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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF APIXABAN IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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Abstract:
In the present work a simple, accurate and precise UV Spectrophotometric method has been developed for determination of Apixaban[1-2] in bulk and pharmaceutical formulations. The optimum conditions for the analysis of drug are established and Apixaban is found to exhibit maximum absorption at 282 nm with DMSO as a solvent. The present method is validated as per guidelines of the International Conference on Hormonization (ICH) guidelines[3-5] including parameters like linearity, accuracy, precision, limit of detection and limit of quantification. Drug obeyed Beer's law in concentration range of 5-20 µg/ml and the regression equation is found to be Y=5.741X-0.071 with correlation coefficient 0.999. From the results it is observed that good correlation exist between drug concentration and absorbance. The percent recovery of Apixaban is found to be 98.5-99.5. The precision is evaluated and relative standard deviation (RSD) is less than 2%, LOD & LOQ are 0.295 & 0.895 respectively. The method is applied to marketed formulation (Eliquis) and Apixaban content is found to be 99.35 with respect to labeled claim. The results suggest that this method can be employed for routine analysis of Apixaban in bulk and commercial pharmaceutical formulations.

Key words: *Apixaban, DMSO, Spectrophotometric method and Validation.*

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INTRODUCTION:

Apixaban chemically known as 1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl)phenyl]-1H,4H,5H,6H,7H-pyrazolo[3,4-c]pyridine-3-carboxamide. Apixaban is an anticoagulant for the treatment of venous thromboembolic events. Apixaban is a reversible and selective factor Xa inhibitor, which does not require antithrombin III for antithrombotic activity. Apixaban inhibits both free and clot bound factor Xa, as well as inhibiting prothrombinase activity. Apixaban decreases thrombin generation and the development of a thrombus through the inhibition of factor Xa. Apixaban indirectly inhibits platelet aggregation through the inhibition of thrombin via the inhibition of factor Xa. Literature survey reveals few analytical methods like UV Spectrophotometric method [6,7], HPLC[8-10] and LCMS Methods[11,12].The aim of the present work is to develop a simple accurate and precise and Spectrophotometric method for the estimation of Apixaban in bulk and pharmaceutical formulation and to validate the developed method as per ICH guidelines.

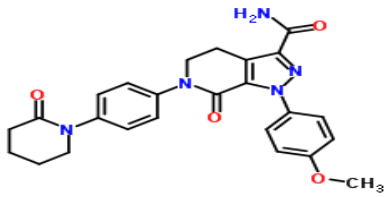

Fig 1: Chemical structure of Apixaban

MATERIALS AND METHODS:

UV Spectrophotometer from Shimadzu, Model no: UV-1800.

Apixaban pure drug is obtained as a gift sample from Dr.Reddy Labs, Hyderabad and Dimethyl Sulfoxide (DMSO) from Finar Reagents. Eliquis is a marketed product of Pfizer and it is obtained from local pharmacy.

Experimental details

Preparation of stock solution:

Standard stock solution is prepared by dissolving 100mg of Apixaban in 100ml DMSO to get a concentration of 1mg/ml (1000ug/ml), 10ml from above stock solution is transferred to a 100ml volumetric flask and the volume is adjusted to 100ml with DMSO to give final strength(100µg/ml). The standard solution of Apixaban is prepared and scanned from 200-400nm to determine λ_{max}. The absorption maxima is found to be at 282nm as shown in Figure 2.

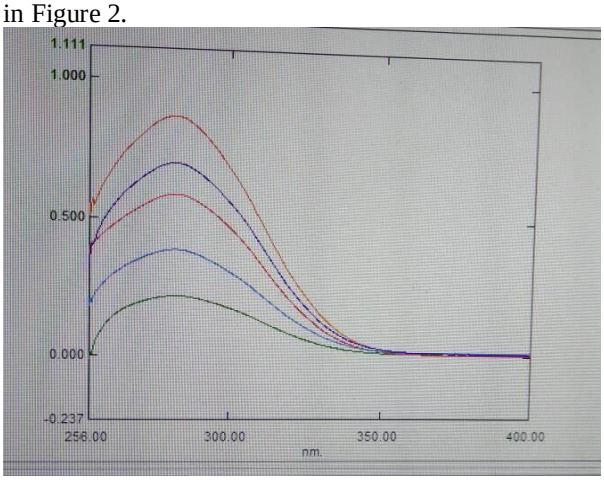

Fig 2: Absorption maxima

Method:

