

# Development and Validation of Ultra Violet-Visible Spectrophotometric Method for Estimation of Rivaroxaban in Spiked Human Plasma

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## Abstract:

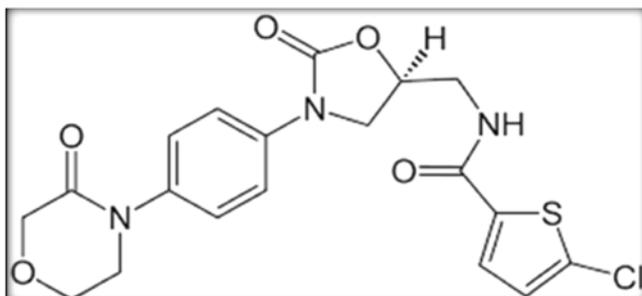
To develop a simple, economic and specific validated UV method for estimation of rivaroxaban in human plasma. The diluent used is acetonitrile: water (60:40% v/v). The absorption maxima were found at 252nm. The bioanalytical procedure involves deproteination of plasma with protein precipitation extraction. The percentage of relative recovery and correlation coefficient of accuracy and precision were within acceptable limits. The method proved in simple, cost effective and sensitive for estimation of rivaroxaban in spiked human plasma.

**Keywords:** Human Plasma, Method Development, Protein Precipitation, Rivaroxaban, Validation.

## INTRODUCTION:

Rivaroxaban(RIV) 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. Anticoagulants are used to treat and prevent blood clots that may occur in your blood vessels. Blood clots can block blood vessels (an artery or a vein). A blocked artery stops blood and oxygen from getting to a part of your body. RIV is a small molecule, that is almost insoluble in water and exhibits high plasma protein binding (92–95%) in humans, with serum albumin being the main binding component. RIV also binds highly selectively to the S1 and S4 pockets of serine endopeptidase, responsible for the potency of factor Xa inhibition.

According to the literature, the pharmacokinetic profile of RIV in healthy subjects has been established and demonstrated a favourable safety and tolerability profile. The bioavailability of a 10-mg dose of RIV is high (80–100%), and RIV is rapidly absorbed, reaching a maximal plasma concentration ( $C_{max}$ ) within 2–4 h after oral administration.



**Figure 1: chemical structure of rivaroxaban.**

Rivaroxaban, sold under the brand name Xarelto among others, is an anticoagulant medication (blood thinner) used to treat and prevent blood clots.

**Iupac name:** 5-chloro-N-[[[(5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl] methyl] thiophene-2-carboxamide.

**Chemical formula:**  $C_{19}H_{18}ClN_3O_5S$ .

**Molecular weight:** 435.89g/mol.

## UV SPECTROSCOPY:

UV spectroscopy is type of absorption spectroscopy in which light of ultra-violet region (200-400 nm.) is absorbed by the molecule. Any molecule has either n,  $\pi$  or  $\sigma$  or combination of these electrons. These bonding ( $\sigma$  and  $\pi$ ) and non-bonding (n) electrons absorb the characteristic radiation and undergoes transition from ground state to excited state. By the characteristic absorption peaks and the nature of the electron present the molecular structure can be elucidated UV spectroscopy obeys the Beer-Lambert law.

**Beer law:** This law can be stated as follows: “When a beam of monochromatic radiation is passed through a solution of absorbing substances, the intensity of a beam of monochromatic light decreases exponentially with the increase in concentration of the absorbing substances exponentially”.

$$I = I_0 * e^{-k_1 * c} \dots\dots\dots 1$$

Where,

$I_0$  = intensity of light incident upon sample cell,

$I$  = intensity of light leaving sample cell,

$C$  = molar concentration of solute,

$K_1$  = constant.

**Lambert's law:** This law can be stated as follows “When a beam of light is allowed to pass through a transparent medium, the rate of decrease of intensity with the thickness of medium is directly proportional to the intensity of the light”.

$$I = I_0 * e^{-k_2 * l} \dots\dots\dots 2$$

Where,

$I_0$  = intensity of light incident upon sample cell,

$I$  = intensity of light leaving sample cell,

$L$  = length of sample cell (cm.),

$K_2$  = constant.

