Force Degradation High Performance Liquid Chromatographic HPLC Assay Method of Apixaban in Pharmaceutical Preparations

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ABSTRACT

The current paper describes the measure of *apixaban* was accomplished on a Thermo BDS, C8, 150 X 4.6, 5 μ , and buffer (pH 4.5) and Acetonitrile at 60:40%v/v ratio as the mobile phase, at a stream pace of 1.0 ml min⁻¹. HPLC recognition of elute was gotten by a photodiode cluster detector (DAD) which was set at 259nm. ICH rules were followed for approval of proposed strategy with respect to particularity, affectability, exactness, linearity, precision, framework appropriateness and vigor. Alignment bends of *apixaban* were made in the scope of 8.0-48 μ g.mL⁻¹ with LOD and LOQ of 0.20 μ g.mL⁻¹ and 0.24 μ g.mL⁻¹ separately. *Apixaban* strength was assessed under different ICH constrained corruption conditions and it was discovered to be effortlessly debased in acidic and basic conditions. The assurance of *apixaban* in drug measurements structures was directed with the portrayed technique and showed mean rate recuperation of 100.54 (n = 3), individually.

KEY WORDS: RP-HPLC, APIXABAN, STABILITY-INDICATING, STRESS DEGRADATION.

INTRODUCTION

Apixaban, 1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl) phenyl]-4,5,6,7-tetrahydro-1H-pyrazolo [3, 4-c] pyridine- 3- carbo-xamide (Figure 1) is an oral anticoagulant used to treat the stroke caused by a blood clot with heart rhythm disorder (Atrial fibrillation) (Cada et al., 2013 (Apixaban, 2019 & 2021) (Raghavan et al., 2009). Scarcely only three insightful techniques (Prabhune et al., 2014) (Katari and Jonnalagadda 2020) (Ani et al., 2021) detailed in the writing for the substance assessment of apixaban in drug substances utilizing liquid chromatography (HPLC). The primary aim of introduced paper is to assess apixaban soundness in accordance with the relevant rules of ICH (ICH Q2 (R1) 2005) using a full approved RP-HPLC technique in exposing the examined medication to a wide scope of stress conditions.

MATERIAL AND METHODS

Instrumentation: Agilent 1120 series consisting 2 channels. Auto sampler with one rack it has 100 vials. Auto injector has capacity to inject 0.1 μ L to 100 μ L. UV-Vis Detector. Thermostat column compartment connected it has a capacity to maintain 4°C to 80°C column temperature. Agilent 1120 HPLC System is equipped with Ezchrome Elite software. Orthophosphoric acid, Acetonitrile (HPLC grade), Milli-Q water. Hydrochloric acid (AR), sodium hydroxide (AR-grade), Hydrogen peroxide were procured from Merck-R, and Sigma -J.

Mobile phase preparation: Mix buffer (pH 4.5) and Acetonitrile at 60:40%v/v ratio was used. Prior to use it was sonicated and degassed using vacuum filtration through 0.45 micron membrane filter.

Buffer preparation (pH 4.5): Prepare 1.625gm (0.02M) Potassium di hydrogen orthophosphate & 0.3gm of di-Potassium hydrogen orthophosphate in 550ml of HPLC water. The pH of the buffer adjusted to 4.5 with diluted orthophosphoric acid.

Standard stock solution preparation: Weigh and transfer 20mg of Apixaban working standard into 50mL

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volumetric flask, add 25mL of diluent and sonicate to dissolve and dilute to volume with diluent.

Standard preparation: Transfer 1.0mL of standard stock solution into 10mL volumetric flask and dilute to volume with diluent. The concentration of apixaban was calculated by calibration graph prepared with stock solution.

Figure 1: Structure of apixaban

Sample Preparation: No pretreatment like time-consuming extraction or evaporation step was performed for specimen preparation. Finely grind pre weighed 20 tablets of apixaban. Transfer grinded sample quantitatively equivalent to 20mg of apixaban into 50mL volumetric flask add 25mL of diluent, sonicate to dissolve for 10 minutes and dilute to volume with diluent. Further filter the solution through filter paper. Dilute 0.8 ml of filtrate to 10ml with mobile phase. All solutions were filtered through 0.45 µm Millipore membrane filters before injections into the HPLC system. Experiments were realized under the same conditions as employed for the apixaban pharmaceutical preparations.

Figure 2: Validated chromatogram of apixaban

Stress studies: Stability indicating analytical method of apixaban has been developed according to the ICH guideline. Degradation was carried out in following conditions:

Acidic –Drug was subjected to 1 M HCl in a water bath at 100°C for 3 hrs. Solution was neutralized by sodium hydroxide and diluted with mobile phase. Peak area of apixaban was observed when chromatographed after exposing at 70°C for 8 hr.

Figure 3: Linearity plot

Alkaline –Drug was also subjected to 0.5 M NaOH at 100° C for 3hrs. Solution was neutralized by 0.5 M HCl diluted with mobile phase.

Oxidation – Drug was subjected to 6 % hydrogen peroxide for 48 hrs at room temperature.

Thermal – Drug was subjected to thermal degradation at 80°C for 8 days.

Photo-stability condition–Drug was subjected to degradation in photostability chamber for 8 days.

RESULTS AND DISCUSSION

HPLC Development And Optimization: Several parameters that can affect chromatographic separation were evaluated and optimized. These parameters included scanning of different wavelengths, experimenting with various types of the aqueous phase, changes of the mobile phase pH, different types and ratio of organic modifier added and flow rate.

