EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 4
929-933
Year: 2018

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHODS FOR APIXABAN IN TABLETS

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Article Received on 20/02/2018 **Article Revised on 12/03/2018** **Article Accepted on 01/04/2018**

ABSTRACT
Two sensitive zero order (D^0) and first order derivative (D^1) UV spectroscopic methods have been developed and validated for the determination of Apixaban in pharmaceutical formulations using phosphate buffer pH 5.0 (Method A), and phosphate buffer pH 7.0 (Method B). The drug showed maximum absorbance at 276 nm in zero order and the amplitude was measured in the range of 264 nm - 305 nm in first order derivative. Beer-Lambert's law was obeyed in the range of 1 – 60 µg/mL. Accuracy of the methods were established by standard addition and the recovery was found to be in the range of 98.3 - 101.1 % (% RSD < 0.66, zero order), 99.06 -100.4 % (% RSD <1.57, first order derivative). Precision of the methods were studied in terms of repeatability and intermediate precision (% RSD < 1.75). The developed methods were successfully applied for the assay of Apixaban in tablets and there was no interference from the excipients. The developed methods were found to be sensitive as observed from the optical characteristics. These methods were validated as per ICH guidelines and can be suitably used for the analysis of Apixaban in API and tablets.

KEYWORDS: Apixaban, Zero Order (D^0), First Order Derivative (D^1), Validation, ICH guidelines.

INTRODUCTION
Chemically Apixaban[1] is 1- (4 – methoxyphenyl) – 7 – oxo – 6 – [4 – (2 – oxopiperidin – 1- yl) phenyl] – 4, 5 – dihydropyrazolo [3, 4 – c] pyridine – 3 – carboxamide which is used as a novel anticoagulant. Its molecular formula is $C_{25}H_{25}N_5O_4$, with a molecular mass of 459.497 g/mol. and pKa value 13.2. Literature review revealed very few UV spectroscopic[2], HPLC[3-6], LC-MS/MS[7] and HPTLC[8] methods for the determination of Apixaban in tablets. Hence an attempt has been made to develop simple and economical UV spectroscopic methods for assay of the drug in formulations.

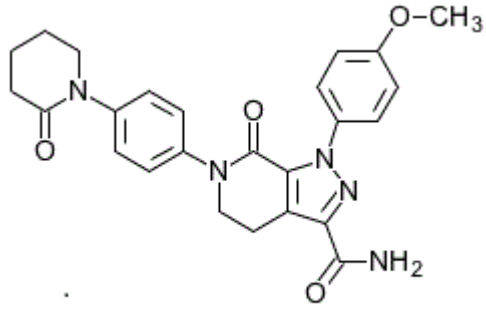

Fig. 1: Chemical Structure.

EXPERIMENTAL
Instrument
UV-1800 SHIMADZU Double beam UV-Visible Spectrophotometer with spectral bandwidth of 1 nm with a pair of 10 mm matched quartz cells. All samples were weighed on electronic balance (Shimadzu).

Chemicals and reagents
Pure sample of Apixaban was gifted by Milestone Health Care, Gujarat. All the chemicals (AR grade) used in the study like disodium hydrogen phosphate, potassium hydrogen phosphate, glacial acetic acid, potassium hydroxide and methanol were procured from MERCK. Marketed formulation of Apixaban (ELIQUIS-2.5 mg, Pfizer) was purchased from the local pharmacy.

Preparation of buffer solutions
Phosphate buffer pH 5.0: Accurately weighed 6.8 gm of potassium dihydrogen phosphate was dissolved in sufficient water to produce 1000 mL and the pH was adjusted to 5.0 with 10M potassium hydroxide.

Phosphate buffer pH 7.0: Accurately weighed 0.50 gm of anhydrous disodium hydrogen phosphate and 0.301 gm of potassium dihydrogen phosphate were dissolved in sufficient water to produce 1000 mL.

Preparation of stock and working standard solutions
25 mg of Apixaban was dissolved in methanol in a 25 mL volumetric flask (1000 µg/mL). Working standard solutions (100 µg/mL) were prepared by suitably diluting the stock solution with phosphate buffers (pH 5.0 and pH 7.0).