After combining equation 1 and 2 and deriving we get the following equation 3 of Beer-Lambert law as:

$$A = \log (I_0/I) = \epsilon cb \dots\dots\dots 3$$

Where,

A = absorbance

$I_0$  = intensity of light incident upon sample cell,

I = intensity of light leaving sample cell,

c = molar concentration of solute,

b = length of sample cell (cm.),

$\epsilon$  = molar absorptivity.

A literature search has shown that there are only few quantitative analytical methods for estimation of Rivaroxaban further, very few methods were available that shows the quantification of Rivaroxaban in biological fluids, these methods include LC-MS, GC-MS, which needs high end instrumentation which are costly and not available in conventional bioanalytical laboratory. Thus, the conclusion was to develop a rapid, simple and economical method which was based on protein precipitation for sample preparation and UV detection for quantification of Rivaroxaban from spiked human plasma.

#### MATERIALS AND METHOD:

**Chemicals:** Rivaroxaban standard is obtained from Gland Pharma Pvt. Ltd., Rivaroxaban(Xarelto) tablets label claim 10mg manufactured by Bayer, were purchased from local market, chemicals used acetonitrile: water (60:40) obtained from SB Fine chemicals.

**Instruments:** Double beam UV spectrophotometer; Model: SL 210; Make: ELICO. The data was obtained using Spectra Treats 3.11.01Rel 2b.

Vortex mixer; Model:CM 101; Make: REMI

The analysis was performed using UV SL120 using UV detector used for method development and validation. The output signal was checked and the acquisition and integration of data was performed using spectral threats. Software on a computer. The diluents are filtered through 0.25 $\mu$ m detection was monitored at 294nm.

#### Preparation of standard stock solution:

Weighed 10mg of Rivaroxaban was taken in to 10ml volumetric flask and then dissolve with small amount of acetonitrile: water (60:40), then sonicated for 2 min and made up to the mark with acetonitrile: water (60:40). (1000ppm)

#### Preparation of working standard solution:

From the standard stock solution take 1ml of the solution and was transferred into another volumetric flask and made up to the mark with acetonitrile: water (60:40) (100ppm) Take 1ml of the solution from the stock solution into another volumetric flask and made up to the mark with same diluent acetonitrile: water (60:40) (10ppm).

#### Determination of wavelength of maximum absorption:

10ppm standard stock solution was prepared by using the diluent acetonitrile: water (60:40) and scanned under the UV spectroscopy within the range 200-400nm using the diluent as blank. The absorption maxima were found at 252nm.

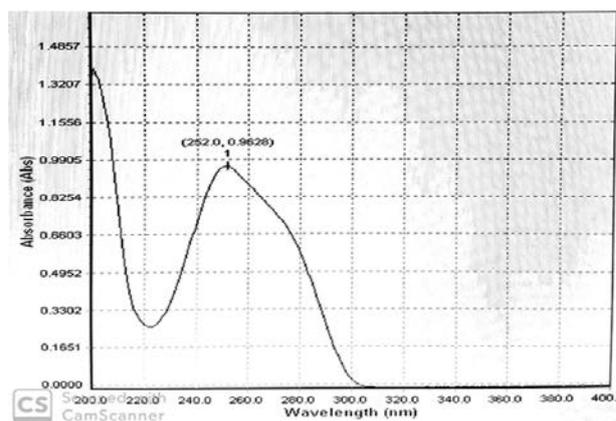


Figure 2: absorption maxima of rivaroxaban.

#### ASSAY:

##### Standard preparation:

10mg of Rivaroxaban drug was accurately weighed and transferred into 10ml of volumetric flask and the volume was made with small amount of acetonitrile: water (60:40), then sonicate it for 2min and made up to the mark with acetonitrile: water (60:40) to get concentration of 1000ppm. From this 0.1 ml was pipetted out and transferred into 10ml of volumetric flask and the volume was made up to the mark with acetonitrile: water (60:40) to get 10ppm solution and its absorbance was measured at 252nm.

