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Two Novel Validated RP-HPLC and UV Spectrophotometric Methods for Estimation of Apixaban in Bulk and Pharmaceutical Dosage Forms

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ABSTRACT

Two novel methods having requisite precision, accuracy, specificity and robustness were developed and validated for quantitative determination of Apixaban in pharmaceutical dosage forms. The first method was based on isocratic reverse phase liquid chromatography using Sunfire C18, 150mm×4.6mm, 5µ and mobile phase consists of Buffer: acetonitrile (60:40) at a flow rate 1ml/min and detection was achieved photodiodide array detector set at 280 nm. The response was linear range of 5-50 µg/ml (R^2 =0.9998). The second spectrophotometric method involves detection at 280 nm. The calibration curve range between 5-50 µg/ml (R^2 =0.9999). Validation of method was carried out fulfilling ICH guidelines. Both the methods were applied without any interference from excipients, for determination of drug in coated tablets. It is suggested that the proposed HPLC and UV spectrophotometric methods could be used routine quality control and dosage form assay of Apixaban.

Keywords: HPLC**,** UV Spectrophotometry, Apixaban, Method development, Validation.

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INTRODUCTION

Apixaban is an anticoagulant for the treatment of venous thromboembolic events. ELIQUIS (apixaban), is chemically described as 1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl)phenyl]-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxamide(Figure 1).

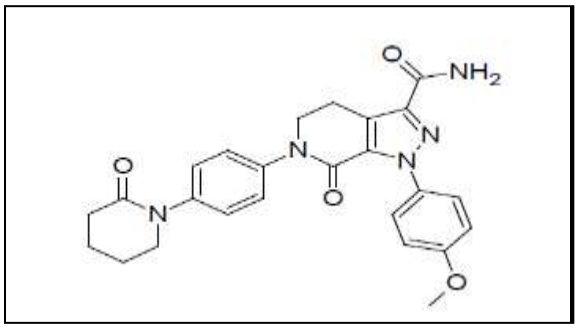

Figure 1: Chemical Structure of Apixaban

Its molecular formula is C25H25N5O4, which corresponds to a molecular weight of 459.5. Apixaban[1, 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 with the following inactive ingredients: anhydrous lactose, microcrystalline cellulose, croscarmellose sodium, sodium lauryl sulfate, and magnesium stearate. The film coating contains lactose monohydrate, hypromellose, titanium dioxide, triacetin, and yellow iron oxide (2.5 mg tablets) or red iron oxide (5 mg tablets). Apixaban has been available in Europe since may 2012.The medical use of apixaban is to lower the risk of stroke and embolism in patients with nonvalvular a trial fibrillation. Apixaban is recommended by the national institute for health and clinical excellence for the prevention of stroke and systemic embolism in people with non valvular a trial fibrillation and at least one of the following risk factors. Apixaban and other newer anticoagulants (dabigatrand and rivaroxaban) appear equally effective as war far in in preventing non-hemorrhagic stroke in people with a trial fibrillation and are associated with lower risk of intracranial bleeding. Apixaban is highly selective, orally bioavailable, and reversible direct inhibitor of free and clot-bound facto Xa. There are some methods of estimation of apixaban from human plasma by LC-MS[3-9], 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. The current HPLC and UV Spectrophotometric methods were developed and validated as per the ICH guidelines. The RP-HPLC and 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 as mentioned in the below.

MATERIALS AND METHOD

Instrumentation

RP-HPLC:

HPLC analysis was performed on Shimazu LC-10AT VP Liquid chromatograph comprising a LC-10 AT PUMP, Shimazu PDA detector and a reverse phase Sunfire C18,(150×4.6 mm:5µ). A auto injector with 50µl sample loop was equipped with the HPLC system. The HPLC system was controlled with Class VP software.

UV Spectro Photometery:

Double Beam UV-visible spectrophotometer (Shimadzu, model 1800) having two matched quartz cells with 1 cm light path and loaded with UV probe software (version 2.41) was used for recording of spectra and measuring absorbance.

