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Determination of Apixaban from Bulk and Tablet Dosage Form by Area Under Curve and First Order Derivative Spectrophotometric Methods

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Abstract : A simple, new, precise and reproducible two UV - spectrophotometric methods has been developed for the estimation of Apixaban in bulk and tablet dosage form. Methanol was used as an solvent to prepare standard and sample solutions. For quantitative determination of apixaban by Method A that is area under curve (AUC) values measured at 269.00nm – 289.00nm and Method B that is first order derivative spectroscopy values measured at λmin = 266.21nm, λmax = 304.62nm and 279.09nm = zero cross. Calibration curve was observed with concentrations 5 – 30 µg/ml (R^2 = 0.9998 and R^2 = 0.9999) for methods A and B respectively. Both methods were validated as per ICH guidelines, limit of detection (LOD) and limit of quantitation (LOQ) were determined for respective methods. Accuracy, precision, assay and repeatability studies produce satisfactory results for both methods. The results of all validation parameters was found to be within acceptable limit. Both method A and B has been used to quantify apixaban from bulk and tablet dosage form successfully.
Keywords : Apixaban, Area under curve, First order derivative, Analytical method validation, ICH Q2 (R1) guideline.

1. Introduction

Apixaban (fig no. 1) is a antithrombotic agent. Chemically is an 1-(4 – methoxyphenyl) – 7 – oxo – 6 – [4 – (2 – oxopiperidin – 1- yl) phenyl] – 4, 5 – dihydropyrazolo [3, 4 – c] pyridine – 3 – carboxamide.The molecular formula and molecular weight of Apixaban is C25H25N5O4and 459.506 g/mol respectively.[20]Apixaban is white to pale – yellow in colour and available in powder form. And it is store into 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.[2, 6, 20]According to literature survey studies, only few HPLC methods are established for determination of Apixaban from pure and pharmaceutical formulations.[21, 23, 25]

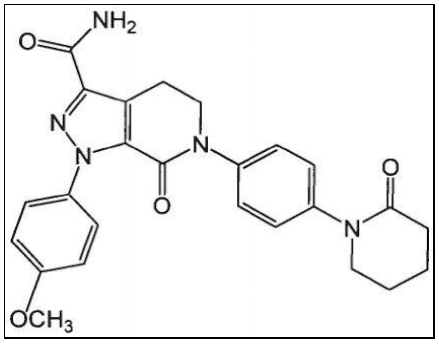

Figure No.1- Chemical Structure of Apixaban

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

Experimental:

Materials and Methods:

Apixaban was provided as a gift sample by Lupin pharmaceuticals Ltd. Aurangabad, India. HPLC grade methanol was used to prepare solutions, Apixaban 5 mg tablets were purchased from local pharmacy in Pune. Shimadzu UV 1800 (Japan) with matched quartz cells, connected to computer loaded with UV Prob Software. Single pan electronic balance (Shimadzu, ATY 224) was used for weighing purpose. Sonication of the solutions was carried out using an Ultrasonication (Spectralab UCB 40, India).Calibrated volumetric glasswares (Borosil) were used to perform study.

Method Development:

Preparation of Standard Solution:

Accurately weighed 100mg quantity of Apixaban was transferred into 100 ml volumetric flask, to this 70 ml of methanol was added and sonicated until all drug get dissolved. After that volume was make up by methanol to obtained 1000 µg/ml solution. From resulting solution 10 ml solution pipetted out into 100 ml volumetric flask and volume adjusted with methanol toobtained 100 µg/ml standard stock solution. This solution was further diluted with methanol to obtained desired concentrations of working standard solutions in the range of 5 – 30 µg/ml.

Wavelength Selection:

Apixaban 15µg/ml working standard solution scanned between 400.00 nm – 200.00 nm in UV spectrophotometer by using methanol as blank after baseline correction. 279.00nm wavelength was selected for further analysis.

(Method A): Area Under Curve (AUC):

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

Area calculation$(\alpha + \beta) = \frac{\lambda 1}{\lambda 2} Ad\lambda$

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,$\lambda 1$ and $\lambda 2$are wavelengths representing start and end point of curve region. In this study area was integrated between wavelength ranges from269.00nm - 289.00nm.

(Method B): First Order Derivative Spectrophotometry:

Solutions of Apixaban 5 – 30µg/ml were prepared and scanned in the spectrum mode from 400.00 nm – 200.00nm. The resulting absorption spectra were analysed by first order derivative method, the absorbance were measured at zero cross = 279.09nm. Absorbance were plotted against their respective concentrations to calculate regression equation.