##### Test preparation:

To determine the content of Rivaroxaban from marketed formulation, 5 tablets were weighed, powdered and average weight was calculated and amount of tablet powder equivalent to 10mg of Rivaroxaban was weighed accurately transferred to a 10ml volumetric flask. Sufficient amount of acetonitrile: water (60:40) was added and sonicated for 10 minutes and the solution was diluted up to the mark with the same solvent and filtered through what mann filter paper. From the filtrate, measured volume was taken and diluted with acetonitrile: water (60:40) to get the final concentration. The absorbance's were measured at selected wave length.

Weight of 5tablets = 4.48gm

Average weight = 4.48/5

Average weight of each tablet =0.896gm $\approx$ 896mg

Each tablet 896mg of rivaroxaban contains in 10mg of tablet

Weight to be taken =896mg in 10ml diluent for 1000ppm

Absorbance of sample= 0.8896

Absorbance of standard=0.9628

Concentration of standard= 10ppm

**Concentration of sample= (Absorbance of Sample/ Absorbance of Standard) x concentration of Std.**

=0.8896/0.9628 x 10

conc. of sample =9.2ppm

**%Assay= (Absorbance of Sample/ Absorbance of Standard) x (Concentration of Standard / Concentration of Sample) x 100**

% Assay= (0.8732/0.9628) x (10/9.2) x 100

= (0.9239 x 1.08) x 100

**%Assay =99.7%**

**Extraction of plasma from blood:**

Blood was collected into an EDTA containing tube and then it was centrifuged for 10 min at 3000rpm blood was separated into two layers after centrifugation. The supernatant which contains stray yellow colour (plasma) was collected and used for sample preparation.

**Preparation of Plasma Solution:**

0.5ml plasma was deproteinated to this 1ml of Rivaroxaban was taken in a centrifuge tube and the contents are vortex for 1 minute. To that add 1ml of acetonitrile and vortex it for 1 minute in cyclomixer. Then the solution was centrifuged for 10 min at 10000rpm. Then the organic layer was transferred and the aliquot was collected and absorbance was measured at 252nm.

**METHOD VALIDATION PARAMETERS****Method validation:**

ICH guidance for industry was followed for validation of the method. linearity, accuracy, robustness, LOD, LOQ were assessed during method validation.

**Linearity:**

Calibration standard solutions were prepared in plasma from the working solutions. Calibration curves ranging from the 1 to 20 ppm were run to establish the linearity by using linear regression analysis. From the working standard solution 0.1ml, 0.2 ml, 0.3ml, 0.4ml, 0.5ml, 0.6ml, 0.7ml, 0.8ml, 0.9ml, 1.0ml, 1.1ml, 1.2ml, 1.3ml, 1.4ml, 1.5ml, 1.6ml, 1.7ml, 1.8ml, 1.9ml, 2.0ml was pipetted out and transferred into 10ml volumetric flask and the volume was made up to 10ml with acetonitrile: water (60:40) were gives us the 1ppm, 2ppm,3ppm, 4ppm, 5ppm, 6ppm, 7ppm, 8ppm, 9ppm, 10ppm, 11ppm, 12ppm,13ppm, 14ppm, 15ppm, 16ppm, 17ppm, 18ppm, 19ppm, 20ppm, concentrations respectively and absorbance was measured at 252nm using acetonitrile: water (60:40) as blank and the calibration curve is plotted.

