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DEVELOPMENT AND VALIDATION OF SIMPLE UV SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF APIXABAN IN API AND ITS BULK DOSAGE FORM

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ABSTRACT

Simple, rapid, sensitive, precise and specific UV spectrophotometric method for the determination of Apixaban in bulk drug and pharmaceutical dosage form were developed and validated. In this method solution of Apixaban was prepared in methanol. Apixaban standard solution was scanned in the UV rang (400-200nm) in a 1cm quartz cell in a double beam UV spectrophotometer. The standard solution of Apixaban showed maximum absorption at wavelength 280 nm. The method obeys Beer's law in the concentration range from 2 µg/ml - 10µg/ml. The correlation coefficient was found to be 0.9967 and regression of the curve was found y = 0.0995x + 0.0149 with excellent recovery 99-104%. Limit of detection and limit of quantitation were found to be 0.186 µg/ml and 0.566µg/ml respectively. The method was validated for several parameters like accuracy, precision as per ICH guidelines. Statistical analysis proved that the methods are repeatable and specific for the estimation of the said drug. These methods can be adopted in routine assay analysis of Apixaban bulk or tablet dosage form.

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INTRODUCTION

Apixaban, sold under name of the tradename Eliquis, is an anticoagulant for the treatement of venous thromboembolic events. It is a direct factor Axe inhibitor. Apixaban is highly selective, orally bioavailable,and reversible direct inhibitor of free and clot –bound factor Xa.Factor Xa catalyses the conversion of prothrombin to thrombin,the final enzyme in the coagulation cascade that is responsible for fibrin clot formation.apixaban has no direct effect on platelet aggregation ,but by inhibiting factor Xa it indirectly decreasesclot formation induced by thrombin.[1]

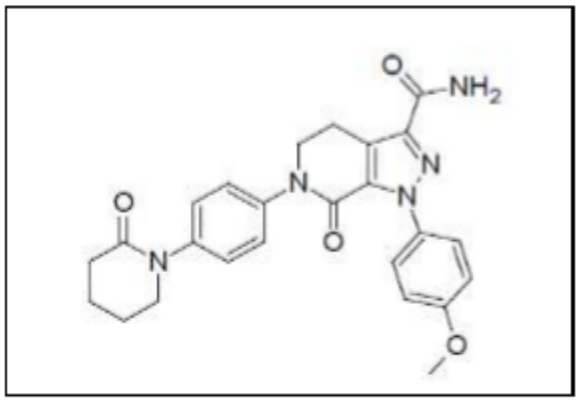

Fig. 1: Chemical Structure of Apixaban.

ELIQUIS (apixaban), is chemically described as 1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1yl)phenyl]-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxamide(Figure 1).
Its molecular formula is C25H25N5O4, which corresponds to a molecular weight of 459.5. Apixaban1, 2 is a white to pale-yellow powder. At physiological pH (1.2-6.8), apixaban does not ionize; its aqueous solubility across the physiological pH range is ~0.04 mg/mL. ELIQUIS tablets are available for oral administration in strengths of 2.5 mg and 5 mg of apixaban. Apixaban has been available in Europe since May 2012.[2]

There are some methods of estimation of apixaban from human plasma by LC-MS, but there is no assay method for apixaban by HPLC and UV Spectrophotometry. Further, apixaban is not officially reported in any pharmacopeia (USP, EP, JP & IP) to date.[7]
The current UV Spectrophotometric methods were developed and validated as per the ICH guidelines. The UV Spectrophotometric methods described here is simple, sensitive, and reproducible for apixaban determination in formulation with low background interfences. An attempt has been made to develop and validate to ensure their accuracy, precision and other analytical method validation parameters .[3] we have made an attempt to develope more precise, simple and economical spectrophotometric method with greater precision,accuracy and sensitivity for analysis of apixaban in bulk and dosage form. Uv visible spectrophotometry is one of the most frequently in pharmaceutical analysis.It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution.

