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DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR RIVAROXABAN ANALYSIS IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A novel, accurate, and efficient zero-order derivative UV spectroscopic method has been developed and validated for the quantification of Rivaroxaban in both bulk drug and pharmaceutical dosage forms. Rivaroxaban exhibits maximum absorbance at 277 nm in 0.1N Hcl, and its concentration follows Beer's Law within the range of 2–12 µg/mL. The method showed strong linearity, with a correlation coefficient (R^2) of 0.9996, indicating high consistency and reliability across the studied range. Recovery rates were observed between 99.07%and 101.03%, while the limits of detection (LOD) and quantification (LOQ) were found to be 0.0458 µg/mL and 0.139 µg/mL, respectively. The method also demonstrated excellent precision, with relative standard deviation (%RSD) values below 2%. All validation parameters—linearity, accuracy, precision, robustness, ruggedness, LOD, and LOQ—were assessed according to ICH guidelines. This validated spectroscopic approach proves to be a dependable and reproducible method for routine analysis of Rivaroxaban in various pharmaceutical preparations.

KEYWORDS: Rivaroxaban, Zero order derivative spectroscopy, Validation, Pharmaceutical formulations.

INTRODUCTION

Rivaroxaban is an anti-clotting medication that acts at a vital stage in the clotting process to prevent blood clots from forming. It is also used of safeguarding persons with atrial fibrillation who do not have heart valve impairment from strokes or severe blood clots. It works by suppressing the activity of a natural substance that assists in the production of blood clots. Rivaroxaban is an enantiomer in its most pure form.[1] It's an odourless, non-hygroscopic powder that ranges from white to yellowish in appearance. It dissolves in organic solvents such as methanol, DMSO, and acetonitrile. Rivaroxaban, also known as (S)-5-chloro-N-((2-oxo-3-(4-(3-oxomorpholino)phenyl)oxazolidin-5-yl)methyl)thiophene-2 carboxamide is a new, oral, selective, and very effective direct Factor Xa inhibitor. Factor Xa is a critical component of the blood coagulation cascade, which causes thrombin activation and clotting. Rivaroxaban's chemical formula is $C_{19}H_{18}ClN_3O_5S$ and its molecular weight is 435.881g/mol. Its melting point varies from 228°C to 232°C.[2] In recent years, direct oral anticoagulants that target a single coagulation factor (e.g. Factor Xa or thrombin) have been developed to address limitations of traditional anticoagulants. Factor Xa is essential for blood clotting and is activated by both the intrinsic and extrinsic coagulation pathways. Factor Xa directly transforms prothrombin to thrombin via the prothrombinase complex, causing fibrin clot formation and platelet activation by thrombin.[3] Rivaroxaban works by blocking free FXa, FXa attached to prothrombinase, and FXa linked to a clot in a concentration-dependent manner. Adenosine diphosphate, collagen, and thrombin cause platelets to aggregate, although it has no direct effect on this process.[4]

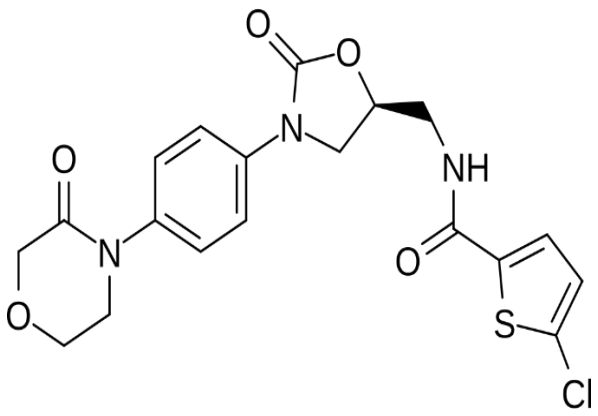

Figure 1: Chemical Structure of Rivaroxaban.

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 Zero order derivative UV Spectrophotometric technique 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: Rivaroxaban pure drug was obtained as a gift sample from Medreich limited (R&D Centre), Bengaluru and its pharmaceutical dosage Rivaroxabin 20 tablets (Xarelto) labelled claim 20mg from Bayer zydus pharma private limited.

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. The absorption spectra obtained and show maximum absorbance at 277 nm, as the wavelength for detection.

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: Zero order derivative spectroscopy

Linearity: The linearity of an analytical method is its capacity to show the test results that are directly proportional to the concentration to the analyte in the sample within the range.

