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METHOD DEVELOPMENT AND VALIDATION OF A ZERO-ORDER UV SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF APIXABAN

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Abstract:

A simple, accurate, and precise zero order derivative UV spectroscopic method has been devised and validated for the estimation of Apixaban in bulk and tablet dosage form. According to Beer's law, the concentration of Apixaban in methanol ranges from 4 to 24 µg/ml, and its absorbance reaches its maximum at 280 nm. The area under the curve in absorption spectra is measured between 275 and 285 nm. The regression coefficient (r^2) was found to be 0.999 following a linearity investigation, demonstrating acceptable linearity and precision over this concentration range. The percentage recovery was found to be 99.2%, 101%, and 99.6%, while the limits of detection (LOD) and quantitation (LOQ) were found to be 1.09 and 3.3 micrograms per milliliter, respectively. Furthermore, excellent precision was indicated by the methodologies' relative standard deviation (% RSD) values, which were less than 2%. All validation metrics, including linearity, accuracy, precision, ruggedness, LOD, and LOQ, were assessed in accordance with ICH requirements. The developed and proven method can be used to regularly estimate the dosage of Apixaban in both bulk and tablet form.

KEYWORDS: *Apixaban ;Quantification;Validation; RP-HPLC; HPTLC, Anticoagulant.*

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INTRODUCTION:

Apixaban is a new generation of oral anticoagulant drug that selectively inhibits coagulation factor Xa. It {is chemically 1-(4-methoxyphenyl)-7-oxo-6-[4 (2-oxopiperidin-1-yl) phenyl]-4,5,6,7-tetrahydro-1H-pyrazolo[3,4 c]pyridine-3-carboxamide. It is used in thromboprophylaxis in patients following total knee replacement surgery with a desired efficacy and safety profile[1]. Apixaban belongs to the anti-coagulant category of drug which acts by directly inhibiting the Factor Xa involved in the conversion of prothrombin to thrombin in the coagulation cascade. Thus helps in inhibiting the clot formation. Apixaban is used in the treatment of venous thrombosis and reduce the risk of stroke and systemic embolism[2]. Apixaban dissolves readily in organic solvents like acetonitrile and methanol but is essentially insoluble in water. Acetonitrile is frequently chosen as a solvent for UV spectrophotometric measurement because of its solubility properties. Achieving clear spectra, a well-shaped peak, and repeatable absorbance values depends heavily on the choice of solvent.

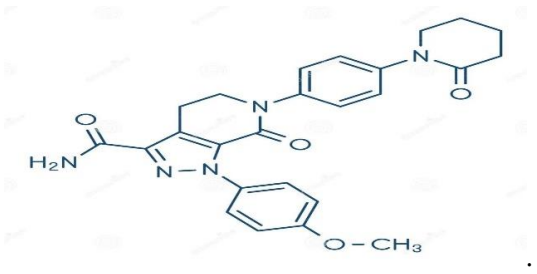

Figure 1: Chemical Structure of Apixaban.

Few analytical methods using UV, HPLC, RP-HPLC, and HPTLC have been described for determining Apixaban pure medication and pharmaceutical dosage forms, according to review of the literature. The current endeavor attempts to create and validate a novel, fast, simple, precise, and specific Zero order derivative UV Spectrophotometric method for estimating Apixaban in tablet and pharmaceutical dose form.

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:

Apixaban pure drug gift sample was given by Ce-Chem Pharmaceuticals Pvt Ltd and its pharmaceutical dosage Apixaban 20 tablets obtained from Apollo Pharmacy .

Solvent:

Acetonitrile is used as a solvent.

Selection of analytical wavelength:

Appropriate dilutions of Apixaban 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 280 nm, as the wavelength for detection.

Preparation of standard stock solution:

100mg of Apixaban was weighed accurately transferred into 100 ml of volumetric flask and diluted in Acetonitrile upto the mark. From this, the solution was further diluted into 100µg/ml and pipetted out 0.4, 0.8, 1.2, 1.6, 2.0 and 2.4 ml into 10 ml individual volumetric flask and diluted in Acetonitrile up to the mark, this gives 4, 8, 12, 16, 20 and 24µg/ml concentration.

Preparation of sample solution:

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

METHOD AND VALIDATION:

The method was validated according to the ICH guidelines.