**Acceptance criteria:** Correlation coefficient ( $r^2$ )  $\geq$  0.999

**Precision:**

10ppm standard solution of Rivaroxaban pure drug is selected for Precision study. From the working standard solution 1ml was pipetted out and transferred into 10ml volumetric flask and the volume was made up to 10ml using acetonitrile: water (60:40) to give 10ppm solution. This procedure is repeated 6 time and observances of all were measured at 252nm using acetonitrile: water (60:40) as blank and its %RSD was calculated by using the formula

**%RSD = (standard deviation of the measurement / mean value of measurement) \*100**

**Acceptance criteria:** %RSD should be within the limits less than 2

**Accuracy:**

Quality control of samples was prepared at three different levels. The concentration of Rivaroxaban was calculated from a standard calibration curve that was concurrently obtained. Accuracy was analysed at each level by comparing the observed concentration as a mean percentage recovery. Standard quantity equal into 50%, 100% and 150 % is to be added in sample.2ml of 1.5ppm of standard solution was spiked with 2ml of 3ppm of

sample solution,2ml of 3ppmof standard solution was spiked with 2ml of 3ppm of sample solution,2ml of 4.5ppm of standard solution was spiked with 2ml of 3ppm of sample solution. Absorbance was measured for three times at 252nm.Repeated three times and their absorbance are measured at 252nm and the %recovery is calculated by using the formula:

**% Recovery = (amount found / amount added) \*100**

**Acceptance criteria:** Accuracy % recovery should be within the limits in the range of 98-102%

**Limit of detection:**

The detection limit (DL) may be expressed as:

$$DL = 3.3 \cdot \sigma / S$$

where  $\sigma$  = the standard deviation of the response, S = the slope of the calibration curves the slope S may be estimated from the calibration curve of the analyte.

**Limit of quantification:**

The quantitation limit (QL) may be expressed as:

$$QL = 10 \cdot \sigma / S$$

where  $\sigma$  = the standard deviation of the response, S = the slope of the calibration curves the slope S may be estimated from the calibration curve of the analyte.

**Robustness:**

3 aliquots of 10ppm of standard solution was prepared and it was scanned at wavelength at ( $\pm$ )1nm of  $\lambda_{max}$ . The absorbance was noted down

**Acceptance criteria:** %RSD should be within the limits less than 2.

**Ruggedness:**

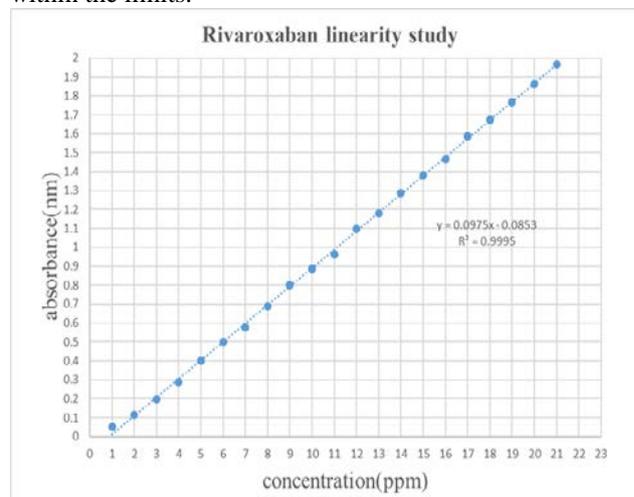
10ppm standard solution was prepared and scanned for 6 times by different analyst and different instruments.

**Acceptance criteria:** %RSD should be within the limits less than 2.

**RESULTS AND DISCUSSION:**

Method development and optimization of chromatographic condition:

**Linearity:** The correlation coefficient( $r^2$ ) was found to be within the limits.



**Figure 3: Linearity curve of Rivaroxaban**

**Table-1: Conc. Vs Abs. table for Linearity Study.**

S.No	Concentration	Absorbance
1	1ppm	0.1161
2	2ppm	0.1989
3	3ppm	0.2877
4	4ppm	0.3989
5	5ppm	0.4999
6	6ppm	0.5776
7	7ppm	0.6879
8	8ppm	0.7991
9	9ppm	0.8874
10	10ppm	0.9628
11	11ppm	1.0963
12	12ppm	1.1786
13	13ppm	1.2863
14	14ppm	1.3797
15	15ppm	1.4675
16	16ppm	1.5874
17	17ppm	1.6732
18	18ppm	1.7684
19	19ppm	1.8648
20	20ppm	1.9652