The linearity was established in the range of 2-12µg/ml was measured at 277nm and absorbance values are shown in table 1. The calibration curve was prepared by plotting graph against the concentration and absorbance and therefore the graph shown in Fig-3 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. Thelimit 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.

$$LOD = 3.3(SD)/S \text{ and } LOQ = 3(LOD)$$

LOD and LOQ value of Rivaroxaban were found be 0.458 µg/mL and 0.139 µg/mL.

Table 1: Results of calibration curve at 277nm by zero order derivative spectroscopy.

Sl No	Concentration in µg|ml	Absorbance±Standard deviation
1	0	0
2	2	0.136±0.0021
3	4	0.251±0.0017
4	6	0.358±0.0016
5	8	0.486±0.0017
6	10	0.616±0.0013
7	12	0.716±0.0018

**Average of six determinations*

Table 2: Regression parameters of Rivaroxaban by Zero order spectroscopy.

Regression Parameter	Results
Range	2-12 µg/ml
λ□ ax	277nm
Regression equation	Y=0.0597x+0.008
Slope(b)	0.0597
Intercept (a)	0.008
Correlation coefficient	0.9996
Sandell's sensitivity	0.016
LOD(µg/ml)	0.045
LOQ(µg/ml)	0.139

Y=bx+a**

Table 3: Determination of Precision results for Rivaroxaban at 277nm by Zero order spectroscopy.

Concentration (µg|ml)	Intra-day Absorbance ±Standard deviation*	%RSD**	Inter-day Absorbance ±Standard deviation*	%RSD**
2	0.136±0.0021	1.544	0.135±0.0017	1.259
4	0.251±0.0017	0.677	0.255±0.0014	0.549
6	0.358±0.0016	0.446	0.360±0.0038	1.055
8	0.486±0.0017	0.349	0.490±0.0026	0.530
10	0.616±0.0013	0.211	0.609±0.0022	0.361
12	0.716±0.0018	0.251	0.719±0.0029	0.403

Average of six determinations, Percentage relative standard deviation.*

Table 4: Determination of accuracy results for rivaroxaban at 277nm by Zero order spectroscopy.

Spiked levels	Amount of sample (µg|ml)	Amount of standard (µg|ml)	Amount recovered	%Recovery± Standard deviation*	%RSD**
50	6	3	9.063	100.69%±0.340	0.3379
100	6	6	11.89	99.07%±0.276	0.2788
150	6	9	15.13	101.03%±0.259	0.2566

Average of six determinations, Percentage relative standard deviation.*

Table 5: Determination of ruggedness results of rivaroxaban at 277nm by Zero order spectroscopy.

Analysts	Analyst 1	Analyst 2
Mean absorbance	0.358	0.359
± Standard deviation*	0.0016	0.0018
%RSD**	0.446	0.501

Average of six determinations, Percentage relative standard deviation.*

FIGURES

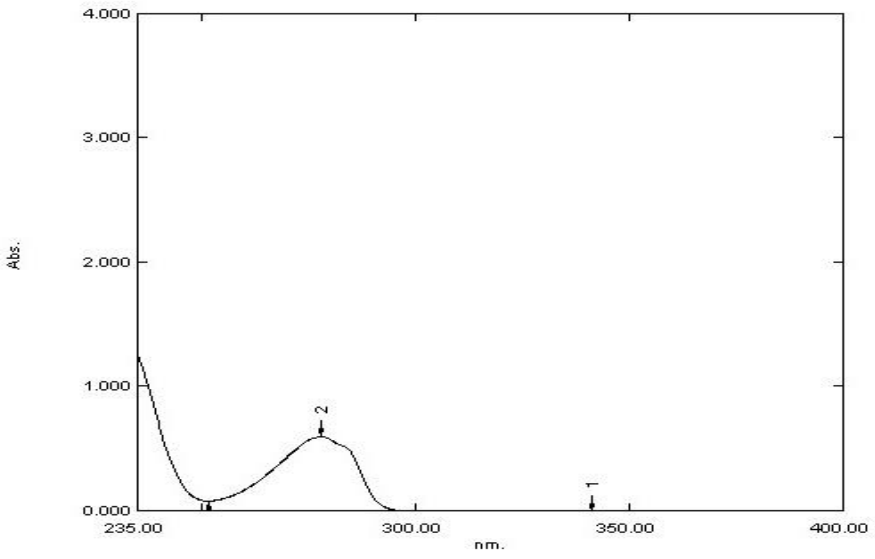

Fig. 2: Zero order spectrum of Rivaroxaban at 277nm.