Preparation of Calibration Curve:

Solutions of Apixaban was prepared of concentrations5, 10, 15, 20, 25 and 30µg/ml from 100µg/ml standard stock solution using methanol as an solvent. For method A: Above solutions were scanned from 400.00 nm – 200.00nm and Area under curve was integrated in the range of 269.00nm – 289.00 nm. Calibration curve was plotted for area under the curve against concentration. For method B: All solutions were analysed at 279.09nm = zero crossing wavelength and absorbance were recorded. Calibration graph was plotted for absorbance against concentration.

Assay of Apixaban (5 mg) Tablets:

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

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 – 30 µg/ml working standard solutions linearity was determined. Method A: Calibration plot constructed for area under curve against concentration and regression equation calculated. Area under curve integrated in the range of 269.00 nm - 289.00nm. Method B: at zero cross = 279.00nm absorbance were measured and calibration plot of absorbance against concentration constructed to obtained regression equation.

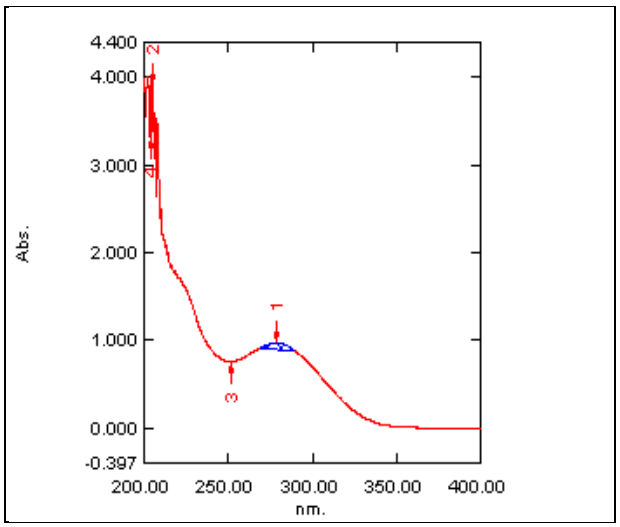

Figure No.2 : Area Under Curve Graph of 25 µg/ml Apixaban Solution.

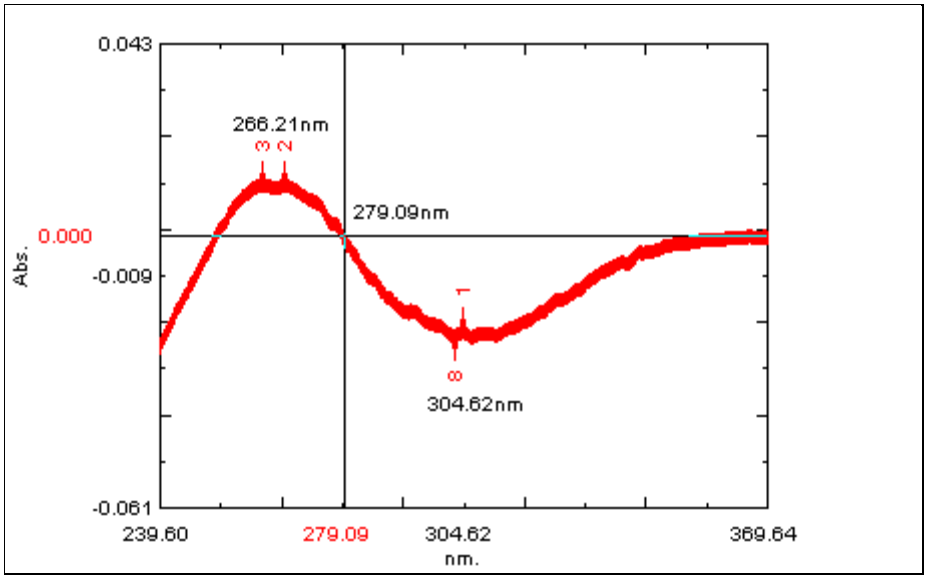

Figure No.3 : First Order Derivative Spectra of Apixaban (25 µg/ml).