**Precision:** The %RSD for precision was found to be within the limits

**Table-2: Evaluation data of precision study**

S.no	Concentration	Absorbance
1	10ppm	0.9628
2	10ppm	0.9632
3	10ppm	0.9626
4	10ppm	0.9624
5	10ppm	0.9622
6	10ppm	0.9624
7	mean	0.9626
8	SD	0.000357
9	%RSD	0.0370

**Accuracy:** Accuracy %recovery was found to be within the limits in the range of 99.04-100.74%

**Table-3: Accuracy data**

%Level	Absorbance's (nm)	%Recovery	Mean% Recovery
50% (3ppm+1.5ppm)	0.4385	98.32%	99.04%
	0.4409	99.76%	
	0.4397	99.04%	
100% (3ppm+3ppm)	0.5542	99.68%	99.70%
	0.5544	99.75%	
	0.5542	99.68%	
150% (3ppm+4.5ppm)	0.7211	100.73%	100.74%
	0.7209	100.72%	
	0.7212	100.78%	

**Limit of detection(LOD):** The limit of detection was found to be

$$LOD = 3.3 * \sigma / S$$

$$LOD = 3.3 * 0.000357 / 0.0968$$

$$LOD = 0.0011781 / 0.0968$$

$$LOD = 0.0121 \mu\text{g/mL}^{-1}$$

**Limit of Quantification(LOQ):** The limit of quantification was found to be

$$LOQ = 10 * \sigma / S$$

$$LOQ = 10 * 0.000357 / 0.0968$$

$$LOQ = 0.00357 / 0.0968$$

$$LOQ = 0.0368 \mu\text{g/mL}^{-1}$$

**Robustness:** The %RSD for robustness was found to be within the limits

**Table-4: Robustness data.**

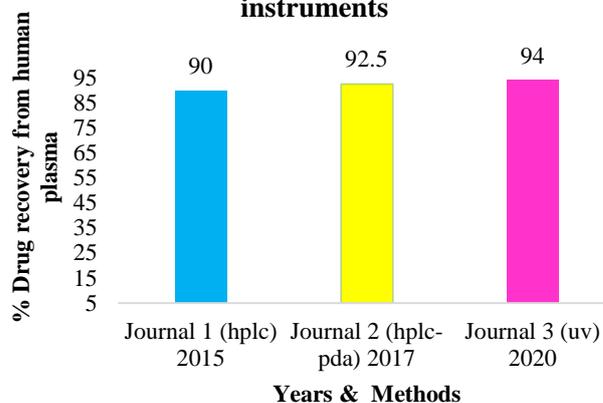
S.no	Concentration	Absorbance	
		251nm	253nm
1	10ppm	0.9576	0.9704
2	10ppm	0.9578	0.9706
3	10ppm	0.9575	0.9702
4	Mean	0.9576	0.9704
5	SD	0.0001527	0.0002
6	%RSD	0.01595	0.02061

**Ruggedness:** The %RSD for ruggedness was found to be within the limits

**Table-5: Ruggedness data**

S.no	Concentration	Absorbance	
		Analyst-1	Analyst-2
1	10ppm	0.9628	0.9598
2	10ppm	0.9622	0.9596
3	10ppm	0.9620	0.9594
4	10ppm	0.9624	0.9592
5	10ppm	0.9622	0.9592
6	10ppm	0.9625	0.9594
7	Mean	0.9623	0.9595
8	SD	0.000281	0.000209
9	%RSD	0.0292	0.0218

The percentage of protein binding in rivaroxaban was found to be 94%

**Drug recovery from plasma in analytical instruments****Figure-4:** Percentage drug recovery from human plasma

**CONCLUSION:**

The results and the statistical parameters demonstrate that the proposed UV spectrophotometric method for the estimation of rivaroxaban in spiked human plasma is simple, rapid, specific, accurate and precise. Therefore, this method can be used for the determination of Rivaroxaban either in bulk or in the dosage formulations without interference with commonly used excipients and related substances.

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