Chemicals and Reagents

Pure drug Apixaban was provided by our APL Research Centre-II. (A Division of Aurobindo Pharma Ltd). All the reagents and chemicals used were of analytical grade from Merck Chemicals, India.

Selection of wavelength

Suitable wavelength for the HPLC and UV analysis was determined by recording UV spectrum in the range of 200-400 nm for drug solution .Wavelength selected for simultaneous estimation is 280nm

Chromatographic conditions

The developed method uses a reverse phase Sun fire C18,(150×4.6 mm:5µ).,mobile phase consisting of Buffer: acetonitrile in the proportion of 60:40v/v. The mobile phase was set a flow rate of 1.0 ml/min and the volume injected was 10µl for every injection .The detection wavelength was set at 280 nm.

Buffer preparation

1.50 g of sodium acetate in 1000 ml buffer bottle added 100 ml water sonicate to dissolve and make up to the volume with water. The buffer was filtered through 0.45µ filters to remove all fine particles and gases.

Mobile phase preparation

The mobile phase was prepared by mixing buffer and acetonitrile 60:40v/v and later it was sonicated for 10 minutes for the removal of air bubbles**.**

Diluent:

Methanol used as a diluent

Preparation of stock and working standard solution for HPLC and UV SPECTRO Photometery

10 mg of apixaban was accurately weighed in 100 ml volumetric flask add 10 ml of methanol sonicate to dissolve and make up to the volume with methanol (0.1mg/ml). 4ml of this stock solution was pipetted out and made up to 20 ml to get a concentration 20µg/ml, treated as working standard, 100% target concentration.

Preparation of stock and working sample solution for HPLC and UV Spectro Photometery

10 tablets were weighed and taken into a mortal, crushed and then uniformly mixed. Test stock solution of apixaban 100 µg/ml were prepared by dissolving weight equivalent to 10 mg of apixaban weighed in 100 ml volumentric flask add 10 ml methanol sonicated for 5 minutes and make up to the volume with methanol then filtered the solution using 0.45µ syringe filter.2 ml of above stock solution was pipette out and made up to 20 ml volumetric flask to get a concentration 20µg/ml to get a working sample solution.

RESULTS AND DISCUSSION

Method development

A reverse phase HPLC method was developed keeping in mind the system suitability parameters i.e, theoretical plates (N), run time and Asymmetry. The optimized method developed resulted in the elution of apixaban at around 4 min. (figure 2-3) represents chromatogram of standard solution and blank solution respectively. The total run time is 10 minutes. System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), number of theoretical plates (N), and peak asymmetry (A) were evaluated for six replicate injections of the standards at working concentration. The results given in (table 1) were with acceptable limits.