From the above working standard solution a series of standard solution are prepared by pipetting 0.5, 0.75, 1, 1.25, 1.50, 2ml into 5 different 10ml volumetric flasks. The volume is made up to 10 ml with DMSO and the absorbance is measured against blank at 282nm.

Application of proposed method for formulation

Procedure for assay of drugs in dosage forms: Ten tablets of commercial samples of Apixaban are accurately weighed and powdered. A quantity of powder equivalent to 25mg of drug is taken and transferred to a 25ml volumetric flask. The sample is first dissolved in DMSO (25 ml) and sonicated for about 10-15 min, finally up the volume is made up to the mark with water. The solution is filtered and10ml from above stock solution is transferred to a 100ml volumetric flask and the volume is adjusted to 100ml with DMSO to give final strength(100µg/ml). Final dilution of the sample (12.5µg/ml) is prepared and the absorbance is measured against blank at 282nm.

RESULTS AND DISCUSSION:

Validation of the method.

The proposed method is validated as per ICH guidelines .The method is validated in terms of linearity, accuracy and precision.

Linearity

A series of standards with 5µg/ml,7.5µg/ml, 10µg/ml,12.5µg/ml,15µg/ml and 20µg/ml respectively are prepared. The absorbance of all the standards is measured at 282nm against blank. The calibration curve is plotted by taking absorbance on Y axis and concentration in µg/ml on X-axis.

Table 1: Linearity Data

S.NO	CONCENTRATION (µg /ml)	ABSORBANCE
1	5	0.22
2	7.5	0.343
3	10	0.505
4	12.5	0.655
5	15	0.791
6	20	1.070

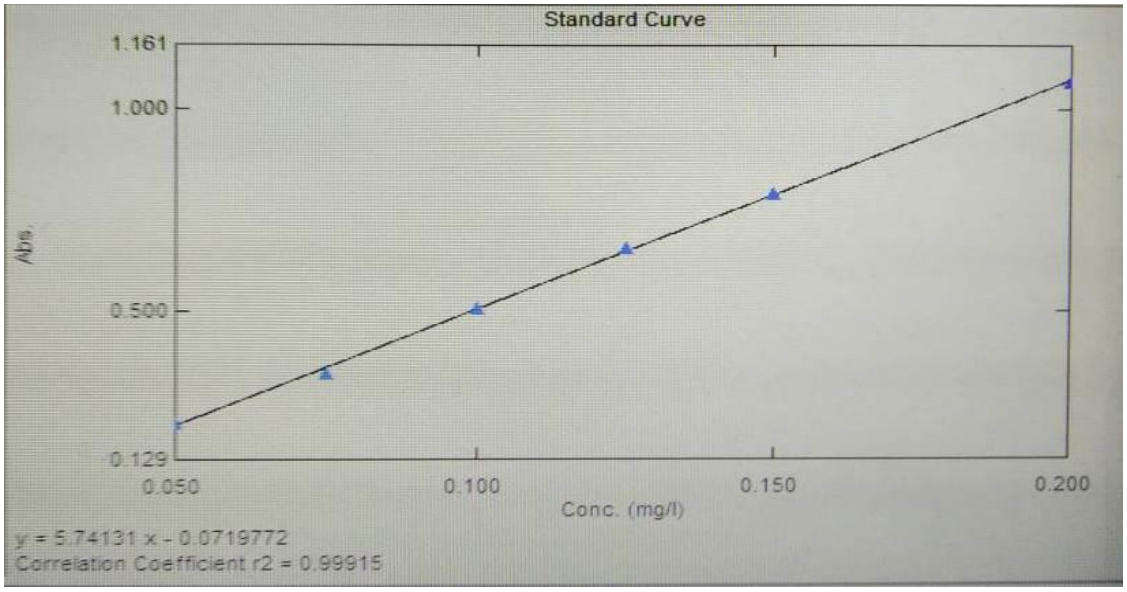

Fig 3: Calibration curve of Apixaban

Accuracy:

To the preanalyzed sample solutions, a known amount of standard stock solution is added at different levels i.e. 50%, 100% and 150 %. The solutions are reanalyzed by proposed method as per ICH guidelines and statistically analyzed.

