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# DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR THE ESTIMATION OF RIVAROXABAN IN BULK AND TABLET DOSAGE FORM

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**Abstract:**

*The quantification of rivaroxaban in pharmaceutical dosage forms and bulk medication has been accomplished through the development and validation of a new, precise, and effective Area under curve derivative UV spectroscopic approach. The absorbance of rivaroxaban reaches its maximum at 277 nm in 0.1N Hcl, and the Area under curve in absorption spectra measured between wavelength range in 272nm and 282nm , its concentration falls between 2 and 12 µg/mL according to Beer's Law. A correlation value (R2) of 0.9993 demonstrated the method's great linearity, demonstrating high consistency and dependability throughout the spectrum under study. While the limits of detection (LOD) and quantitation (LOQ) were determined to be 0.0331 µg/mL and 0.1003µg/mL, respectively, recovery rates ranged from 98.84% to 100.95%. Additionally, the method's relative standard deviation (%RSD) values were less than 2%, indicating exceptional accuracy. The ICH recommendations were followed in evaluating the following validation parameters: linearity, accuracy, precision, robustness, ruggedness, LOD, and LOQ. For regular analysis of rivaroxaban in different pharmaceutical formulations, this verified spectroscopic methodology proved to be a reliable and repeatable method.*

***Keywords:*** *Rivaroxaban , Area under curve [AUC], Validation , Pharmaceutical formulations.*

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## INTRODUCTION:

Rivaroxaban is a drug that inhibits the formation of blood clots by acting at a critical point in the clotting process. People with atrial fibrillation who do not have heart valve dysfunction can also take it to protect themselves from serious blood clots of stroke. A natural chemical that aids in the formation of blood clots is suppressed in its function. An enantiomer in its purest form is rivaroxaban. [1] . It is a white to yellowish powder that has no smell and is not hygroscopic. Methanol, DMSO, and acetonitrile are among the organic solvents in which it dissolves. Rivaroxaban is a novel oral, selective, and highly efficient direct Factor Xa inhibitor. It is often referred to as (S)-5-chloro-N-((2-oxo-3-(4-(3-oxomorpholino)phenyl)oxazolidin-5-yl)methyl)thiophene-2-carboxamide. The blood coagulation cascade, which triggers thrombin activity and clotting, depends critically on factor Xa. Rivaroxaban has a molecular weight of 435.881g/mol and the chemical formula is $C_{19}H_{18}ClN_3O_5S$. It has a melting point between 228°C and 232°C.[2] . Direct oral anticoagulants, which target a specific coagulation factor (such as thrombin or Factor Xa), have been developed in recent years to overcome the drawbacks of conventional anticoagulants. Both the intrinsic and extrinsic coagulation pathways stimulate factor Xa, which is necessary for blood clotting. Through the prothrombinase complex, Factor Xa directly converts prothrombin to thrombin, which results in the production of fibrin clots and platelet activation by thrombin. [3]. In a concentration-dependent manner, rivaroxaban inhibits free FXa, FXa bound to prothrombinase, and FXa associated with a clot. Although it has no direct influence on the process, platelets aggregate due to adenosine diphosphate, collagen, and thrombin [4] .

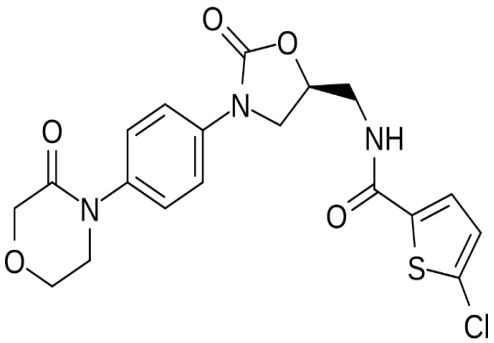


**Figure 1 . Chemical Structure of Rivaroxaban**

According to a literature review, few analytical techniques have been published for determining Rivaroxaban in pure medication and pharmaceutical dosage forms employing UV[5-8], HPLC[9-19], RP-HPLC[20-22], and HPTLC[23-25]. The current effort aims to develop and verify a new Area under curve for estimating Rivaroxaban in tablet and bulk dose form that is quick, easy, accurate, and specific.