MATERIALS AND METHODS:

Instruments:

For Weighing, a calibrated weighing balance (Make- Shimadzu) of 1mg sensitivity was used. For analytical purpose UV Spectrophotometer Shimadzu-UV 2450 PC was used. All other glasswares and apparatus were made of Borosilicate and were calibrated.

Chemicals:

API-Apixiban is pure drug gifted by MVPs college of pharmacy, Nashik . Methanol was used in this study.

Preparation of standard stock solution:

The standard stock solution of Apixiban was prepared by transferring, accurately weighed 100 mg of apixaban to 100 ml volumetric flask containing 50ml distilled water. Dissolved drug properly. Then volume was made up to the mark by using distilled water to gives concentration 1000µg/ml. From this 10 ml of the solution was transferred to a 100 ml volumetric flask and make up the volume with distilled water to gives a concentration of 100µg/mL it is standard stock solution and which is further diluted with distilled water to get concentration 2-10µg/ml.[5]

Determination of Absorption Maxima:

The appropriate dilution of standard stock solution with methanol, solution contain 10µg/ml of Apixaban was scanned in the range of 400-200nm to determine the wavelength of maximum Absorption. Drug showed Absorption maxima at 280nm.

Preparation of Calibration Curve

For the preparation of standard calibration curve, concentration of 2-10µg/ml were prepared by pipetting out 0.2, 0.4, 0.6, 0.8 and 1 ml from the 100µg/ml solution in to a 10ml volumetric flask and made up the volume with methanol. The absorbance of each solution was measuredat 280nm against methanol as blank. Calibration curve of the Apixiban was plotted by taking the absorbance obtained on y-axis and the concentration of the solution on x-axis (Fig. 2). The curve showed linearity in the range of 2-10 µg/ml with correlation coefficient 0.9967.[5]

Table No.1: Calibration Curve.

Sr.no	conc ppm	Absorbance
1	2	0.210
2	4	0.411
3	6	0.562
4	8	0.770
5	10	0.985

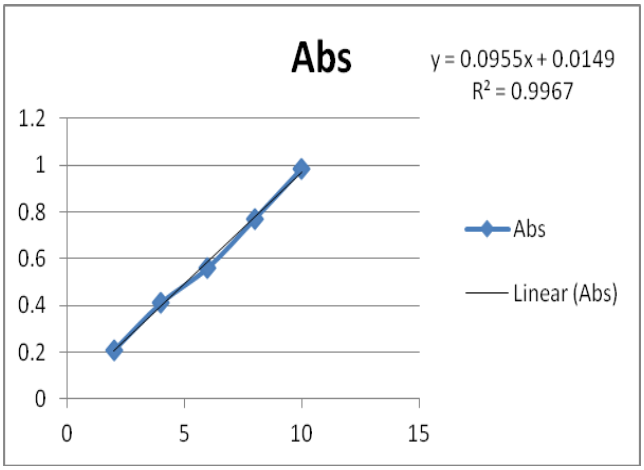

Fig. 2: Calibration Curve of Apixaban.

METHOD VALIDATION:

The developed method was validated as per ICH guidelines for following parameters [2,3]

Linearity:

Aliquots of standard stock solution were further diluted with water to get the solutions of concentration within range from 2-10 µg/mL. The absorbance was measured at wavelength 280 nm. Linear calibration graph was obtained by plotting the absorbance value versus concentration of Apixiban.

Range:

The Range of analytical procedure is interval between upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure as suitable level of precision,accuracy,linearity.

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision of the method was determined in terms of repeatability, intraday and interday precisions.

Intraday and Interday Precision (Intermediate Precision):

Intraday precision was determined by analyzing the drugs at concentrations (10µg/mL) and each concentration for three times, on the same day. Interday precision was determined similarly, but the analysis being carried out daily, for three consecutive days.