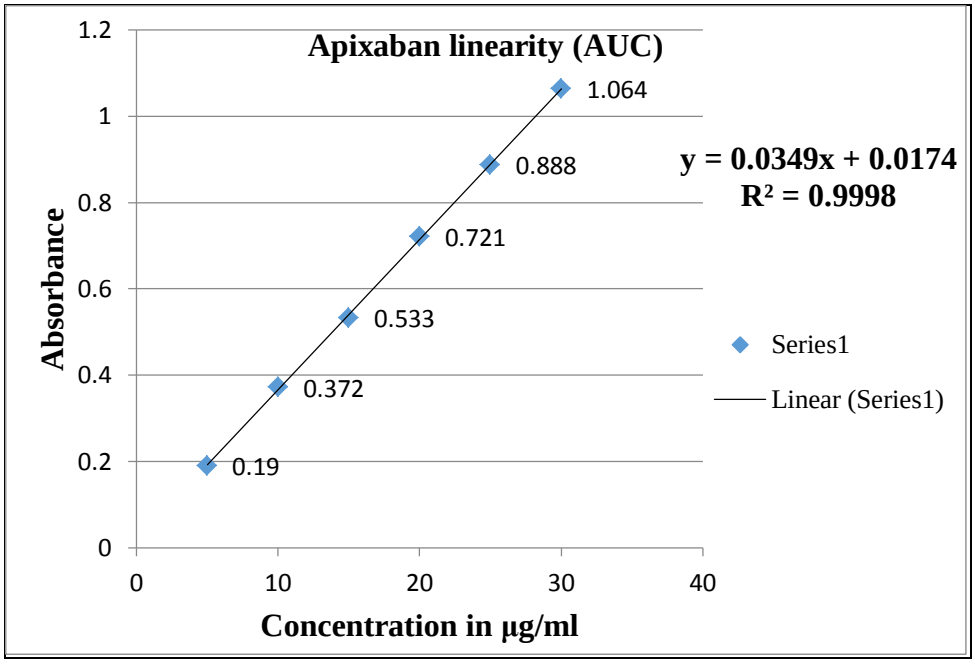

Figure No.4 : Calibration curve of Apixaban method A: Area under curve.

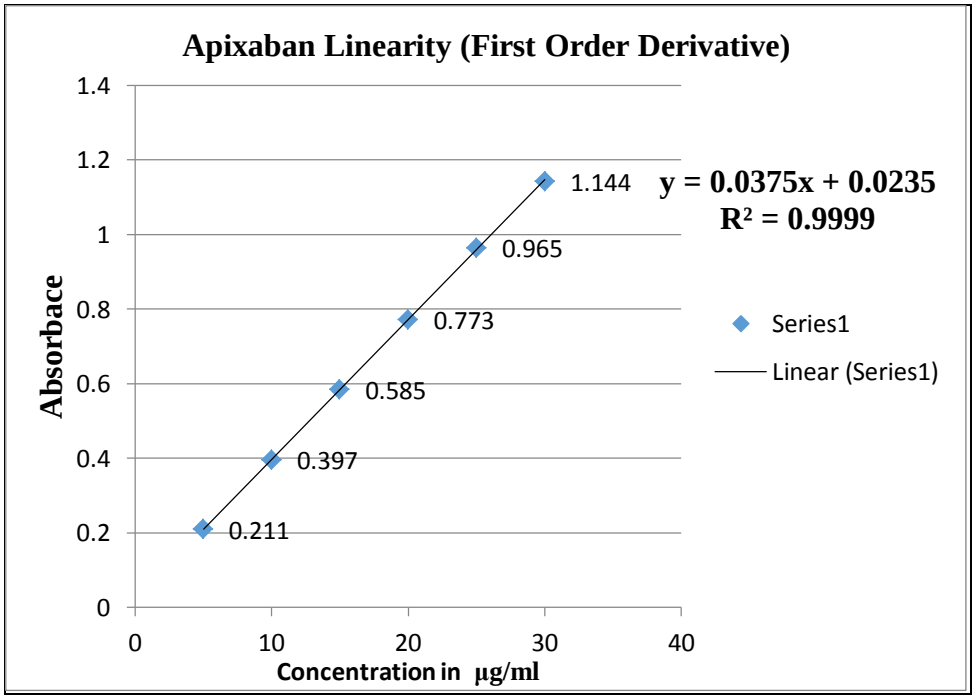

Figure No.5 : Calibration curve of Apixaban method B: First Order Derivative.

Table No. 1: Assay of Marketed Tablets of Apixaban

Method	Label claim	Amount taken	Amount found (mg/tab)	% Assay
A	5 mg	10 mg	9.999 mg	99.99 %
B	5mg	10 mg	9.975 mg	99.75 %

Table No. 2: Apixaban Calibration Data

Concentration (µg/ml)	Absorbance: Method A (Area Under curve)	Absorbance: Method B (First Order Derivative)
5	0.190	0.211
10	0.372	0.397
15	0.533	0.585
20	0.721	0.773
25	0.888	0.965
30	1.064	1.144

Table No. 3: Precision data of Apixaban

Precision	Method A (% RSD)	Method B (%RSD)
Repeatability	0.86	1.74
Intraday	1.31	1.03
Interday	1.53	1.34

Method Precision:

Repeatability:

The repeatability study was carried out by repeatedly analysing (n = 6) working standard solutions of Apixaban (15 µg/ml). at 269.00nm – 289.00nm range area under curve (AUC) measured and percent relative standard deviation (% RSD) was determined.