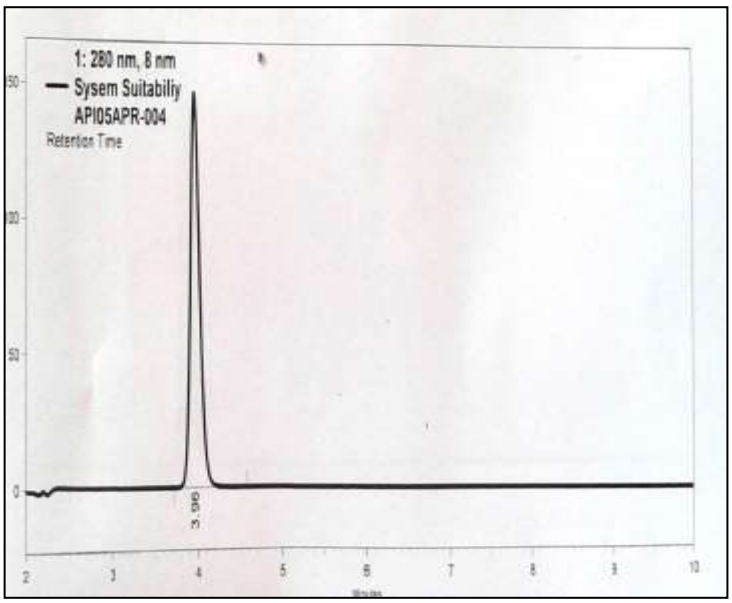

Figure 2: Chromatogram of standard

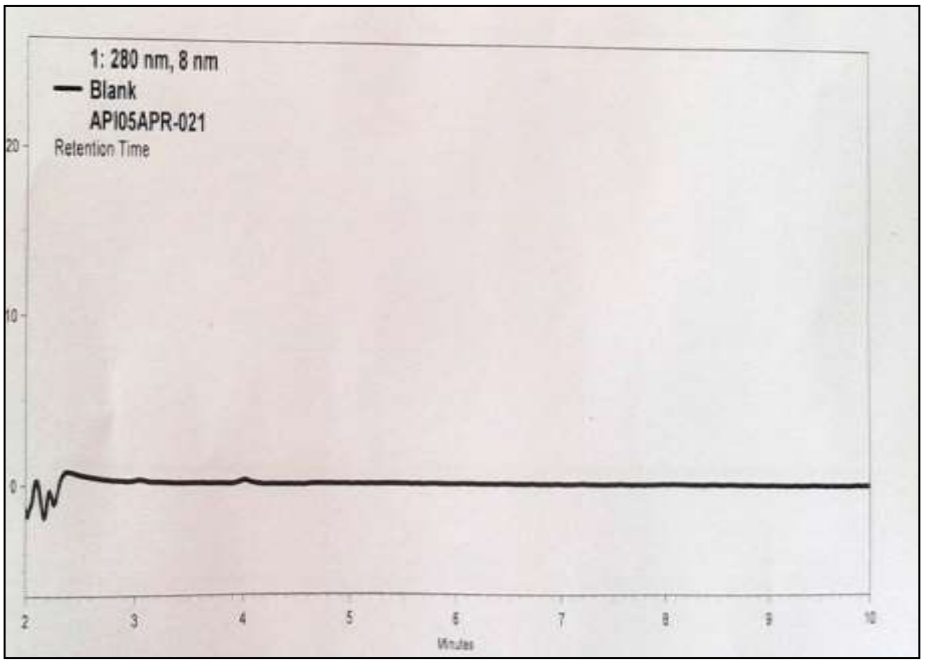

Figure 3: Chromatogram of Blank

Table 1: System suitability studies results (HPLC Method)

S. No	Parameter	HPLC Method
1	Retention time(RT)	3.96
2	Number of theoretical plates(N)	5674
3	Peak asymmetry(A)	1.21

System suitability test for HPLC

System suitability parameters like asymmetry (A), theoretical plates etc were calculated and compared with standard value.

Method validation

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. HPLC and UV Spectrophotometric methods were developed and validated according to International Conference on Harmonization (ICH) guidelines[23] for validation of analytical procedures. The methods were validated for parameters like linearity, accuracy, system precision, intra day precision ,limit of detection(LOD) and limit of quantitation (LOQ).

Specificity

For blank, mixture of standard drug solution and sample chromatogram reveals that the peaks obtained in the standard solution and sample solution at working concentration are only because of the drugs as blank has no peak at the retention time of apixaban .accordingly it can be concluded that, the method developed is said to be specific.

PRECISION

System precision

Six replicate recording of area of standard solution (HPLC Method), absorbance of standard solution (UV Method) at working concentration showed % RSD (Relative Standard Deviation) less than 1 , which indicates the acceptable reproducibility and thereby the precision of the system. System precision results are tabulated in (Table 2).

Table 2: System precision results of Apixaban

S. No	HPLC Method(Area)	UV Method(Absorbance)
1	931308	0.80
2	937062	0.798
3	936473	0.797
4	938276	0.799
5	926865	0.796
6	944462	0.802
Mean	935741	0.799
SD^	6053	0.002
%RSD*	0.65	0.25

^ Standard deviation

* Relative standard deviation

Method precision

Method precision was determined by preparing assay of sample under the test of repeatability at working concentration for both the methods (Table 3).