Recovery:

The accuracy studies were performed by using 80%, 100% and 120% of the solution 0f 10 ppm. Thus 8, 10 and 12 ppm concentration were used in triplicates for accuracy. Accuracy is reported as % recovery which was calculated from the expression as equation given below,

$$\%\ Recovery = \frac{\text{Observed value}}{\text{True value}} \times 100$$

Repeatability:

Repeatability of the method was determined by analyzing six samples of same concentrations of drug (10µg/mL). Spectra were recorded, and the absorbance of each spectrum was measured.

Robustness:

The robustness of developed method is its capacity to remain unaffected by small changes in altered conditions. To determine the robustness of the method, the wavelength of analysis was deliberately altered and assay was evaluated. The effect of detection wavelength was studied at ± 5nm.

Solution Stability:

The stability of the solution was studied by analyzing the standard solution at 1, 2, 3, 4 and 5 days intervals.

RESULT AND DISCUSSION:

Determination of wavelength of maximum absorption:

The wavelength of maximum absorption was found to be 280nm

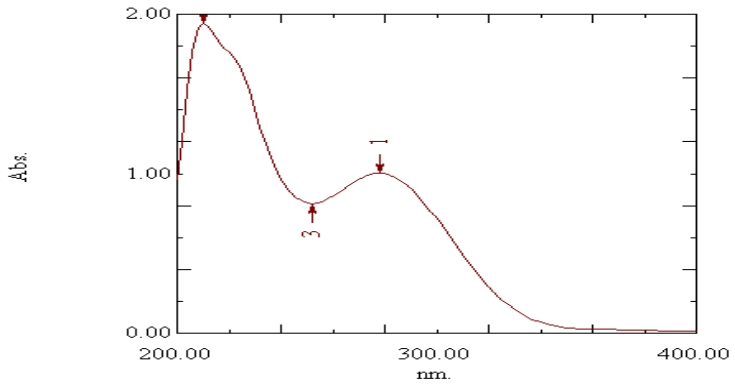

Fig 3: Wavelength of maximum absorption of Apixaban.

Linearity:

The linearity of this method was determined at ranging from 2-10µg/ml for Apixaban. The regression equation were found to be y = 0.0995x + 0.0149, r^2= 0.9967.

Table.2: Linearity table.

Sr.No	Concentration (ppm)	Absorbnace
1	2	0.210
2	4	0.411
3	6	0.562
4	8	0.770
5	10	0.985

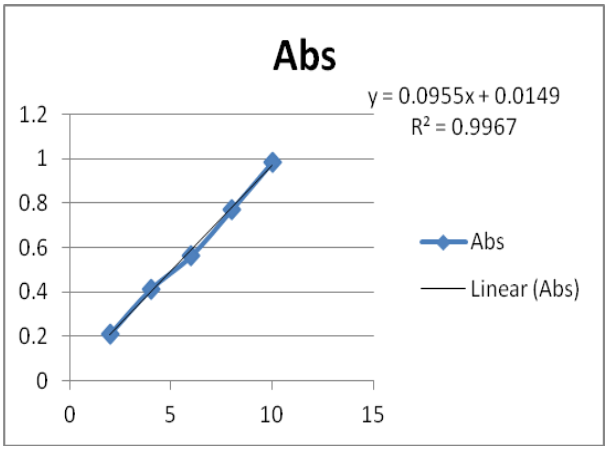

Fig 5: Linearity graph of Apixiban.

The method for Apixaban was found to be linear in the range of 2-10 ppm with $R^2 = 0.9967$ and the straight line equation as: $y = 0.0955x – 0.0149$

Precision:

The precision (measurement of intraday, interday, repeatablity) results showed good reproducibility with percent relative standard deviation (% RSD) was below 2.0%. This indicated that method was highly precise.

Table.3: Intraday morning precision.

	Concentration	Morning absorbance	(Y-Ȳ)	$(Y-\bar{Y})^2$	SD	%RSD
1	10	0.561	-0.006	0.000036	0.00538	0.948%
2	10	0.571	0.004	0.000016		
3	10	0.568	0.001	0.000001		
4	10	0.576	0.009	0.000081		
5	10	0.569	0.002	0.000004		
6	10	0.561	0.006	0.000036		
		Ȳ=0.567		Σ=0.000174		

Intraday precision:

Table.4: Intraday Afternoon precision.

