



COMPARATIVE STUDIES OF APIXABAN IN BULK AND ITS FORMULATIONS BY UV-SPECTROSCOPY (ZERO DERIVATIVES AND AREA UNDER CURVE)

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ABSTRACT

A simple, rapid, precise and highly selective spectrophotometric method was developed for estimation of Apixaban in tablet dosage form by Zero Order Derivative and Area Under Curve. Zero order derivative and Area Under Curve methods involve the measurement of absorbances of Apixaban at the wavelength of 279 nm and 269-289 nm respectively. Methanol was used as solvent. Linearity was observed in the concentration range of 5-25 µg/ml for Apixaban. The accuracy of the Zero order derivative method and AUC were confirmed by recovery studies of tablet dosage forms and were found to be 100% and 100% for Apixaban respectively. The Zero order derivative method and AUC showed good reproducibility and recovery with % RSD less than 0.790% and 0.988%. The LOD for Zero order derivative method and AUC of Apixaban were found to be 0.23 µg/ml and 0.335 µg/ml and LOQ for Zero order derivative method and AUC of Apixaban were found to be 0.713 µg/ml and 1.015 µg/ml respectively. Thus, the proposed method was found to be rapid, specific, precise, accurate and cost-effective quality control tool for the routine analysis of Apixaban in bulk and tablet dosage form. Drug stability studies have been determined for the formulation under specified conditions and it was found stable.

KEY WORDS

Apixaban, Area Under Curve, analytical method validation, ICH Q2 (R1) guideline, zero order derivative, comparative studies.

INTRODUCTION

Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behavior of matter. The purposes of chemical analysis are together and interpret chemical information that will be of value to society in a wide range of contexts. Quality control in manufacturing industries, the monitoring of clinical and environmental samples, the assaying of geological specimens, and the support of fundamental and applied research are the principal applications. Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess

qualitative, quantitative and structural information on the nature of matter¹.

Spectroscopy is the measurement and interpretation of electromagnetic radiation absorbed or emitted when the molecules or atoms or ions of a sample move from one energy state to another energy state. Spectroscopy is a general methodology that can be adapted in many ways to extract the information you need (energies of electronic, vibrational, rotational states, structure and symmetry of molecules, dynamic information). Ultraviolet-Visible spectrophotometry is one of the most frequently employed techniques in Pharmaceutical analysis. It involves the measurement of

the amount of Ultraviolet (190-380 nm) radiation by a substance in a solution. A compound or drug which possesses conjugated double bond absorbs UV radiation at a specific wavelength and this character of the drug is specific for a fixed solvent system. The wavelength at which maximum absorption occurs is called λ_{max} . It is independent of concentration. For a drug to be measured by the ultraviolet analytical method, it should follow the Beer's-Lambert's law^{2,3}.

Apixaban is an anti-coagulant drug. Chemically it is 1-(4-methoxy phenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl) phenyl]-4,5,6,7-tetrahydro pyrazolo [3,4-c] pyridine-3-carboxamide. The molecular formula and molecular weight of Apixaban is $\text{C}_{25}\text{H}_{25}\text{N}_5\text{O}_4$ and 459.497 g/mol respectively. Apixaban is white to pale-yellow in color and available in powder form and it is stored between 20 °C to 25 °C temperature. Apixaban is an inhibitor of coagulation factor Xa, thereby interfering with the conversion of prothrombin to thrombin and preventing formation of cross-linked fibrin clots. The drug is indicated for the prophylaxis of deep vein thrombosis. According to literature survey studies, only few HPLC methods are established for determination of Apixaban from pure and pharmaceutical formulations^{4,5}.

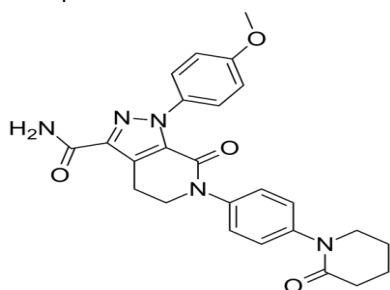


Fig. 1: Chemical Structure of Apixaban

This study has established new, precise and reproducible spectrophotometric methods for quantification of Apixaban from bulk and its tablet dosage form.