Table 3: Method precision results of apixaban drug substance

S.No	HPLC Method	UV Method
1	99.90	100.00
2	100.97	100.13
3	99.61	100.63
4	99.61	100.63
5	100.99	99.87
6	99.88	100.13
Mean	100.16	100.23
SD^	0.65	0.32
%RSD*	0.65	0.32

Linearity

The calibration curve was constructed by preparing methanolic solution of the drug with concentration range between 5-50µg/ml for both the methods (Figure 4 and 5).

Linearity was evaluated by linear regression analysis, which was calculated by least sware regression method concentration v/s peak area (HPLC Method) and concentration v/s absorbance (UV Method) was used for plotting linearity graph (Table 4).

Limit of Detection (LOD) & Limit of Quantification (LOQ)

LOD and LOQ Were estimated from linearity curve data for both the methods. The sensitivity of measurement of assay by use of the proposed method was estimated in terms of the limit of quantitation (LOQ), limit of detection (LOD). The limit of detection (LOD) and limit of quantitiation (LOQ) were found to be 1.16 µg/ml and 3.52 µg/ml (HPLC method), 0.73 µg/ml and 2.21 µg/ml (UV method) respectively. Optical characteristics results are summarized in in (Table 5).

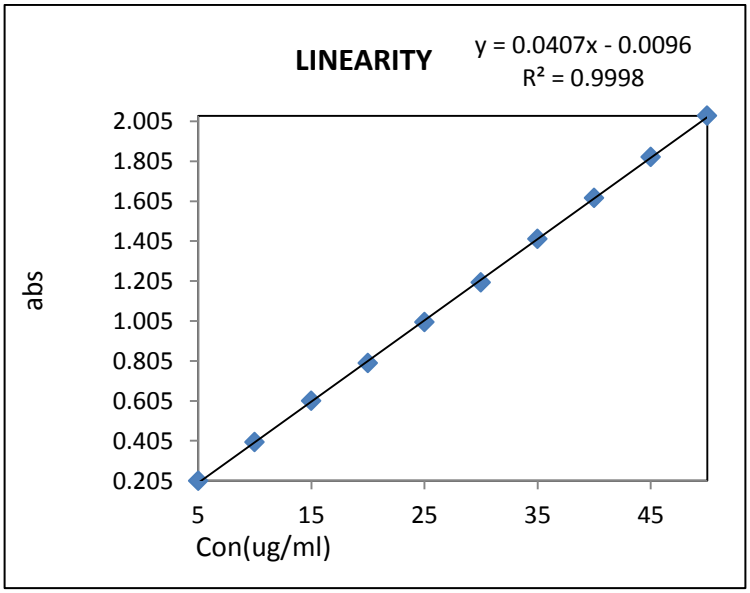

Figure 4: Linearity graph of apixaban(UV Method)

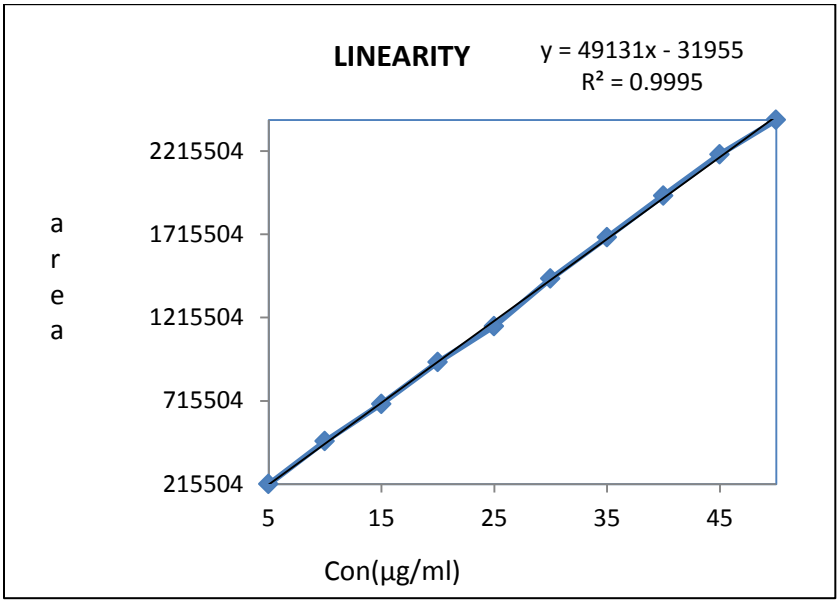

Figure 5: Linearity graph of apixaban(HPLC Method)