Sr.No	Concentration	Afternoon absorbance	(Y-Ȳ)	$(Y-\bar{Y})^2$	SD	%RSD
1	10	0.567	-0.003	0.000009	0.0056	0.895%
2	10	0.588	0.003	0.000009		
3	10	0.570	0.010	0.0001		
4	10	0.568	0.008	0.000064		
5	10	0.561	0.001	0.000001		
6	10	0.561	0.002	0.000004		
		Ȳ=0.569		Σ=0.000187		

Table.5: IntradayEvening precision.

Sr.No	Concentration	Evening absorbance	(Y-Ȳ)	$(Y-\bar{Y})^2$	SD	%RSD
1	10	0.568	0.00	0.00	0.0017	0.3049%
2	10	0.570	0.002	0.000004		
3	10	0.571	0.003	0.000009		
4	10	0.569	0.001	0.000001		
5	10	0.566	0.002	0.000004		
6	10	0.568	0.00	0.00		
		Ȳ=0.568		Σ=0.000018		

Interday precision:

Table.6:Interday morning precision Study.

Sr.No	Concentration	Morning absorbance	(Y-Ȳ)	$(Y-\bar{Y})^2$	SD	%RSD
1	10	0.568	0.003	0.000009	0.00371	0.658%
2	10	0.569	0.004	0.000016		
3	10	0.566	0.001	0.000001		
4	10	0.570	0.005	0.000025		
5	10	0.561	0.004	0.000016		
6	10	0.561	0.004	0.000016		
		Ȳ=0.565		Σ=0.000083		

Table.7: Interday Afternoon precision Study.

Sr.No	Concentration	Afternoon absorbance	(Y-Ȳ)	$(Y-\bar{Y})^2$	SD	%RSD
1	10	0.567	0.004	0.000016	0.00410	0.718%
2	10	0.571	0.00	0.00		
3	10	0.570	0.001	0.000001		
4	10	0.567	0.004	0.000016		
5	10	0.579	0.008	0.000064		
6	10	0.573	0.002	0.000004		
		Ȳ=0.571		Σ=0.000101		

Table.8:Interday Evening precision.

Sr.No	Concentration	Evening absorbance	(Y-Ȳ)	$(Y-\bar{Y})^2$	SD	%RSD
1	10	0.571	0.002	0.000004	0.00367	0.641%
2	10	0.574	0.001	0.000001		
3	10	0.571	0.002	0.000004		
4	10	0.579	0.006	0.000036		
5	10	0.573	0.00	0.00		
6	10	0.573	0.00	0.00		
		Ȳ=0.573		Σ=0.000081		

Repeatability:

Table.9: Repeatability study.

Sr.No	Concentration	Absorbance	(Y-Ȳ)	$(Y-\bar{Y})^2$	SD	%RSD
1	10	0.625	-0.004	0.000016	0.00430	0.683%
2	10	0.630	0.001	0..000001		
3	10	0.636	0.007	0.000049		
4	10	0.625	-0.004	0.000016		
5	10	0.627	-0.002	0.000004		
6	10	0.634	0.005	0.000025		
		Ȳ=0.629		Σ=0.000111		

Accuracy:

The accuracy for the analytical method for Apixaban was determined at 80%, 100% and 120% levels of standard solution. Absorbance was measured at 280nm and results were expressed in terms of % recoveries.

Table.10:Accuracy Study.

Sr.No	%concentration	Concentration in ppm	Volume of API stock (ml)	Absorbance (nm)	Mean
1	80	8	0.8	0.770 0.772 0.770	0.770

The concentration is calculated by using straight line equation:

$$y = mx + c$$

y = 0.095x +0.014

$$x = (y - c)/m$$

$$x = \frac{0.770 - 0.014}{0.095}$$

X= 7.95 ppm

% Recovery from 8 ppm solution: $\frac{x}{8} * 100$

= (7.95/8)* 100

= 99.47%

Table.11: Recovery Study.