MATERIALS AND METHODS

Apixaban was provided as a gift sample by Honour Lab Limited, Visakhapatnam, Andhra Pradesh, India. HPLC grade methanol was used to prepare solutions, Apixaban 5mg tablets were purchased from local pharmacy in Hyderabad. Shimadzu UV 1800 (Japan) with matched quartz cells, connected to computer loaded with UV Prob Software and Single pan electronic balance (Shimadzu, ATY 224) was used for weighing purpose. Sonication of the solutions was carried out using an Ultrasonication (Spectra lab UCB 40India).

Calibrated volumetric glassware (Borosil) was used to perform study.

Method Development

Preparation of Standard Solution

Accurately weighed 100 mg quantity of Apixaban was transferred into 100 ml volumetric flask, to this 70 ml of methanol was added and sonicated until all the drug got dissolved. After that, volume was made up by methanol to obtained 1000 $\mu\text{g}/\text{ml}$ solution. From resulting solution 10 ml solution was pipetted out into 100ml volumetric flask and volume was adjusted with methanol to obtained 100 $\mu\text{g}/\text{ml}$ standard stock solution. This solution was further diluted with methanol to obtain desired concentrations of working standard solutions in the range of 5 – 25 $\mu\text{g}/\text{ml}$.

Wavelength Selection

Apixaban 10 $\mu\text{g}/\text{ml}$ working standard solution was scanned between 400.00 nm – 200.00 nm in UV-spectrophotometer by using methanol as blank after baseline correction. 279.00 nm wavelength was selected for further analysis.

Methods:

(Method A): Zero Order Derivative (ZOD) Spectrophotometry

Solutions of Apixaban 5–25 $\mu\text{g}/\text{ml}$ were prepared and scanned in the spectrum mode from 400.00 nm–200.00 nm. The resulting absorption spectra were analyzed by zero order derivative method, the absorbance were measured at zero cross=279.00 nm. Absorbances were plotted against their respective concentrations to calculate regression equation.

(Method B): Area Under Curve (AUC) Spectrophotometry

This method involves calculation of integrated value of absorbance with respect to wavelength in indicated range. Area calculation processing it calculates the area bounded by the curve and horizontal axis. Here horizontal axis represents baseline.

Whereas, α is area of portion bounded by curved at a and a straight line connecting the start and end point, β is area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis, λ_1 and λ_2 are wavelengths representing start and end point of curve region. In this method 5, 10, 15, 20 and 25 $\mu\text{g}/\text{ml}$ solutions were used to study area, within the wavelength ranges from 269.00 nm–289.00 nm.

Preparation of Calibration Curve

Solutions of Apixaban were prepared of concentrations 5, 10, 15, 20 and 25 µg/ml from 100 µg/ml standard stock solution using methanol as a solvent.

For Method A:

All solutions were analyzed at 279.00 nm=zero crossing wavelength and absorbance were recorded. Calibration graph was plotted for absorbance against concentration.

For Method B:

Above solutions were scanned from 400.00 nm-200.00 nm and Area Under Curve was integrated in the range of 269.00 nm-289.00 nm. Calibration curve was plotted for area under the curve against concentration.

Assay of Apixaban (5 mg) Tablets

Twenty tablets were weighed, and their average weight was determined. Tablets were crushed into a fine powder; from this 10 mg powder was weighed and transferred into 100 ml volumetric flask. To this, 70ml of methanol was added and sonicated for 30 minutes to dissolve the drug completely. After attaining room temperature, volume was made up with same solvent, and shaken well to obtain a homogeneous solution. Resulting solution was filtered by 0.45 µ syringe filter after discarding first 5 ml of solution. Resulting solution was 100µg/ml sample stock solution, which was further diluted with methanol to obtain working stock solutions. Working stock solutions were prepared in triplicate and scanned at 279.00nm.

Table 1: Assay of marketed tablets of apixaban

Method	Label Claim	Amount Taken	Amount found (mg/tab)	% Assay
A	5mg	10mg	9.96	99.6%
B	5mg	10mg	9.944	99.4%

Analytical Method Validation

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. Present method was validated according to ICH Q2 (R1) guideline for range, linearity, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ).

Linearity and Range

By using 5–25 µg/ml working standard solutions linearity was determined.

Method A: at zero cross=279.00 nm, absorbance was measured and calibration plot of absorbance against concentration was constructed to obtain regression equation.

