transferred to a 50 mL calibrated flask and the solution was prepared as described under "Procedure for analysis of placebo blank", and aliquots of extracts containing three different concentrations of RXN were subjected to analysis by the procedure described under "General procedure for the analysis of rivaroxaban".

RESULTS AND DISCUSSION

3.1. Choice of solvent

The solubility of the RXN was tested in different solvents such as methanol, DMSO, 0.1 N HCl and 0.1N NaOH. The RXN was completely insoluble in 0.1 N HCl & 0.1N NaOH and soluble in methanol and DMSO. However the highest absorbance value was obtained with DMSO as the solvent. Therefore, DMSO was chosen as the solvent.

3.2. Detection of Absorption maximum

Into a 5 mL volumetric flask, 1 mL (20 µg mL^{-1}) of RXN standard solution was transferred and diluted to the mark with DMSO. Absorption maximum of RXN in DMSO was determined by scanning the RXN solution from 200-400 nm. The absorption maximum was found at 270 nm (Figure 2).

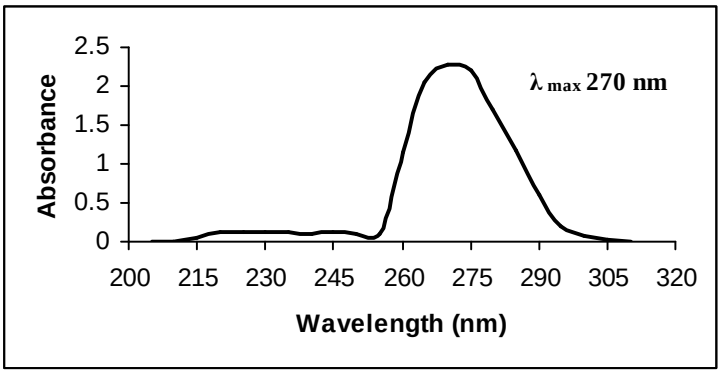

Figure 2: Absorption spectra of 20 µg mL^{-1} concentration of rivaroxaban in dimethyl sulphoxide

3.3. 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 (A) versus concentration of RXN (C) in the range of 2-20 µg mL^{-1}. The regression equation was A = 0.1086C + 0.0154. The regression coefficient (R^2 = 0.9991) was very much significant.

3.4. Sensitivity

The limits of detection (LOD) and quantitation (LOQ) were determined as specified by the ICH guidelines [16]. The LOD and LOQ values obtained respectively are 0.212 µg mL^{-1} and 0.642 µg mL^{-1}. The calculated molar absorptivity and Sandell's sensitivity are 4.825×10^4 L mol^{-1} cm^{-1} and 2.262×10^{-3} µg cm^{-2}, respectively. The values obtained for the LOD, LOQ, Sandell's sensitivity and molar absorptivity point to the highly sensitive nature of the proposed method for the determination of RXN in bulk.

3.5. Selectivity

The analysis of placebo blank was performed to study the interference by excipients such as talc, starch, methyl cellulose, sodium citrate, magnesium stearate and sodium alginate. The absorbance values of the placebo blank at 270 nm were almost equal to zero which revealed the significant selectivity of the proposed method.

3.6. Precision

The precision of the proposed method was determined by repeatability (intra-day precision) and intermediate precision (inter-day precision). The precision was expressed as relative standard deviation of a series of measurements. The repeatability was evaluated by analyses of RXN, at the three different concentration levels (2, 10, 20 µg mL^{-1}) during the same day. 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 presented in Table 1 and 2, respectively. The relative standard deviation values of the proposed method were in the range of 0.204-0.351% in intra-day precision and 0.074- 1.076% in inter-day precision. The relative standard deviation values found for the proposed method were within the acceptable range demonstrating that this method has good repeatability and intermediate precision.

Table 1: Results of intra-day precision study

Concentration of RXN (µg mL^{-1})		SD (n=5)	% RSD
Taken	Found (n=5)		
2	2.001	0.007071	0.351
10	10.174	0.028970	0.284
20	19.968	0.040866	0.204

Table 2: Results of inter-day precision study

Concentration of RXN (µg mL^{-1})		SD (n=5)	% RSD
Taken	Found (n=5)		
2	2.020	0.011547	0.571
10	10.040	0.108080	1.076
20	19.986	0.015875	0.074

3.7. Accuracy

The evaluation of the accuracy of the proposed method was performed by analyzing RXN in synthetic mixture at three different concentration levels (2, 10, 20 µg mL^{-1}). The accuracy of the proposed method was expressed as mean percentage recovery and percentage of error. The mean percentage recovery was in the range of 99.82 – 100.50% with percentage error of 0.01 – 0.50%. The results are summarized in Table 3. The excellent mean percentage recovery values and their percentage error values revealed that RXN concentration could be accurately determined by the proposed method. The results also confirm the selectivity of the proposed method with no interference from the added excipients.

Table 3: Results of analysis of rivaroxaban in synthetic mixture

Concentration of RXN (µg mL^{-1})		Recovery	% Error
Taken	Found (n=5)		
2	2.010	100.50	0.50
10	9.982	99.82	0.18
20	19.998	99.99	0.01

3.8. Robustness

The robustness of the proposed method was assessed with changes in the analytical wavelength (270 ±1 nm). Robustness was carried out at two different concentration levels (2 and 20 µg mL^{-1}). The results are expressed as standard deviation and relative standard deviation and are compiled in Table 4. The results revealed that the slight changes in the analytical wavelength did not adversely influence the absorbance intensity and indicate acceptable robustness of the proposed method.

Table 4: Results of robustness study

Concentration of RXN (µg mL^{-1})	Wavelength (nm)	Absorbance	SD (n=3)	% RSD
2	269	0.216	0.00321	1.502
	270	0.217		
	271	0.211		
20	269	1.874	0.02542	1.366
	270	1.876		
	271	1.831		

3.9. Ruggedness

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 two different concentration levels (2 and 20 µg mL^{-1}). The results are expressed as standard deviation and relative standard deviation. The results are presented in Table 5. The low values of the standard deviation and relative standard deviation indicates the ruggedness of the method.

Table 5: Results of ruggedness study

Concentration of RXN (µg mL^{-1})	Analyst	Absorbance	SD (n=2)	% RSD
2	I	0.222	0.001410	0.630
	II	0.221		
20	I	1.874	0.165463	0.882
	II	1.876		

CONCLUSION

A simple, rapid, economical UV spectrophotometric method has been developed for the quantification of RXN. The proposed method has been validated as indicated by the ICH guidelines and found to be linear, sensitive, selective, precise, accurate, robust and rugged for the detection and quantification of RXN. This method is suitable for routine analysis and quality control of RXN.

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