+%concentration	Sr.No	Concentration in ppm	Volume of API stock (ml)	Absorbance (nm)	Mean
100	1	10	1.00	0.980	0.981
	2			0.984	
	3			0.981	

The concentration is calculated by using straight line equation:

$$y = mx + c$$

y = 0.095x+ 0.014

$$x = (y - c)/m$$

$$x = \frac{0.981 - 0.014}{0.095}$$

X= 10.17 ppm

% Recovery from 10 ppm solution: $\frac{x}{10} * 100$

= (10.17/10)* 100

= 101.7 %

Table.12:Recovery Study.

Sr.no	Concentration %	Volume of API stock in (ml)	Absorbance(nm)	Mean
1	120	1.2	1.19	
			1.20	1.20
			1.20	

The concentration is calculated by using straight line equation:

$$y = mx + c$$

y = 0.095x +0.014

$$x = (y - c)/m$$

$$x = \frac{1.20 - 0.014}{0.095}$$

X= 12.48ppm

% Recovery from 12 ppm solution: $\frac{x}{12} * 100$

= (12.48/12)* 100

= 104.03%

Limit of Detection and Limit of Quantification:

It is calculated by using slope and standard deviation from linearity and precision respectively:

Limit of detection (LOD):

$$LOD = 3.3 \times SD/Slope$$

$$LOD = 3.3 \times 0.00538/0.095$$

$$LOD = 0.186\ ppm$$

Limit of quantification (LOQ):

$$LOQ = 10 \times SD/Slope$$

$$LOQ = 10 \times 0.00538/0.095$$

$$LOQ = 0.566\ ppm$$

Robustness:

Table.13: Robustness Study.

Sr.No	Wavelength(nm)	Absorbance	(Y-Ȳ)	$(Y-\bar{Y})^2$	SD	%RSD
1	275	0.560	0.003	0.000009	0.00277	0.493
2	276	0.561	0.002	0.000004		
3	277	0.561	0.02	0.000004		
4	278	0.565	0.002	0.000004		
5	279	0.559	0.04	0.000016		
6	280	0.561	0.002	0.000004		
7	281	0.564	0.001	0.000001		
8	282	0.566	0.003	0.000009		
9	283	0.566	0.003	0.000009		
10	284	0.568	0.005	0.000025		
11	285	0.566	0.003	0.000004		
		Ȳ=0.563				

Result and Discussion: summary

Sr No.	Validation Parameters	Results
1	Absorption maxima(nm)	280nm
2	Beers range (µg/ml)	2-10µg/ml
4	Standard Regression Equation	y = 0.0995x +0.0149
5	Correlation Coefficient (r2)	0.9969
6	Accuracy(8,10&12 ppm)	99.47%, 101.07% & 104.03%
7	Precision (%RSD)	0.948
8	LOD &LOQ(µg/ml)	0.186&0.566
9	Robustness(%RSD)	0.493

CONCLUSION

The UV-spectrophotometric method was developed and it is found to be simple, accurate, precise, highly sensitive, reproducible and inexpensive. The proposed method was found suitable for determination ofApixaban in in API and its bulk dosage form without any interference from the excipients. The validation procedure confirms that this is a workable method for their quantification in the raw material and also in the formulations. Hence it can be effectively applied for the routine analysis of Apixaban in bulk drug. Its advantages are low cost of reagents, speed and simplicity of sample treatment, satisfactory precision and accuracy.

ABBREVIATIONS:

UV : Ultra Violet
µm : micrometre
nm : nanometre
ml : millilitre
UV- Vis : Ultraviolet-Visible
API : Active Pharmaceutical Ingredient
% : Percentage
Ppm : Parts per million
API : Active Pharmaceutical Ingredient
HCl : Hydrochloride

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CONFLICT OF INTEREST:

The authors do not report any conflict of interest.

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