## MATERIALS AND METHODS :

**Instrument:**

UV-Visible double beam spectrophotometer,SHIMADZU (model UV-1800) with UV probe software . All weights were taken in analytical balance .

**Chemicals :**

The pharmaceutical dosage of Rivaroxaban was 20 tablets (Xarelto) with a claim of 20 mg from Bayer Zydus Pharma Private Limited, while the pure medicine was acquired as a gift sample from Medreich Limited (R&D Centre), Bengaluru.

**Solvent :**

0.1N Hcl is used as a solvent

**Selection of analytical wavelength :**

Appropriate dilutions of rivaroxaban were prepared from standard stock solution and using spectrophotometer solution was scanned in the wavelength range 200-400 nm Area under Curve[AUC] in absorption spectra were measured between the wavelength range 272nm and 282nm , as the wavelength for detection(Fig-2).

**Preparation of standard stock solution**:

100mg of rivaroxaban was weighed accurately transferred into 100 ml of volumetric flask and diluted in 0.1N Hcl upto the mark. From this, the solution was further diluted into 100µg/ml and pipetted out 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml into 10 ml individual volumetric flask and diluted in 0.1N Hcl up to the mark, this gives 2, 4, 6, 8, 10 and 12µg/ml concentration .

**Preparation of sample solution** :

20 tablets of rivaroxaban marketed formulations was weighed and powdered. A quantity of tablet powder equivalent to 100mg of Rivaroxaban was transferred into 100ml volumetric flask then it was diluted with 0.1N Hcl and make upto the mark.

## METHOD AND VALIDATION

The method was validated according to the ICH guidelines[26-28] .

## RESULT AND DISCUSSION:

**Method : Area under curve spectroscopy.**

**Linearity:**

The linearity of an analytical method is its capacity to show the test results that are directly proportional to the concentration of the analyte in the sample within the range. The linearity was established in the range of 2-12µg/ml and Area under curve [AUC] in absorption spectra were measured between the wavelength of 272nm and 282nm as absorbance values are shown in table-1(fig-3). The calibration curve was prepared by plotting graph against the concentration and absorbance and therefore the graph shown in (Fig-4). Statistical variables like slope, intercept, regression equation, correlation coefficient and sandell's sensitivity were determined and shown in( table-2).

**Precision :**
The precision of an analytical method express the closeness of series of individual analytical measurement obtained from the multiple sampling of equivalent sample. Precision was established by intra-day and inter-day was determined by analysing the same concentration for six times in a same day. Inter-day precision was analysing the same concentration daily for six days shown in (table-3).

**Accuracy:**
The accuracy of an analytical method says that closeness of test results obtained by that method of the true value .To assess the accuracy of the developed method ,recovery studies were carried out at three different levels at 50% ,100% and 150% .In which the formulation concentration holds it constant and varied pure drug concentration Shown in (table -4).

**Ruggedness:**
The ruggesdness is defined as the reliability of results when the method is performed under the variation in condition. This includes distinct analyst,laboratories,instruments , temperature etc.Ruggedness was determined between disteinct analyst, the value of %RSD was found to be less than 2.(Table-5)

**LOD and LOQ:**
The limit of detection is an individual analytical method is the smallest amount of analyte in the sample whuch can be reliably detected by the analytical method. The limit of quantification is a descrete analytical procedure is the smallest amount of analyte in the sample which can be quantitatively determined. LOD and LOQ were calculated by using following formula

LOD = 3.3(SD)/S and LOQ = 3(LOD)

LOD and LOQ value of Rivaroxaban were found be 0.033 µg/mL and 0.1003 µg/mL.