Method B: Calibration plot constructed for area under curve against concentration and regression equation calculated. Area under curve integrated in the range of 269.00 nm-289.00 nm.

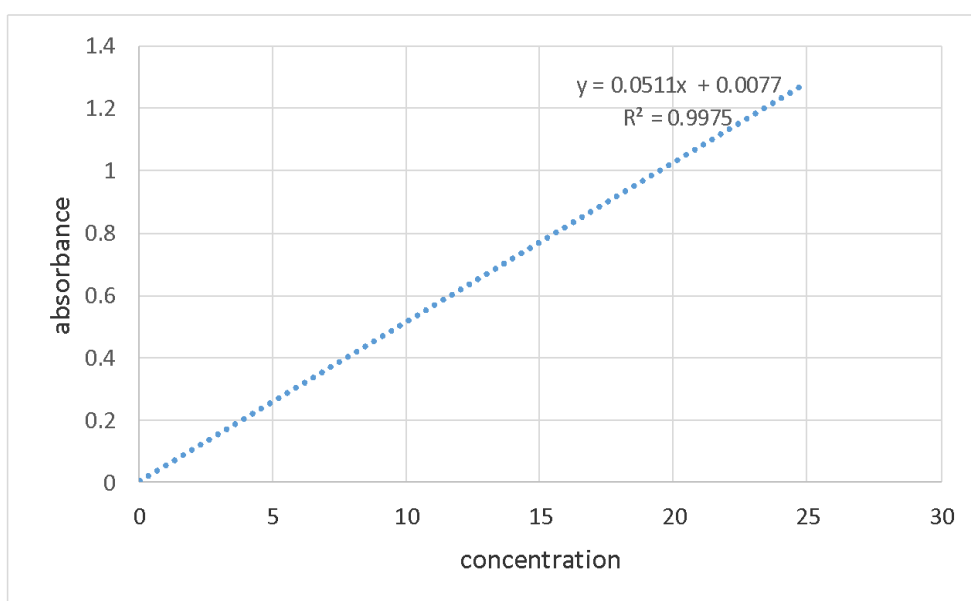


Fig. 2: Calibration curve of Apixaban Zero Order Derivative

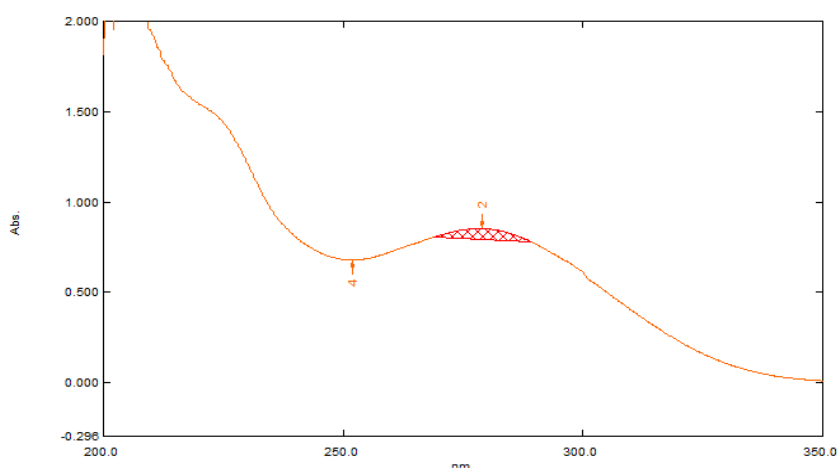


Fig. 3: Calibration curve of Apixaban Area Under Curve

Table 2: Apixaban Calibration Data

Concentration (µg/ml)	Absorbance (ZOD)	Absorbance (AUC)
5	0.274	0.245
10	0.542	0.522
15	0.753	0.758
20	0.999	0.984
25	1.312	1.304

Intermediate Precision (Reproducibility)

The three concentrations of Apixaban i.e., 10 µg/ml, 15 µg/ml and 20 µg/ml each were analyzed in triplicate on same day (Intraday precision) and same solutions were

analyzed in triplicate on different day (Interday precision). The results were calculated and % RSD was determined. Results were tabulated in (Table 3).