Table No.4: Results for Recovery of Apixaban

Test sample (µg/ml)	Accuracy Level (%)	Amount of standard drug added (µg/mL)	% Recovery (Method A)	Amount of standard drug added (µg/mL)	% Recovery (Method B)
10	80	8	99.74	8	99.13
	100	10	99.55	10	99.48
	120	12	100.3	12	99.64

Table No. 5: LOD and LOQ Data of Apixaban

Method	Method A (Area Under Curve)	Method B (First Order Derivative)
LOD(µg/ml)	0.66	0.33
LOQ(µg/ml)	2.00	1.01

Intermediate Precision (Reproducibility) :

The three concentrations of apixaban that is 10 µg/ml, 15 µg/ml and 20µg/ml each were analysed in triplicate on same day (Intraday precision) and same solutions were analysed in triplicate on different day (Interday precision). The results were calculated and % RSD determined. Results aretabulated in (Table no.3).

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

Solutions of concentrations 5 µg/ml – 30 µg/ml were prepared six times (six sets) and calibration curves were determined for each set. The values ofLOD and LOQ were calculated by using following formula:

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

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

Where,SD is standard deviation of y-intercept of the calibration curves, S is mean slope of six calibration curves.

TableNo. 6 : Results of Validation Parameters of Apixaban by UV –Spectroscopic Method:

Validation Parameter	Method A (Area Under Curve)	Method B (First Order Derivative)
Rang	269.00 nm – 289.00 nm	279.00 nm
Linearity range	5 – 30 µg/ml	5 – 30 µg/ml
Regression equation ($y = mx + c$)	0.0349x + 0.0174	0.0375x + 0.0235
Slope (m)	0.0349	0.0375
Intercept (c)	0.0174	0.0235
Correlation coefficient (R^2)	0.9998	0.9999
Repeatability (% RSD)	0.86	1.74
Intraday (% RSD)	1.31	1.03

Interday (% RSD)	1.53	1.34
Accuracy (Mean % Recovery)	99.86	99.41
LOD (µg/ml)	0.66	0.33
LOQ (µg/ml)	2.00	1.01

Accuracy:

Accuracy studies was carried out at 80%, 100% and 120% levels of standard solutions. At 269.00 nm – 289.00 nm area under curve values were measured and percent recoveries were calculated for respective levels. (% RSD) was calculated by analysing each level in triplicate. The results are tabulated in (Table no.4).

Result and Discussion:

A specific and reproducible area under curve and first order derivative spectroscopy methods were attempted to develop for determination of apixaban in tablet dosage form. The following regression equation were obtained,

Method A = $\int_{269}^{289} Ad'\lambda$ 0.0349x + 0.0174 R^2 = 0.9998

Method B = $\frac{d'A}{d'\lambda}$ 0.0375x + 0.0235 R^2 = 0.9999

Where, $\int_{269}^{289} Ad'\lambda$ is area under curve between 269.00nm – 289.00nm, $\frac{d'A}{d'\lambda}$ is amplitude difference, x is concentration and R^2 is correlation coefficient. The R^2 values were 0.9998 and 0.9999 for method A and B respectively showed that both methods are linear.

Both method A and B were precis as % RSD for intraday and interday precision are within limits. In accuracy studies percent recovery were satisfactory for each 80%, 100% and 120% level, that is in the range of 99.00% – 100.00%. from these values both methods A and B found to be accurate.the LOD and LOQ values found to be 0.66µg/ml and 2.00µg/ml for method A and 0.33µg/ml and 1.01µg/ml for method B respectively. Assay was found to 99.99% for a pharmaceutical tablet dosage form which is consistent with the label claim. From all over studies it was shown that present methods are reproducible and precise to carry out routine analysis of Apixaban in tablet dosage form. Results for methodA and B validation studies are summarised in (Tableno.6).

Conclusion:

There was no methods were reported for determination of apixaban from bulk and pharmaceutical dosageform, by area under curve (AUC) and first order derivative spectrophotometry. So, from present research work it is concluded that economical and reproducible area under curve and first order derivative spectrophotometric methods are developed and validated as per ICH Q2 (R1) guideline. The proposed methods can be employed for routine analysis of apixaban from pharmaceutical dosage form.

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