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Spectrophotometric Determination of Apixaban in Bulk Drug and Oral Dosage Formulation

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ABSTRACT

A simple, rapid, cost effective and extractive UV-Vis spectrophotometric method has been developed for the determination of Apixaban (AP) in bulk drug and pharmaceutical formulation. It was based on UV-Vis spectrophotometric measurements in which the drug reacts chromogenic reagent (N-(1-napthyl) ethylene diamine dihydrochloride solution) in acidic medium and give stable pale yellow colored complex which exhibits absorption maximum at 680 nm. Beer's law was obeyed in the concentration range of 5 - 50 µg /ml. This method was tested and validated for various parameters according to ICH guidelines. The proposed method was successfully applied for the determination of AP in oral formulation. The results demonstrated that the procedure is accurate, precise and reproducible (relative standard deviation < 2 %). As it is simple, cheap and less time consuming, it can be suitably applied for the estimation of LA in dosage forms in quality control labs.

Keywords : Apixaban, Spectrophotometric, UV-Vis, Atrial fibrillation, DOAC

I. INTRODUCTION

Atrial fibrillation (AF) is an uneven heart rhythm. It is measured the most frequent cardiac arrhythmia and increases the risk of strokes [1]. In order to avoid strokes, anticoagulants are routinely prescribed, particularly along with the CHA2DS2- VASc score greater than or equal to double [2]. CHA2DS2-VASc and CHADS2 risk models are preferred tools to estimate the risk of embolic strokes in patients with atrial fibrillation [3, 4]. Warfarin reduces the risk of strokes by 68% and however requires regular international normalized ratio testing and has frequent interactions with multiple drugs and food. In recent years, researchers have overcome these limitations by introducing a new class of anticoagulants called nonvitamin K oral anticoagulants (NOACs) or as "direct oral anticoagulants" (DOACs). Clinical trials have demonstrated that NOACs are equivalent to warfarin in effectiveness and safety and therefore are now routinely used in practice [5–8].

DOACs have the advantage of a quick response time than warfarin. However, they also have relatively short elimination half-lives. [9,10] This is a challenge because nonadherence can be expected to be even more common for DOACs than for warfarin since the patients are not regularly monitored. Exclusion of only a few doses will increase the risk of thromboembolic events. Moreover, although DOACs have a lower pharmacokinetic and pharmacodynamic variability than warfarin and also fewer drug–drug interactions, reduced hepatic metabolism and

impaired renal function may cause supratherapeutic drug concentrations with an increased risk of bleeding.[11] Subsequently, even when using recommended doses, a certain number of those treated with DOACs will either have high plasma concentrations with an increased risk of bleeding or low plasma concentrations with an increased risk of developing a thromboembolic episode.[12] As there seems to be a direct association between the plasma concentration of the DOAC and the anticoagulation effect,[13] the existence of therapeutic plasma concentration ranges where the risk of these endpoints is lower can be predicted.

Apixaban is a pyrazole derivative small-molecule, act as a potent, oral, and reversible agent. [14]. It represents a new oral anticoagulant molecule, and it received approval in European in 2011, being approved in prevention of stroke and systemic embolism in adults with non-valvular atrial fibrillation, for treatment of deep venous thrombosis, pulmonary embolism and for the prevention of recurrence of DVT and PE after elective hip or knee replacement surgery [15]. The available proof provided by the pre- approval studies and various analysis of emerging clinical experience suggest that apixaban is non-inferior to existing standard anti-thrombotic therapies and that it has improved safety, expressed as reduced hemorrhagic risk [16-18].

At therapeutic doses, the absolute bioavailability of apixaban is approximately 50%, with low to moderate within subject and inter-subject exposure variability and with linear pharmacokinetics.

Cmax is reached approximately 3 h post dose in healthy volunteers. Apixaban's plasma protein binding is approximately 87% and it has a half-life of approximately 12 hours. It has multiple routes of elimination, with 27% of the total clearance being attributed to the renal excretion. The main metabolic pathway is through CYP3A4/5, P-glycoprotein and breast cancer resistance protein (BCRP) being involved in its transport . Because Apixaban is metabolized by the liver (partially by CYP 3A4) there are few recommendations to prescribe it to patients with hepatic impairment. No dosage adjustment of Apixaban is necessary in patient with mild hepatic impairment, but it should be used with cautions in patients with moderate liver disease (Child class Pugh A or B) and a discontinuation period up to 5 days can be considered before elective surgery in such patients [19]. The present clinical experience allows its administration in renal impairment with a creatinine clearance ≥ 15 ml/min. This is best done by calculating creatinine clearance using Cockcroft-Gault formula (some calculators are also available online), but with some adjustments regarding body-mass index [19]. Strong inhibitors or inducers of both CYP3A4 and P-glycoprotein can markedly influence the plasmatic concentrations of apixaban if co-administered. There are no interactions between food and pharmacokinetics and pharmacodynamics of apixaban [20]. Apixaban has high oral bioavailability and it is absorbed throughout the gastrointestinal tract. Despite this feature, currently there are no warnings or recommendations regarding its use in special populations at high risk for developing venous thromboembolism (after gastric bypass surgery, lap-band weight loss surgery or extended resection of the small bowel) [20].

Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is the reference method used in therapeutic monitoring of novel direct oral anticoagulants due its precision, accuracy, sensitivity and robustness [21-24]. There are several pharmacokinetics studies of apixaban [25-26] and assessments of different laboratory tests [27] that used LC-MS/MS for quantification of apixaban in human

plasma. The main objective of our study was to develop and validate a specific and quantitative high throughput LC-MS/MS method for the analysis of apixaban in human plasma with applicability in clinical therapeutic drug monitoring, pharmacokinetics, as well as in bioequivalence studies. The protocol study was reviewed and validated by the Ethics Committee of the University of Medicine and Pharmacy of Tirgu Mures and has been performed in the accordance with standards of the Declaration of Helsinki and local regulations. All subjects provided written informed consent.

II. METHODS AND MATERIAL

All employed chemicals were of analytical grade and highly purified water was used. Apixaban (AP) was provided as a gift sample by Lupin pharmaceuticals Ltd. Aurangabad, India., Sodium nitrite, Ammonium sulfamate, B.M reagent (N-(1-napthyl) ethylenediamine dihydrochloride solution) (Merck, Mumbai, India), hydrochloric acid (HCl) ((Sd fine Chem Ltd., Mumbai, India)

Solution Preparations

Sodium nitrite solution (0.1%w/v): 100 mg of sodium nitrite was dissolved in distilled water and made up to 100 ml.

Hydrochloric acid (5N): 425 ml of concentrated HCL was taken and diluted to 1000 ml with distilled water.

Ammonium Sulfamate Solution (0.1%W/V): 500 mg of ammonium sulfamate was dissolved in distilled water and made up to 100 ml with distilled water.

B.M Reagent (N-(1-napthyl) ethylene diamine dihydrochloride solution) (0.1%w/v): 100 mg of B.M reagent was dissolved in 100 ml of distilled water.

Preparation of the standard drug solution (1mg/ml): 100 mg Apixaban (AP) accurately weighed and transferred into 100 ml volumetric flask, dissolved and diluted to 100ml with methanol.

Preparation of working standard solution (100 µg/ml): Accurately 10 ml of standard stock solution was transferred in to 100ml volumetric flask and then diluted up to mark by water.

Analytical Procedure

In 100 ml volumetric flask, required volume of AP working standard solution has transferred. To each flask 1 ml of 5N HCl and 1 ml of 0.1% w/v sodium nitrite were added and shaken for 10 minutes. To this, 1 ml of 0.5 % w/v ammonium sulfamate was added followed by the addition of 1 ml of B.M reagent. The absorbance was measured at 680 nm against the reagent blank.

Validation of Method

Analytical method validation is the systematic steps to confirm that the developed analytical procedure is applicable for a specific analysis. Analytical data of this validation process can be used to evaluate the quality and consistency of developed method.

System suitability

System suitability parameter is not mandatory for UV-Vis spectroscopic systems. However, to develop more dependable analysis method this parameter is important to perform. A 100% concentrate of standard solution of had taken and the absorbance of this is measured at the wavelength of maximum absorbance. The E 1% 1cm values were also calculated and were found to be within specified percentage.

Linear dynamic Range

The linearity of the suggested methods was determined by calibration curve of apixaban in the range of 0.5-50 µg/ml. The calibration curve was made by plotting concentration versus absorbance and carried out linear regression analysis.

Limit of detection (LOD)

The lowest concentration of analyte in sample which can be detected by cannot be quantified under the same experimental condition. Limit of detection was calculated from calibration curve using following equation.

LOD= 3.3 σ / S

Where σ = S.D of y-intercept of calibration curves

S = Mean of slope of calibration curve

Limit of quantification (LOQ)

The lowest concentration of analyte from sample which can precisely and accurately measured under same experimental condition. Limit of quantification was calculated from calibration curve using following equation.

LOQ = 10 σ / S

Where σ = S.D of y-intercept of calibration curves

S = Mean of slope of calibration curve

Precision

Precision was determined by calculating intra- day and inter-day variations of the developed method in 3 replicates at their different concentrations of AP (10, 20 and 30 µg /ml). The absorbances of the solutions were measured at 680 nm. For intra-day precision, analyzed these solutions in triplicates on same day. To determine inter-day precision each of three samples was analyzed on different day.