Assay procedure for tablets
The developed were applies for the assay of Apixaban in commercial tablets (ELIQUIS). The drug was suitably extracted with methanol from the tablets and the resultant solution was sonicated and made up to volume with phosphate buffers (pH 5.0 and pH 7.0) separately. The solutions were also centrifuged to obtain clear solutions (100 µg/mL). Further dilutions were made from this solution with phosphate buffers as per the requirement.

METHODOLOGY

A sensitive zero order (D^0) and first order derivative (D^1) UV spectroscopic method has been developed and validated for the determination of Apixaban in tablets using phosphate buffer pH 5.0 (method A) and phosphate buffer pH 7.0 (method B). A 10 µg/mL solution of Apixaban was scanned in the range of 200 – 400 nm. The absorption maxima was obtained at 276 nm in zero order and the amplitude was measured in the range of 263 nm (λ_{min}) to 305 nm (λ_{max}), 263 nm (λ_{min}) to 304 nm (λ_{max}) respectively in the first order derivative for a series of standard solutions (Method A and B).

METHOD VALIDATION[9]

The validity of the developed methods was studied in terms of linearity, precision and accuracy per ICH guidelines.

Linearity
A series of standard solutions were prepared by diluting working standard aliquots (0.1 – 6 mL) with phosphate buffers (pH 5.0 and pH 7.0) separately to prepare series of concentrations in the range of 1-60 µg/mL. The linearity of the methods was studied in terms of regression analysis and a calibration curve was plotted for concentration vs absorbance.

Precision
The precision studies were carried out in terms of repeatability and intermediate precision. Solutions containing 10, 20 and 30 µg/mL of Apixaban were prepared in phosphate buffers (pH 5.0 and pH 7.0), analysed three times on the same day (intraday precision) and on different days (interday precision). The % RSD for the assay obtained was calculated from the measured absorbance values to establish the precision of the developed methods.

Accuracy
The accuracy was established separately for both the methods by addition of standard drug to the pre analysed sample (from tablets) at three different concentration levels (50, 100 and 150 %) and the % RSD was calculated for recovery studies.

Limit of detection (LOD) and Limit of quantification (LOQ)
The LOD and the LOQ of the drug in both the methods A and B were calculated using the following equations as given by International Conference on Harmonization (ICH) guidelines.
$LOD = 3.3 \times \sigma/S$
$LOQ = 10 \times \sigma/S$
Where, σ = the standard deviation of the response and S = slope of the calibration curve.

RESULTS AND DISCUSSION

An attempt had been made to develop new sensitive zero order (D^0) and first order derivative (D^1) UV spectroscopic methods by using different phosphate buffers (pH 5.0 and pH 7.0) for the determination of Apixaban in tablets. The developed methods have been validated as per ICH guidelines. From the spectra of Apixaban it is evident that developed methods A and B were obeying linearity in the range of 1 - 60 µg/mL. The linearity data of Apixaban is presented in Table 1. Overlain zero order and first order derivative spectra obtained in method A and method B are shown in Fig. 2 and Fig. 3. The % RSD for intra-day and inter-day precision of drug was found to be < 1.8 in both the methods as given in Table 2. The % recovery of the drug as measured by standard addition method was found to be ≤ 101.1 % as given in Table 3. The methods were applied for commercial formulation and the results are presented in Table 4. An overview of optical and validation parameters is given in Table 5.

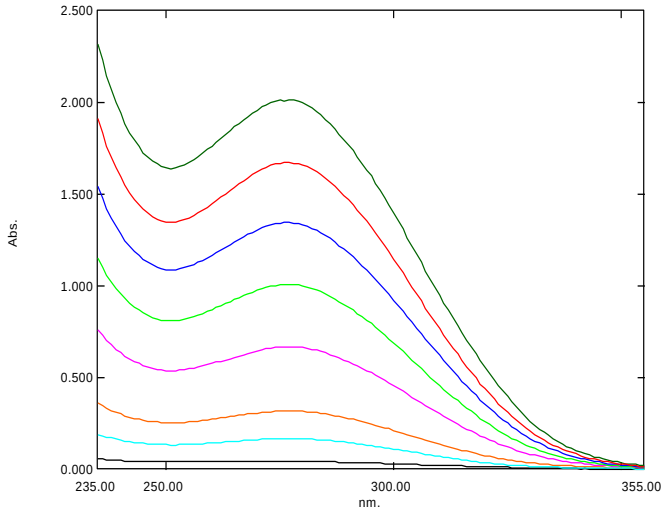

Fig. 2a: Overlain zero order spectra (phosphate buffer pH 5.0)