**Table 1: Results of calibration curve for Rivaroxaban at 272nm-282nm by Area under Curve**

| Sl No | Concentration in µg\|ml | Absorbance±Standard deviation |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 2 | 0.120±0.0019 |
| 3 | 4 | 0.221±0.0021 |
| 4 | 6 | 0.320±0.0026 |
| 5 | 8 | 0.445±0.0038 |
| 6 | 10 | 0.553±0.0014 |
| 7 | 12 | 0.654±0.0015 |

**Average of six determinations*

**Table 2: Regression parameters of Rivaroxaban at 272nm-282nm by Area under Curve**

| Regression Parameter | Results |
|---|---|
| Range | 2-12 µg/ml |
| $\lambda_{max}$ | 272-282nm |
| Regression equation | Y=0.0545x+0.0034 |
| Slope(b) | 0.0545 |
| Intercept (a) | 0.0034 |
| Correlation coefficient | 0.9993 |
| Sandell's sensitivity | 0.01875 |
| LOD(µg/ml) | 0.03312 |
| LOQ(µg/ml) | 0.1003 |

**Y=bx+a****

**Table 3 : Determination of Precision results for Rivaroxaban at 272nm-282nm by Area under Curve**

| Concentration (µg\|ml) | Intra-day Absorbance ±Standard deviation* | %RSD** | Inter-day Absorbance ±Standard deviation* | %RSD** |
|---|---|---|---|---|
| 2 | 0.120±0.0019 | 1.583 | 0.125±0.0015 | 1.200 |
| 4 | 0.221±0.0021 | 0.950 | 0.257±0.0021 | 0.925 |
| 6 | 0.320±0.0026 | 0.812 | 0.325±0.0013 | 0.400 |
| 8 | 0.445±0.0038 | 0.853 | 0.435±0.0017 | 0.390 |

| | | | | |
|---|---|---|---|---|
| 10 | 0.553±0.0014 | 0.253 | 0.540±0.0021 | 0.388 |
| 12 | 0.654±0.0015 | 0.229 | 0.643±0.0019 | 0.295 |

*Average of six determinations,** Percentage relative standard deviation.*

**Table 4: Determination of accuracy results for rivaroxaban at 272nm- 282nm by Area under Curve**

| Spiked levels | Amount of sample (µg\|ml) | Amount of standard (µg\|ml) | Amount recovered | %Recovery± Standard deviation* | %RSD** |
|---|---|---|---|---|---|
| 50 | 6 | 3 | 8.95 | 99.82%±0.339 | 0.340 |
| 100 | 6 | 6 | 11.85 | 98.84%±0.569 | 0.575 |
| 150 | 6 | 9 | 15.13 | 100.95%±0.362 | 0.362 |

*Average of six determinations,** Percentage relative standard deviation.*

**Table 5: Determination of ruggedness results of at 272nm-282nm by Area under Curve**

| Analysts | Analyst 1 | Analyst 2 |
|---|---|---|
| Mean absorbance | 0.320 | 0.321 |
| ±Standard deviation* | 0.0026 | 0.0011 |
| %RSD** | 0.8125 | 0.3426 |