Accuracy

Recovery study has performed to evaluate the accuracy of proposed method. In this method, standard drug was added at three different levels i.e. 50 %, 100 % and 150 % to known pre – analyzed sample solution. By using the proposed method the total concentrations were determined.

Sensitivity parameters

The molar absorptivity and Sandell's Sensitivity has calculated as per their standard formula as given below. Molar absorptivity is a characteristic constant of the species under consideration. It is the absorbance at the specified wavelength of solution of a compound of unit molar concentration measured in 10 mm path length.

Molar absorptivity can be evaluated from the following equation.

Molar absorptivity = Slope x molecular weight x 103 lit/ mol.cm

Sandell's index represents the number of micrograms or nanograms of the determinant per millilitre of a solution having an absorbance of 0.001 for the cell path length of 1 cm and is a suitable parameter for expressing and comparing the sensitivities of developed UV-VIS-spectrophotometric methods [15, 30].

Sandell's sensitivity= Molecular weight / Molar absorptivity

Application of proposed method for formulation

Procedure for assay of drugs in dosage forms:

Twenty tablets of commercial samples of Apixaban are accurately weighed and powdered. A quantity of powder equivalent to 25 mg of drug is taken and transferred to a 50ml volumetric flask. The sample is first dissolved in methanol (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 methanol to give final strength(50µg/ml).

III. RESULTS AND DISCUSSION

In this method in the presence of acidic environment sodium nitrite can diazatitation of drug molecule and this diazonium salt of drug molecule coupled with B.M. regent which can be indicated by dark blue colour

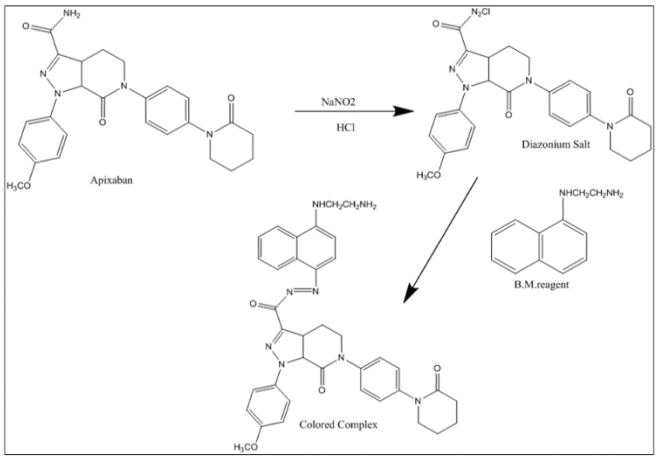

Figure 1 : Mechanism of Colour complex formation

System suitability experiment

Solutions of standard AP having a concentration of 20 µg /ml and 10 µg /ml were taken. The abso rbance value of these solutions was recorded five times and the E1% 1cm values were calculated as per the equation below.

E1%1cm = Absorbance value / Concentration (g/100 ml)

The results of the system suitability experiments are tabulated in Table 1. The % RSD of this analysis was found 1.97 which is below the prescribed limit according to ICH guideline. It reveals that the system is suitable for further analysis.

Calibration curve and Linearity:

For the proposed method the calibration curves were constructed by plotting the absorbance the final concentration of the drug. The correspond ing regression equations were derived.

From the calibration plot it has been found that the linearity of said method is between 5- 50 µg/ml. Results of linearity study reveals that the method follows Beer's Lambert law. Further, limit of detection (LOD) and quantification (LOQ) of this method are 0.006 µg/ml and 0.018 µg/ml respectively.

Precision

The results of precision study show excellent repeatability and good precision of developed method. % RSD value was not more than 2% in both intraday and interday precision study. For the interday precision, the range of RSD (%) value was found between the ranges of 0.3 to 0.55. For intraday precision the % RSD values were found between the ranges of 0.1 to 0.4 for same analyst (Table 2) and when the experiment was carried out by another analyst, the range of RSD (%) was between 0.3 to 0.8 %.

