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Method developed for the determination of apixaban by using U.V. spectrophotometric

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ABSTRACT

The present work is aim to Develop UV spectrophotometry method for the estimation of Apixaban in its dosage forms. Analysed the marketed formulations for their reliability and accuracy and performed the recovery studies for the developed UV spectrophotometric method. The developed method was validate for its accuracy precision reproducibility. On the basis of results the UV spectrophotometric method developed for the determination of Apixaban is found to be precise, accurate and cost effective. Hence this method can be used for routine analysis of Apixaban in bulk and pharmaceutical dosage forms.

Keywords: UV spectrophotometry; apixaban; bulk dosage forms.

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INTRODUCTION

Development of a simple, reliable and accurate method for the assay of apixaban tablet dosage form by UV and validate the method for its repeatability and reproducibility. Apixaban, sold under the trade name Eliquis among others, is an anticoagulant used to treat and prevent blood clots and to prevent stroke in people with atrial fibrillation.[1, 2] Specifically it is used to prevent blood clots following hip or knee replacement and in those with a history of prior clots. It is used as an alternative to warfarin and does not require monitoring by blood tests.

Apixaban was approved for medical use in the United States in 2012. A month supply in the United Kingdom costs the NHS about £57 as of 2019.[2] In the United States the wholesale cost of this amount is about $427.[4] In 2016 it was the 123rd most prescribed medication in the United States with more than 5 million prescriptions.[5]

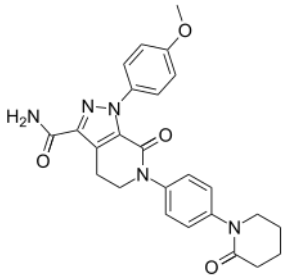

Figure 1: Chemical Structure of Apixaban

Apixaban is a highly selective, orally bioavailable, and reversible direct inhibitor of free and clot-bound factor Xa. Factor Xa catalyzes the conversion of prothrombin to thrombin, the final enzyme in the coagulation cascade that is responsible for fibrin clot formation.[10] Apixaban has no direct effect on platelet aggregation, but by inhibiting factor Xa, it indirectly decreases clot formation induced by thrombin.[6]

MATERIALS AND METHODS

Materials: Apixaban received as a gift sample from Sun Pharmaceuticals pvt. Ltd. and the authenticity of purity of the sample was certified by same. ELIQUIS-2.5mg, ELIQUIS-5mg marketed samples are obtained from the nearby stores.

Instrument: In developing quantitative method for determining unknown concentration of analyte by absorption spectrophotometry, UV visible double beam spectrophotometer, Systronics-2203(smart), Matched quartz cells 1 cm path length.

Solvent Selection: Solvent selection was made on the basis of solubility studies with pure drug several solvents were proposed for solubility they are Water, Sodium Hydroxide, Methanol, Ethanol. From solvents studies it was found that the pure drug was freely soluble in Water, Methanol, Ethanol.

Wave Length selection: Standard stock solution of Apixaban was prepared by dissolving 50 mg of drug in 50ml of water and diluted to required volume with same solvent. Then the solution was further diluted to get concentration of 10µg/ml. The solution was scanned inn U.V. region from 200-400nm.The λ max of drug was found to be at 278nm for Apixaban.

Table 1: Wave length selection

S.no.	Wave length	Absorbance
1	272	0.361
2	273	0.378
3	274	0.384
4	275	0.392
5	276	0.398
6	277	0.410
7	278	0.445
8	279	0.424
9	280	0.397
10	281	0.375

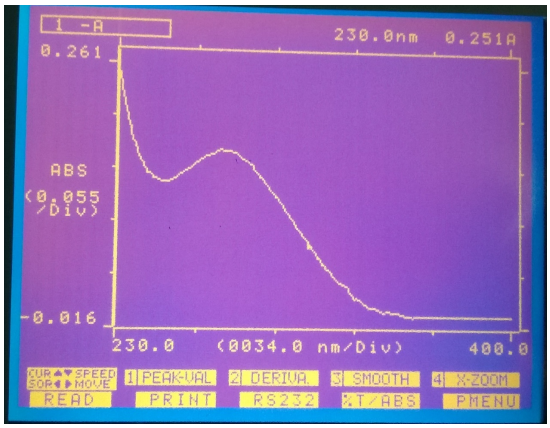

Figure 2: Spectra of Apixaban

Methods:

Preparation of stock solution: Standard stock solution of Apixaban was prepared by dissolving 50 mg of drug in 50ml of water and diluted to required volume with same solvent. Then the solution was further diluted to get concentration of 10µg/ml. The solution was scanned inn UV region from 200-400nm.The λ max of drug was found to be at 278nm for Apixaban.