RESULT AND DISCUSSION:

Method: Zero order derivative spectroscopy

Linearity:

Linearity shows how well the response of the method changes in proportion to the concentration of the drug within a given range. In other words, when the concentration increases, the absorbance should also increase in a consistent and predictable manner. The linearity was established in the range of 4-24µg/ml was measured at 280 nm and absorbance values are shown in table 1. The calibration curve was prepared by plotting graph against the concentration vs 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:

Precision indicates how close the results are when the same sample is tested multiple times under similar conditions. If the variation between repeated measurements is very small, the method is considered precise. 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:
Ruggedness evaluates whether the method gives consistent results when small changes are made, such as using different analysts or performing the test on different days. A rugged method produces similar results despite these minor variations. Ruggedness was determined between distinct analyst, the value of %RSD was found to be less than 2. (Table-5)

LOD and LOQ:
LOD is the smallest amount of drug that the method can detect, even if it cannot measure it accurately. LOQ is the lowest amount of drug that can be measured accurately and precisely using the developed method. LOD and LOQ were calculated by using following formula
LOD = 3.3(σ/S) and LOQ = 10 (σ / S)
LOD and LOQ value of Apixaban were found be 1.09 µg/mL and 3.3 µg/mL.

Table 1: Results of calibration curve at 280nm by zero order derivative spectroscopy.

Sl No	Concentration in µg|ml	Absorbance ± Standard deviation
1	0	0
2	4	0.155±0.000516
3	8	0.309±0.000408
4	12	0.453±0.000408
5	16	0.591±0.000516
6	20	0.721±0.000547
7	24	0.874±0.000516

** Average of six determinations*

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

Regression	Parameter Results
Range	4-24 µg/ml
λ_{max}	280nm
Regression equation	y = 0.036x + 0.0119
Slope(b)	0.036
Intercept (a)	0.0119
Correlation coefficient	0.999
Sandell's sensitivity	0.0265
LOD(µg/ml)	1.09 µg/ml
LOQ(µg/ml)	3.3 µg/ml

Y=bx+a**

Table 3: Determination of Precision results for Apixaban at 280nm by Zero order spectroscopy.

Concentration (µg|ml)	Intra-day Absorbance ± Standard deviation*	% RSD	Inter-day Absorbance ± Standard deviation*	% RSD
2	0.156± 0.0025	0.720	0.158±0.00109	0.693
4	0.313±0.0005	0.121	0.316±0.00098	0.310
6	0.452±0.0005	0.0925	0.455±0.00204	0.447
8	0.591±0.0005	0.071	0.593±0.0005	0.086
10	0.721±0.0007	0.086	0.731±0.00089	0.122
12	0.875±0.0022	0.720	0.872±0.00081	0.093

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

Table 4: Determination of accuracy results for Apixaban at 280nm by Zero order spectroscopy

Spiked levels	Amount of sample (µg|ml)	Amount of standard (µg|ml)	Amount recovered	%Recovery± Standard deviation*	%RSD**
50	12	6	17.89	99.2	0.363
10	12	12	24.26	101	0.393
150	12	18	29.89	99.6	0.109

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

Table 5: Determination of ruggedness results of Apixaban at 280nm by Zero order spectroscopy.

Analysts	Day-1	Day-2
Mean absorbance	0.452	0.454
±Standard deviation*	0.0005	0.00047
%RSD**	0.074	0.113

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

FIGURES

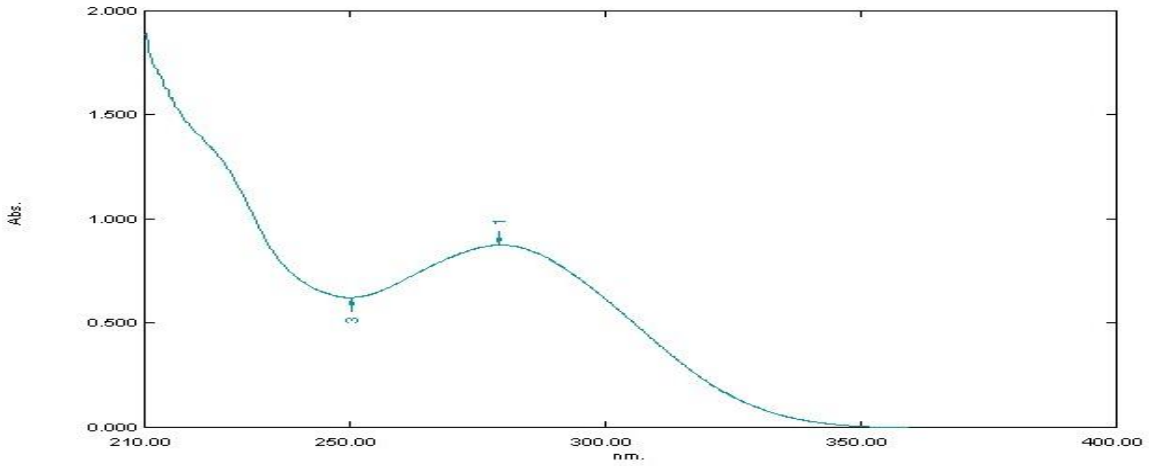

Fig. 2: Zero order spectrum of Apixaban at 280nm.