Table 3: Precision data of Apixaban

Precision (µg/ml)	Zero Order Derivative (% RSD)	Area Under Curve (% RSD)
5	1.982%	0.548%
10	0.790%	0.988%
15	1.22%	1.097%
20	0.978%	0.101%
25	0.242%	0.132%

Accuracy

Accuracy studies were carried out at 80%, 100% and 120% levels of standard solutions. At 279.00 nm, Zero Order Derivative values were measured, and percent recoveries were calculated for respective levels. % RSD was calculated by analyzing each level in triplicate. The results were tabulated in (Table 4).

Table 4: Recovery of Apixaban

Test sample (µg/ml)	Accuracy Level (%)	Amount of standard drug added (µg/ml)	% Recovery (ZOD)	Accuracy Level (%)	Amount of standard drug added (µg/ml)	% Recovery (AUC)
10µg/ml	80%	18µg/ml	100%	80%	18µg/ml	100%
	100%	20µg/ml	100%	100%	20µg/ml	100%
	120%	22µg/ml	100%	120%	22µg/ml	100%

Method Precision

Repeatability

The repeatability study was carried out by repeatedly analyzing (n = 6) working standard solutions of Apixaban (10µg/ml) at 269.00 nm–289.00 nm range area under curve (AUC) measured and percent relative standard deviation (% RSD) was determined.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Solutions of concentrations 5 µg/ml-25 µg/ml were prepared five times (five sets) and calibration curves were determined for each set. The values of LOD and LOQ were calculated by using following formulae:

$$LOD = 3.3 \times \frac{SD}{S}$$

$$LOQ = 10 \times \frac{SD}{S}$$

Where, SD is standard deviation of y-intercept of the calibration curve and

S is mean slope of six calibration curves.

Table 5: LOD and LOQ Data of Apixaban

METHOD	ZOD	AUC
LOD	0.23	0.335
LOQ	0.713	1.015

RESULTS AND DISCUSSION

A specific and reproducible Zero order derivative and Area under Curve spectroscopy methods were attempted for determination of Apixaban in tablet dosage form. The following regression equations were obtained,

$$ZOD = f 0.0511x + 0.0077; R^2 = 0.9975$$

$$AUC = f 0.2562x - 0.0042; R^2 = 0.9965$$

f is amplitude difference, x is concentration and R² is correlation coefficient. The R² values were 0.9975 and 0.9965 for method A and B respectively showed that both methods are linear.

Both methods, A and B were precise as % RSD for intraday and interday precision are within limits. In

accuracy studies percent recoveries were satisfactory for each 80%, 100% and 120% level, which is in the range of 99.00% – 100.00%. From these values both methods A and B were found to be accurate. The LOD and LOQ values found to be 0.23µg/ml and 0.335µg/ml for method A and 0.713µg/ml and 1.015µg/ml for method B respectively. Assay was found to be 100% for a pharmaceutical tablet dosage form which is consistent with the label claim. From overall studies it was shown that present methods are reproducible and precise to carry out routine analysis of Apixaban in tablet dosage form. Results for method A and B validation studies are summarized in (Table 6).

Table 6: Validation parameters of Apixaban by UV–spectroscopic method

Validation Parameter	Zero Order Derivative	Area Under Curve
Range	279.00nm	269.00nm - 289.00nm
Linearity	5-25µg/ml	5-25µg/ml
Regression Equation ($y = mx + c$)	0.0511x + 0.0077	0.2562x – 0.0042
Slope (m)	0.0511	0.2562
Intercept (c)	0.0077	0.0042
Correlation coefficient (R ²)	0.9975	0.9965
Repeatability (% RSD)	0.335	0.5469
Intraday (% RSD)	0.790	0.988
Interday (% RSD)	0.2021	0.438
Accuracy (Mean % Recovery)	100%	100%
LOD (µ/ml)	0.23	0.335
LOQ (µg/ml)	0.713	1.015

CONCLUSION

There was no method reported for determination of Apixaban from bulk and pharmaceutical dosage form by zero order derivative and Area under curve spectrophotometry. So, from present research work it is concluded that the methods are economical and reproducible. Zero order derivative and Area under curve spectrophotometric methods were developed and validated as per ICH Q2 (R1) guidelines. The proposed methods can be employed for routine analysis of Apixaban from pharmaceutical dosage form. The results obtained on the validation parameters met ICH and USP requirements. It is inferred that the methods were found to be simple, accurate, precise and linear. The methods were found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

SPONSORSHIP

Nil.

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