*Average of six determinations,** Percentage relative standard deviation.*

**FIGURES:**

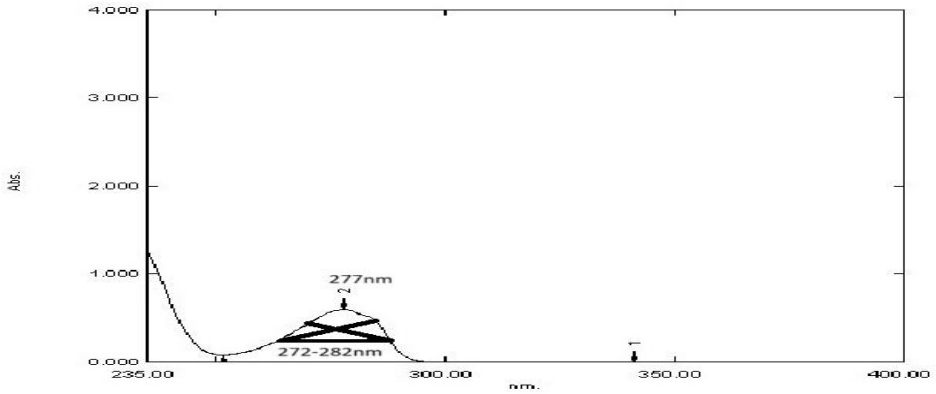


**Fig.2: Area under curve spectrum of Rivaroxaban at 272-282nm.**

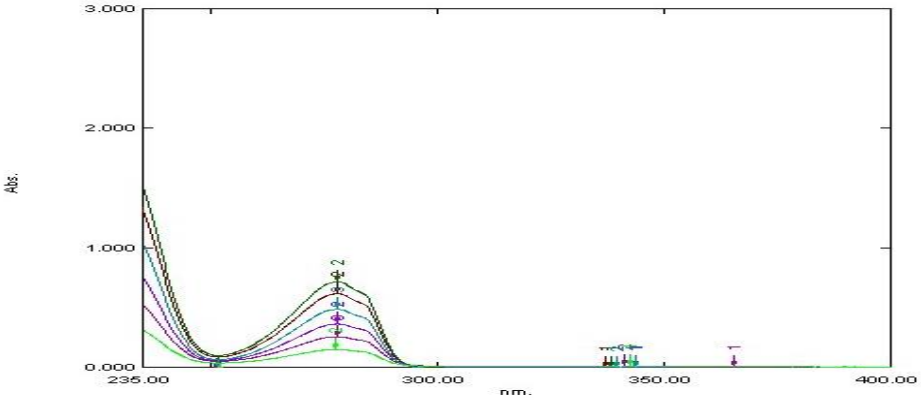


**Figure 3: Area under curve overlain spectra of Rivaroxaban showing absorbance at 272-282nm.**

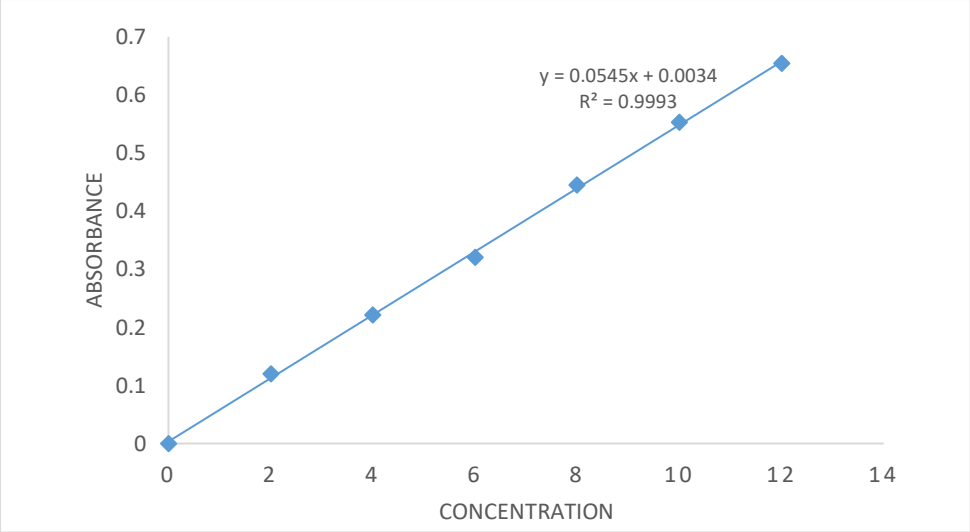


**Figure 4: Calibration curve of Rivaroxaban at 272nm-282nm by Area under curve method .**

## CONCLUSION:

The analytical method developed for Rivaroxaban was validated as per ICH guidelines demonstrating simplicity, specificity, accuracy, economy, and sensitivity. This method is suitable for regular analysis of Rivaroxaban in both bulk form and pharmaceutical preparations.

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