Beers law concentration range: The stock solution was suitably diluted with water to get a concentration range from 10-80µg/ml and their absorbance was measured at 278nm. Using the absorbance values against concentration calibration curve was plotted. From the graph it was found that Apixaban obeys beers law between 10-70 µg/ml.

Analysis of formulation: 10 Tablets were finely powdered. An accurately weighed quantity of tablet powder equivalent to about 50mg of Apixaban was transferred to 50 ml standard flask. The content of flask was mixed with 30 ml water and shaken to dissolve the active ingredients and then make up to the volume with water .The solution was filtered and then filtrate diluted with water to give the concentration range 50 µg/ml. Absorbance values of samples solution was recorded at 278nm.

$$\frac{\text{Sample abs}}{\text{standard abs}} \times \frac{50}{\text{wt of sample}} \times \frac{50}{1} \times \frac{\text{wt of std taken}}{50} \times \frac{1}{50} \times \text{average weight}$$

Table 2: calibration curve of Apixaban

S.no.	Concentration	Absorbance
1.	10	0.103
2.	20	0.164
3.	30	0.265
4.	40	0.316
5.	50	0.425
6.	60	0.500
7.	70	0.573
Slope		**= 0.008131**
Correlation coefficient		**= 0.9957**

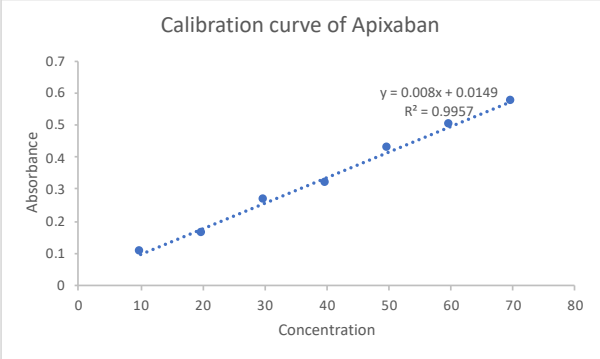

Figure 3: Calibration curve of Apixaban

Precision

The closeness of agreement between a series of measurements. Multiple sampling of homogeneous samples under prescribed condition, precision is two types Repeatability and Reproducibility.

Repeatability (system precision): 50µg/ml concentration solution of Apixaban was prepared whose absorbance of measured six times for which relative standard deviation is calculated.

Acceptance criteria: The relative standard deviation for preparation should not be more than 2%.

Reproducibility(method precision): six individual preparations of Apixaban was prepared with a concentration of 50µg/ml. Whose absorbance measured at 278nm.

Acceptance criteria: The relative standard deviation for preparation should not be more than 2%.

Solution stability: 50µg/ml concentration solution of Apixaban was prepared and the solution whose absorbance is measured for every half an hour for 90

Table 3: Quantitative estimation of apixaban (Eliquis-2.5mg)

S.no.	Concentration (µg/ml)	Label Claim	Amount Present	% of label claim	%deviation
1	30	2.5	0.002453	98.11321	0.0189
2	40	2.5	0.002524	100.9494	-0.0094
3	50	2.5	0.002512	100.4706	-0.0047
4	60	2.5	0.002545	101.8	-0.018
5	70	2.5	0.002641	98.4293	0.0158

Table 4: Quantitative estimation of apixaban (Eliquis-5mg)

S.no.	Concentration (µg/ml)	Label Claim	Amount Present	% of label claim	%deviation
1	30	5	0.005075	101.5094	-0.015
2	40	5	0.004921	98.41772	0.0159
3	50	5	0.004976	99.52941	0.0048
4	60	5	0.00491	98.2	0.018
5	70	5	0.004956	99.1274	0.0088

minutes and the solution was found to be stable up to 90 minutes.

Table 5: Solution stability of apixaban

S.no	Time	Absorbance
1.	1 min	0.524
2.	60 min	0.520
3.	90 min	0.480

Table 6: System precision of apixaban

Sl.no	Repeatability (conc) µg/ml	Absorbance
1	50	0.429
2	50	0.437
3	50	0.441
4	50	0.468
5	50	0.451
6	50	0.499
	Average	**0.445833**
	Standard deviation	**0.013512**
	%RSD	**0.030307**

Table 7: Method precision of apixaban

S.no.	Concentration (µg/ml)	Absorbance	Average
1	50	0.441	0.4325
		0.424	
2		0.434	0.4345
		0.435	
3		0.44	0.44
		0.44	
4		0.46	0.462
		0.464	
5		0.445	0.4555
		0.466	
6		0.45	0.4505
		0.451	
Average			**0.4455**
Standard deviation			**0.012062**
%RSD			**0.027076**

Limit of detection: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Table 8: LOD of Apixaban

Sl.no.	Parameter	Apixaban
1	Slope	0.008131
2	Standard deviation	0.013512
3	LOD	4.895579

Limit of quantitation: The limit of quotation of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with precision and accuracy .the quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrix and is used particularly for the determination of impurities and degradation product.