Table 2: Recovery studies:

Sample	Concentration(µg/ml)		%Recovery of pure drug	Statistical analysis	
	Pure drug	Formulation			
50%	2.5	5	98.7 98.3 98.5	Mean SD %RSD	98.5 0.199 0.203
100%	5	5	99.2 98.9 99.3	Mean SD %RSD	99.1 0.208 0.209
150%	7.5	5	99.3 99.7 99.5	Mean SD %RSD	99.5 0.200 0.201

The results of recovery studies showed that the % amount found is between 98.5% to 99.5%.

Precision:

Precision is the method to check degree of repeatability of results. Precision of the method is carried out by intraday and interday studies. Six samples containing 10µg/ml solution of Apixaban are taken and analysed on the same day and on the consecutive days. The % R.S.D. value is found to be less than 2, so the method developed was precise. The results obtained are presented in the table 3.

Table 3: Intraday precision and Interday precision

S.NO	Intraday precision	Interday precision
1	0.505	0.507
2	0.503	0.505
3	0.509	0.503
4	0.504	0.504
5	0.503	0.506
6	0.501	0.505
Mean	**0.504**	**0.505**
SD	**0.002**	**0.0014**
%RSD	**0.396**	**0.277**

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ decide about the sensitivity of the method.LOD and LOQ were calculated by LOD=3δ/s and LOQ=10δ/s, respectively, where δ is the standard deviation and s is slope of calibration.

Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness is checked by varying the wavelength by ±1nm.

Table 4: Robustness

CONC(µg/ml)	WAVE LENGTH (nm)	ABSORBANCE
5	281	0.219
5	282	0.221
5	283	0.218
	Mean	0.658
	SD	0.0015
	%RSD	0.232

Application of proposed method for pharmaceutical formulation:

Table 5: Analysis of formulation.

Dosage form	Label claim (mg)	Conc (µg/ml)	Amount found	% Recovery	Statistical analysis	
Eliquis	2.5	12.50	12.42 12.41 12.42	99.35 99.30 99.40	Mean SD %RSD	99.35 0.05 0.05

All the results are presented in table 6.

Table 6: Optical characteristics

Parameter	Value
Absorption maximum (nm)	282nm
Beer's law limit (µg/ml)	5-20µg/ml
Correlation coefficient (R^2)	0.999
Regression equation Y= mX+c	Y=5.741x-0.0719
Intercept(c)	0.0719
Slope(m)	5.741
Sandell's sensitivity (µg/cm^2×0.001 absorbance unit)	0.022
Limit of detection(µg/ml)	0.295
Limit of quantification (µg/ml)	0.895

CONCLUSION:

The absorption maximum of Apixaban in DMSO is found to be 282 nm. The regression equation is found to be Y=5.741x-0.0719. The Correlation coefficient is 0.999 which shows that the linear relationship exists between concentration and absorbance. The percent recovery of Apixaban is found to be 98.3-99.57 which suggests this method is accurate. The % Relative standard deviation (RSD) is found to be less than 2% which shows that the method is precise and LOD & LOQ values shows that the method is sensitive. The developed method is applied to marketed formulation (Eliquis) and Apixaban content is found to be 99.35 with respect to labeled claim.

The developed UV spectroscopic method is simple, sensitive with good precision and accuracy. The findings of the work suggest that the method can be applied for quantitative estimation of Apixaban in bulk and pharmaceutical dosage forms. Hence this method can be used in the routine work of quality control aspects.

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