Table 4: Calibration data for Apixaban

S. No	Concentration(µg/ml)	HPLC Area	UV Absorbance
1	5	215504	0.205
2	10	473058	0.399
3	15	697488	0.606
4	20	947961	0.795
5	25	1164331	0.999
6	30	1449075	1.198
7	35	1696996	1.416
8	40	1948152	1.621
9	45	2195960	1.827
10	50	2403046	2.033
Regression equation		y=49131x-31955	y = 0.040x – 0.009
Correlation coefficient (r^2)		0.999	0.999

Table 5: Optical characteristics of Apixaban drug substance

S. No	Parameters	HPLC Method	UV Method
1	Detection wavelength(nm)	280	280
2	Beer's law limits (µg/ml)	5-50	5-50
3	Regression equation (y = mx+c)	y=49131x-31955	y = 0.040x – 0.009
4	Correlation coefficient (r^2)	0.999	0.999
5	LOQ (µg/ml)	3.52	2.21
6	LOD (µg/ml)	1.16	0.73

Accuracy

The recovery studies, also known as standard addition method, is performed by addition of known amount of the standard drugs to a solution of known concentration of previously analyzed

commercial pharmaceutical product. The recovery studies were performed by adding 10, 20 and 30 µg/ml of solution of standard drug in previously analyzed solution of tablet (Table 6 and 7).

Table 6: Results of accuracy studies for apixaban by UV Method

Accuracy	Level-I	Level-II	Level-III
Added(µg/ml)	10.0	20.0	30.0
Found(µg/ml)	9.78	19.96	29.82
Recovery(%)	97.8	99.8	99.40
RSD(%)	0.36	0.40	0.6

Table 7: Results of accuracy studies for apixaban by HPLC Method

Accuracy	Level-I	Level-II	Level-III
Added(µg/ml)	10.0	20.0	30.0
Found(µg/ml)	9.95	19.96	30.28
Recovery(%)	99.5	99.7	100.9
RSD(%)	0.31	0.38	0.0

Robustness

HPLC

The robustness of method was checked by evaluating system suitability parameters date obtained after varying the HPLC pump flow rate(±5%),mobile phase composition (±5%),column temperature (±2°c).

UV

The robustness of an analytical method 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. It is concluded that the method is robust.

Sensitivity

The sensitivity of measurement of apixaban by use of proposed methods were estimated in terms of the limit of quantitation (LOQ) and limit of detection(LOD).LOQ and LOD were calculated by the use of the equation LOD=3.3×σ/S and LOQ= 10×σ/S where σ is the standard deviation of response of calibration plots and s is the slope of the corresponding calibration plot.

CONCLUSION

A reverse phase HPLC isocratic method, UV spectophoto metric methods were developed has been validated as per ICH guidelines in terms of specificity, precision, linearity, limit of detection and limit of quantitation for estimation of apixaban in bulk and pharmaceutical dosage forms. The developed method (HPLC) resulted in apixaban eluting at around 4 min and linear in the range of 5-50 µg/ml. The precision is exemplified by relative standard deviations of 0.65% for apixaban. Percentage mean recoveries were found to be in the range of 98-102, during accuracy studies. The

limit of detection (LOD) for apixaban 1.16µg/ml and limit of quantitation (LOQ) for apixaban was found to be 3.52µg /ml. The developed method (UV) linear in the range of 5-50 µg/ml. The precision is exemplified by relative standard deviations of 0.25% for apixaban .Percentage mean recoveries were found to be in the range of 98-102,during accuracy studies. The limit of detection(LOD) for apixaban 0.73µg/ml and limit of quantitation(LOQ) for apixaban was found to be 2.21µg/ml

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