Table 1 : Selectivity

Sr.No	Conc. (µg /ml)	$E^{1\%}1$ cm	Mean	SD	% RSD
1	20	155.5	156.75	2.89	1.97
2	20	156.0			
3	20	155.5			
4	20	157.0			
5	20	156.5			
6	10	160.0			
7	10	158.0			
8	10	159.0			
9	10	158.3			
10	10	156.3			

Table 1 : Precision Study

Results of Intra-day precision				
Sr.No	Conce. (µg/ ml)	Abs.	SD	%RSD
1	10	0.342	0.00229	0.401
2	20	0.371	0.00143	0.382
3	30	0.421	0.00231	0.523
Results of Interday precision- Same analyst				
Sr.No	Conce. (µg/ ml)	Abs.	SD	%RSD
1	10	0.332	0.00329	0.501
2	20	0.383	0.00153	0.282
3	30	0.434	0.00241	0.323
Results of Interday precision- Different analyst				
Sr.No	Conce. (µg/ ml)	Abs.	SD	%RSD
1	10	0.340	0.00296	0.451
2	20	0.385	0.00152	0.312
3	30	0.429	0.00241	0.323

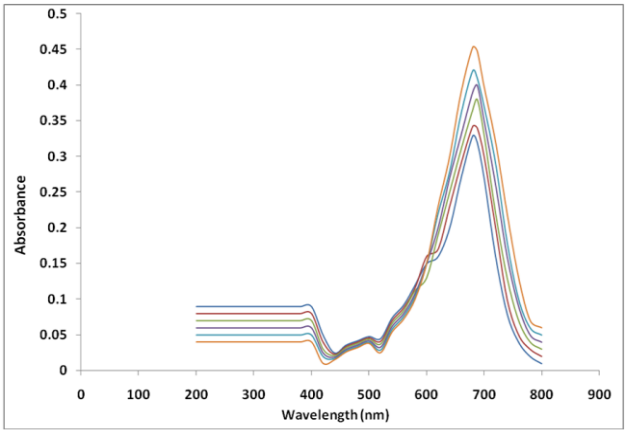

Figure 2: UV spectra of linearity

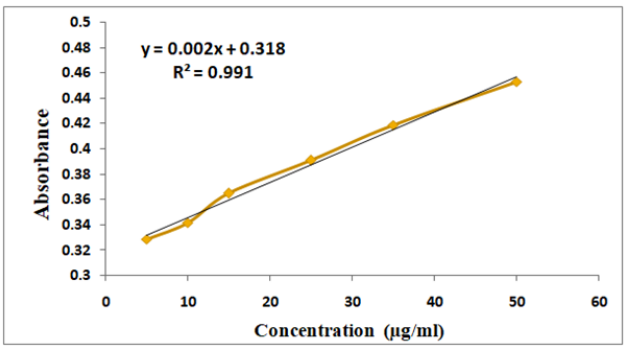

Figure 3: Calibration Curve

Accuracy (Recovery)

The absorbance values of solutions from each of the sets were recorded. Determination of accuracy of this method, different levels of drug concentrations- lower concentration (LC), intermediate concentration (IC) and higher concentration (HC) were prepared from independent stock solutions and analysed. A comparison of these absorbance values with those obtained from the standard AP was made and after applying the appropriate dilution factor, the amounts of AP present we calculated.

Table 2 : Accuracy Study

Sr. No	Wt of Sample taken (mg)	Amount of Std. Added (mg)	Amount found (mg)	% Recovery
1	200.04	50.03	251.2	100.45
2	200.02	50.01	250.7	100.27
3	200.01	100.02	300.08	100.02
4	200.03	100.04	301.02	100.32
5	200.04	150.05	350.18	100.03
6	200.05	150.03	351.01	100.27
Mean	100.23			
SD	0.170			
%RSD	0.169			

Molar Absorptivity

The molar absorptivity value of AP was calculated from the calibration curve using the formula, Molar absorptivity= Slope x mol wt. x 10^3 lit./mol/cm = 919.01 lit/mol/cm

= 9.19 X 10^{-3} lit/mol/cm

Sandell's sensitivity= Molecular weight / Molar absorptivity = 0.050 µg /cm

Assay of marketed formulation

Here we take different Eliquis formulation for analysis in this study. The procedure to made sample solution as describe in experimental section. The absorbance values of these solutions were recorded at 680 nm and it was shown in Table 4. A comparison of these values with those obtained from the standard AP was made and after applying the appropriate dilution factor.

Table 3 : Assay of formulation

Sr. No	Amount taken (mg)	Amount Found	Percentage assay (%)
1	10	10.05	100.5
2	10	10.04	100.4
3	25	25.07	100.28
4	25	24.98	99.92
5	40	40.12	100.3
6	40	40.07	100.2
Mean	100.27		
SD	0.199		
%RSD	0.198		

III. CONCLUSION

The present study reported successful development and evaluation of B.M reagent assisted detection method of Apixaban. The developed method selectively and sensitively deters mines Apixaban in bulk drug and its different formulations. Different validation study such as precision, accuracy, linearity study reveals that the developed method is fully validate as per ICH guidelines. The developed method does not need any expensive sophisticated equipment. The colored complex produce in this method is stable it shows high throughput property of this method. Hence, this method is commercially viable and valuable for its routine application in quality control laboratories for analysis of Apixaban.

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