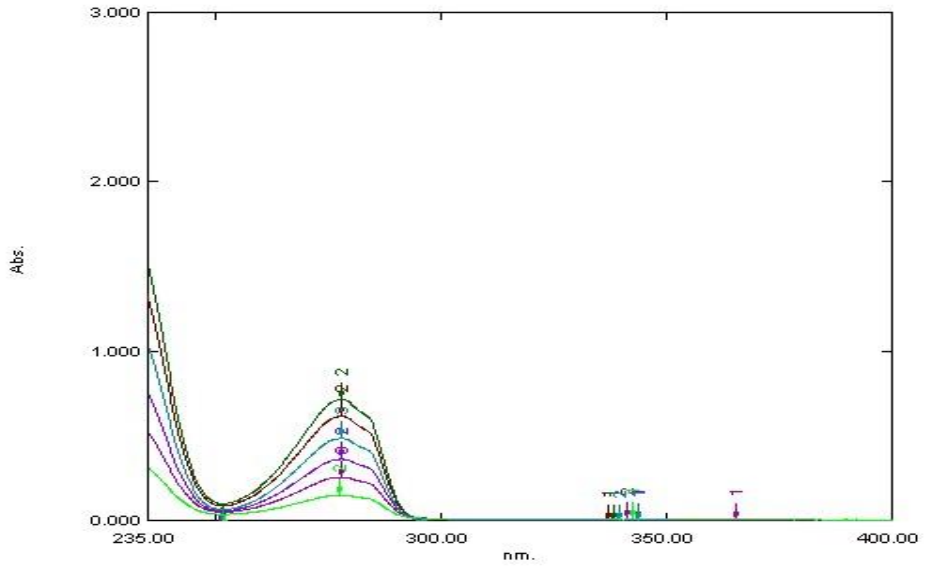

Figure 3: Zero order overlain spectra of Rivaroxaban showing absorbance at 277nm.

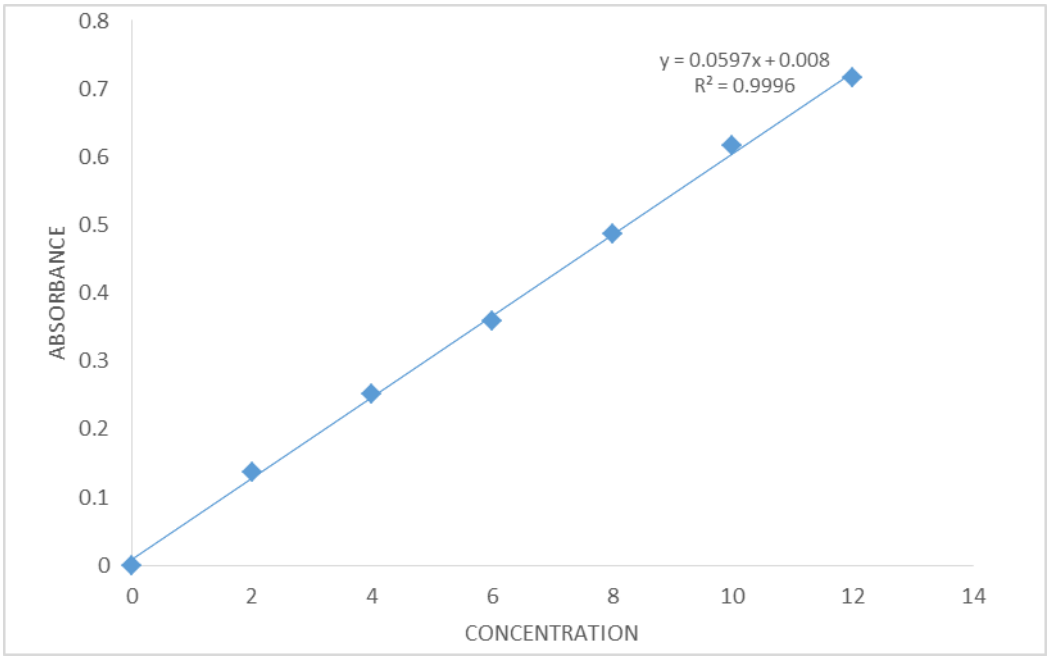

Figure 4: Calibration curve of Rivaroxaban by Zero order derivative spectroscopy.

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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