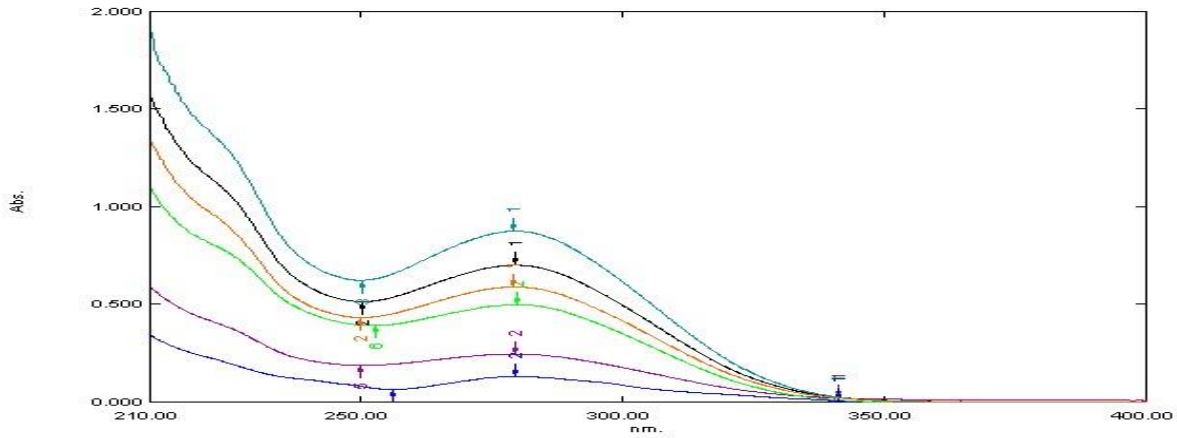

Figure 3: Zero Order Overlain Spectra of Apixaban Showing Absorbance At 280nm.

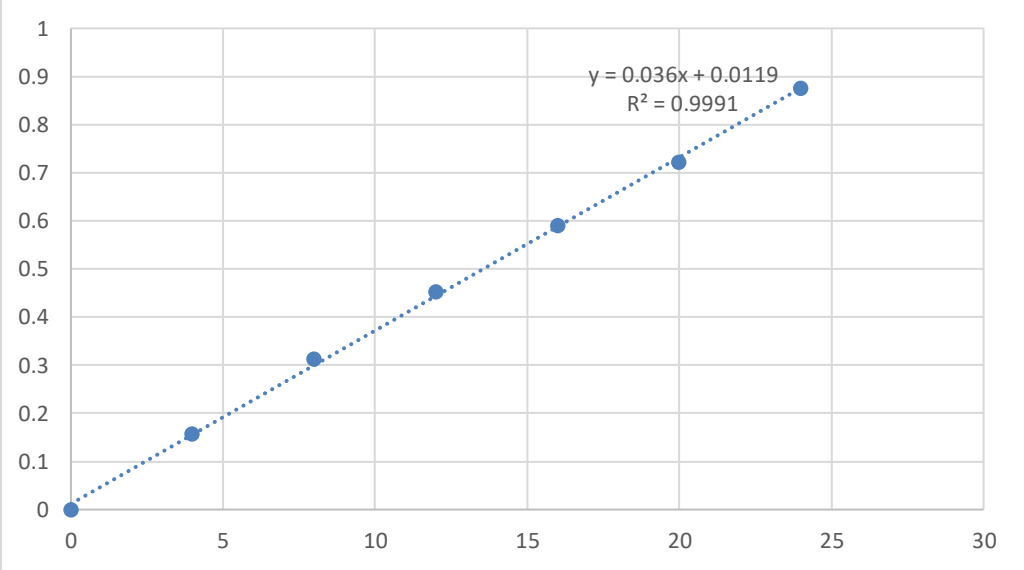

Figure 4: Calibration curve of Apixaban by Zero order derivative spectroscopy

CONCLUSION:

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

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