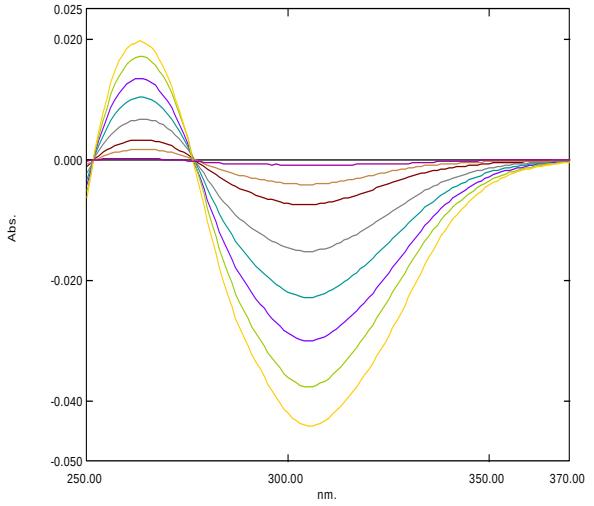

Fig. 2b: Overlain first derivative spectra (phosphate buffer pH 5.0)

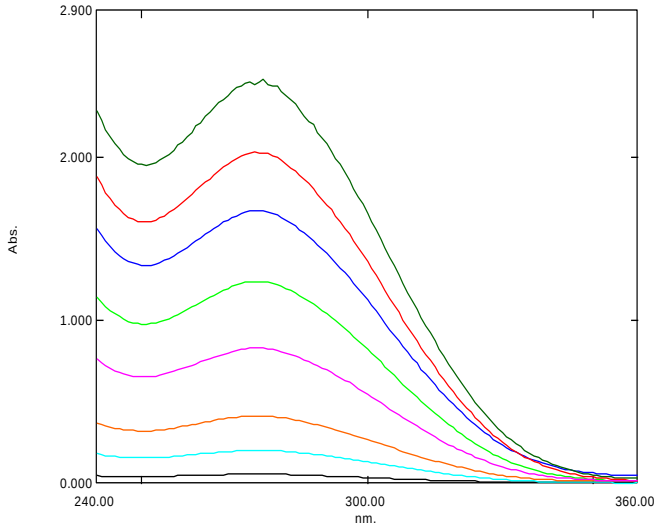

Fig. 3a: Overlain zero order spectra (phosphate buffer pH 7.0)

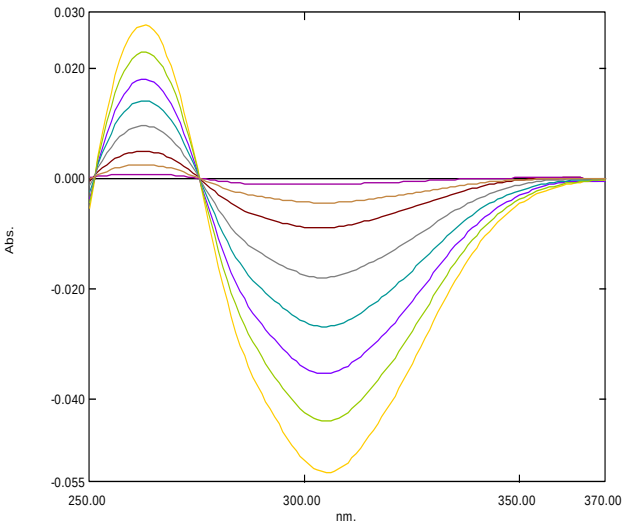

Fig. 3b: Overlain first derivative spectra (phosphate buffer pH 7.0)

Table 1: Linearity data.

Conc. (µg/mL)	Method A		Method B	
	Abs.	Amplitude	Abs.	Amplitude
1	0.045	0.001	0.057	0.002
5	0.168	0.006	0.203	0.006
10	0.318	0.011	0.415	0.014
20	0.670	0.022	0.831	0.028
30	1.008	0.033	1.239	0.041
40	1.345	0.044	1.672	0.055
50	1.672	0.055	2.027	0.067
60	2.011	0.065	2.842	0.081

Table 2: Precision data.