Table 9: LOQ of Apixaban

Sl.no.	Parameter	Apixaban
1	Slope	0.008131
2	Standard deviation	0.013512
3	LOQ	14.83509

Accuracy

Accuracy of method is the closeness of the measured value to the true value for the sample. Accuracy is usually determined by recovery studies. Recovery studies are performed by spiking pure powdered into the sample solution. The spiked samples prepared at a concentration range of 80%, 100%, 120%.

Table 10: Accuracy of Apixaban

S.no	Conn	Mg found	Mg added	% Recovery
1	80	0.005016	0.005	**100.316**
2	100	0.004976	0.005	**99.5294**
3	120	0.00497	0.005	**99.41**

The sample solution was prepared to get a concentrate range of 40µg/ml, 50µg/ml, 60µg/ml, into which 5mg of pure powdered is been added to get 80%, 100%, 120% concentration range. The percentage recovery is calculated for these concentrations from absorbance obtained.

The percentage recovery was calculated by using the following formula;

$$\text{Percentage recovery} = \frac{\text{amount obtained}}{\text{amount added}} \times 100$$

Acceptance criteria: The percentage recovery for the spiked preparation should be within 98-102 %.

Ruggedness: The extent to which is turned precision should be established depends on circumstances which the procedure is intended to be used. Intermediate precision expresses with in laboratory variation ie., different days, different analysts, different equipment's. The procedure followed for this is the same followed in the method precision was repeated on two different days by two different analyst the result for the intermediate precision recorded in the table.

Table 11: Ruggedness of Apixaban

Analyte	Concentration (µg/ml)	Absorbance	% RSD
Analyte-1	50	0.445	**0.27**
Analyte-2	50	0.515	**1.54**

Acceptance criteria: The relative standard deviation for the preparation should not be more than 2%

Robustness: Robustness of the method is its ability to remain unaffected by small ranges in parameter such as changes in wavelength, changes in p^H, changes in temperature etc. Robustness examines the effect of operational parameters on the analytical method. 50µg/ml concentration of Apixaban was prepared. whose absorbance measured in two different wavelength closer to the λmax of the drug.

Table 12: Robustness of Apixaban

Sl.No	Concentration (µg/ml)	Wave length	Absorbance
1	50	276	0.451
2	50	278	0.453
3	50	280	0.450

Table 13: System suitability parameter of apixaban

S.no.	Parameter	Requirement	Results	Acceptance criteria
1	Linearity	Corr. Coefficient	0.998	< 1%
2	Repeatability	% RSD	0.030307	< 2%
3	Reproducibility	% RSD	0.0270	< 2%
4	LOD		4.895579	< 3%
5	LOQ		14.83507	<10%
6	Accuracy	% Recovery	99.4-100.3	98-102%
7	Ruggedness	% RSD analyst-1	0.278	< 2%
		% RSD analyst-2	1.540	< 2%
8	Robustness	No limits	-----	No change in absorbance
Formulation1				98.11-101.8
Formulation2				98.2-101.5

RESULTS AND DISCUSSIONS

Under UV spectral analysis , the absorption maxima (λmax) of Apixaban was observed as 278 nm and tabulated in Table 1. Obeyance to Beer's law was confirmed by the linearity of the calibration curve of Apixaban, which is represented in and Figure 2. Apixaban showed the linearity in the concentration range of 10-70µg/ml. The data regarding the calibration curves are given in Table 2. The quantitative estimation was carried out in tablet formulations by taking concentrations of 10-70 µg/ml. The regarding the quantitative estimation is given in Table 3,4. The brands of formulations shows the percentage purity values range from 98.11-101.8%. The percentage deviation values were found to be between -0.004 to 0.0189. The quantitative results obtained were subjected to statistical analysis to find out standard deviation values. The relative standard deviation values are <2%, indicating the precision of the methodology 3. The repeatability, reproducibility, ruggedness of the method was conformed as per ICH guidelines. The data is given in Tables 6, 7. The results obtained expresses the precision of the given method. The validation of the proposed method was further confirmed by recovery studies. The recovery data is given in Table 10. The recovery values vary from 99.4-100.31% this serves as a good index of accuracy of the method.

CONCLUSION

Method developed for the determination of Apixaban by using U.V. spectrophotometric, On the basis of our experimental results we conclude that the U.V. spectrophotometric method developed for the determination of Apixaban is found to be precise, accurate and cost effective. Hence this method can be used for routine analysis of Apixaban in bulk and pharmaceutical dosage forms.

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