Conc. (µg/mL)	Mean* ± SD (% RSD)			
	Method A		Method B	
	D^0	D^1	D^0	D^1
INTRADAY				
10	99 ± 1.0 (1.01)	99.33 ± 1.15 (1.16)	98.8 ± 0.721 (0.72)	99.13 ± 0.80 (0.81)
20	99.66 ± 0.28 (0.28)	99.5 ± 0.5 (0.50)	99.5 ± 0.5 (0.50)	99.66 ± 1.04 (1.04)
30	99.73 ± 0.23 (0.23)	99.63 ± 0.35 (0.35)	98.8 ± 0.51 (0.51)	99.8 ± 0.43 (0.43)
INTERDAY				
10	100.1 ± 0.80 (0.80)	100.66 ± 0.57 (0.57)	98.33 ± 0.57 (0.58)	99 ± 1.73 (1.74)
20	98.8 ± 0.68 (0.69)	100.66 ± 0.76 (0.75)	99.76 ± 0.11 (0.11)	99.33 ± 0.57 (0.58)
30	99.93 ± 0.55 (0.55)	98.63 ± 0.65 (0.65)	99.86 ± 0.23 (0.23)	99.86 ± 0.23 (0.23)

*Mean of three replicates

Table 3: Accuracy data.

Sample conc. (µg/mL)	Std. drug added (µg/mL)	% Recovery*± SD (% RSD)			
		Method A		Method B	
		D^0	D^1	D^0	D^1
20 (50 %)	10	99.7 ± 0.23 (0.23)	99.06 ± 0.50 (0.50)	99.3 ± 0.65 (0.65)	100.4 ± 0.51 (0.51)
20 (100 %)	20	99.96 ± 0.25 (0.25)	9.33 ± 0.35 (0.35)	01.1 ± 0.57 (0.57)	100.2 ± 1.5 (1.49)
20 (150 %)	30	99.66 ± 0.11 (0.11)	99.46 ± 0.30 (0.30)	98.3 ± 0.31 (0.31)	100.1 ± 1.55 (1.54)

*Mean of three replicates

Table 4: Assay (ELIQUIS, 2.5mg).

Methods	Amount found (mg)		Assay* (% w/w) ± SD	
	D^0	D^1	D^0	D^1
A	2.45	2.45	98.6 ± 0.95	98.6 ± 1.02
B	2.46	2.47	98.3 ± 0.87	98.9 ± 0.65

*Mean of two determinations

Table 5: Summary of validation parameters.

Parameters		Method A		Method B	
		D^0	D^1	D^0	D^1
λ (nm)		276	263 - 305	276	263 - 304
Linearity and range (µg/mL)		1-60			
Sandell's sensitivity (µg/cm^2/0.001abs.unit)		0.0314	-	0.0240	-
Molar extinction coefficient (L mol^{-1} cm^{-1})		14612.0	-	19069.1	-
Correlation coefficient (R^2)		0.9999	0.9998	0.9997	0.9997
Slope (m)		0.0335	0.0011	0.0409	0.0013
Intercept (c)		0.0000	0.0002	0.0082	0.0008
Precision	Intra day	0.23 - 1.01	0.35 - 1.16	0.50 - 0.72	0.43 - 1.04
	Inter day	0.55 - 0.80	0.57 - 0.75	0.11 - 0.58	0.23 - 1.74
Accuracy (% Recovery)		99.6 - 99.9	99.0 - 99.4	98.3 - 101.1	100.1 - 100.4
LOD (µg/mL)		0.054	0.6	0.012	0.639
LOQ (µg/mL)		0.164	1.818	0.037	1.938

CONCLUSION

Two simple, sensitive and economical UV spectroscopic methods have been developed and validated for estimation of Apixaban in bulk and pharmaceutical dosage forms. Both the methods were found to be linear in the given range, precise and accurate. The developed methods can be used for the assay of Apixaban in bulk and pharmaceutical dosage forms without the interference of excipients.

ACKNOWLEDGEMENT

We are grateful to GITAM Institute of Pharmacy, GITAM Deemed to be university, Visakhapatnam India for providing research facilities.

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