Chromatographic conditions:

Column	: Thermo BDS, C8, 150 X 4.6, 5µ.
Flow Rate	: 1.0 ml/min
Wave length	: 259 nm
Column temperature	: 30°C
Injection volume	: 20 µL
Diluent	: Mobile Phase
Elution type	: Isocratic
Needle wash solution	: Water: Acetonitrile (90:10%v/v)

Strategy: In our investigation, chromatographic separation was affected utilizing an isocratic mode with a C18 column. The mobile phase made out of combination

of buffer (pH 4.5) and Acetonitrile at 60:40%v/v ratio at a steady stream pace of 1.0mL/min. The eluate was checked at a set frequency of 259nm at RT. Every infusion run was repeated multiple times, with an infusion volume of 20 μ L. Absolute run season of each example infusion was roughly 8 min, and evaluation of the segments under investigation was accomplished utilizing the all out peak areas of the researched segments.

Figure 4 a: Acid stress studies chromatogram

Figure 4 b: Base degradation chromatogram

Method validation: To demonstrate appropriateness of method for the predetermined purpose, validation of the developed RP-HPLC method was done as per ICH guidelines (ICH Q2 (R1) 2005).

System suitability: The system suitability was checked by infusing six replicate injections of apixaban (40 μ g/mL) under optimized chromatographic conditions. The system was deemed to be suitable for use, as the tailing factors and resolutions for apixaban were less than 1.4 and more than 4.0, respectively. System suitability results are given in Table 1.

Figure 4 c: Oxidative degradation chromatogram

Figure 4 d: Thermal degradation chromatogram

Table 1. System suitability studies

Sample Name	Rt	Area	Theoretical Plates	Asymmetry
Blank	-	-		
Apixaban, Standard	3.133	23535298	3941	1.38
Placebo	-	-		
Sample	3.13	23546852		

Linearity: Accurately measured volumes of the apixaban standard solution (100 μ g/mL) were successively transferred into six 10mL volumetric flasks. 20 μ L aliquots of each solution were chromatographed three times and analysis was performed by optimized method. The regression equation was derived from the plot of average area of apixaban peak against the concentration of apixaban area were submitted to linear regression analysis to obtain the regression equation and correlation coefficient. Table 2 presents the data of linearity and Figure 3 shows calibration curve for apixaban. The calibration graph was linear over the concentration range 8.0- 48 μ g/mL of apixaban ($r^2 = 0.9996$). The

calibration results revealed a good linear relationship of peak area of apixaban over the concentration range of 8.0-48 µg/mL.

Table 2. Linearity studies

Conc(mcg)	Area
8.0	4849783
16.0	9683579
24.0	14371967
32.0	18721575
40.0	23462427
48.0	28151143
r ²	0.9999
LOD	0.600µg/ml
LOQ	2.00µg/ml

Table 3. Stress degradation studies

Degradation Studies	Assay	Assay Value	% Of Net Degradation
Acid	97.87	100.54	2.672
Base	97.114	100.54	3.426
H ₂ O ₂	97.097	100.54	3.443
UV	96.307	100.54	4.233
Thermal	97.635	100.54	2.905

Limit of Detection and Limit of Quantitation: Standard deviation of y intercept of calibration curve of linear lower concentrations (N) and slope (S) of the calibration curve were calculated and using their values LOD and LOQ were estimated as per ICH guideline. LOD and LOQ were estimated as per ICH guidelines using mathematical formula. Obtained. value for LOD was 0.600µg/mL and obtained value for LOQ was 2.00µg/mL respectively.

Stress studies: The forced degradation studies in acid, alkaline, photolytic, heat, and oxidative conditions, resulted in non significant decrease of the area without any detectable eluting degradation product. Under these conditions, 97.87, 97.114, 97.097, 96.307 and 97.635% of apixaban were recovered, respectively. The results (Table 3) of HPLC method for stress samples, including separation of the degradation products produced of apixaban in indicated the proposed method is stability-indicating. Chromatograms of apixaban after degradation with acid, alkaline, oxidative, heat, and photolytic conditions are displayed in Figures 4a -4e.

Repeatability: To assess repeatability of the method, chromatographic analysis of six injections of 40µg/mL apixaban solutions was performed. The average, standard deviation and % RSD for retention time and area of apixaban peak were estimated. Table 4 presents results of repeatability. % RSD of retention time and

area of apixaban determination was found less than 2% that proves the repeatability of the developed HPLC method.

Table 4. Repeatability Studies

S No	Name	RT	Area
1	Method Precision-1	3.14	23522656
2	Method Precision-2	3.163	23647919
3	Method Precision-3	3.133	23549208
4	Method Precision-4	3.113	23526105
5	Method Precision-5	3.13	23436418
6	Method Precision-6	3.157	23507797
Avg		3.139	23531684
Std Dev		0.018	68668.04
%RSD		0.586	0.292

Table 5. Accuracy studies

80% accuracy	RT	Area
Accuracy-80-1	3.13	18762329
Accuracy-80-2	3.14	18741277
Accuracy-80-3	3.107	18735915
Avg	3.126	18746507
		80.35
amt added(mg)		16.00
amt Recoverd(mg)		16.14
%Recovery		100.86
100%accuracy		
Accuracy-100-1	3.103	23307056
Accuracy-100-2	3.102	23343824
Accuracy-100-3	3.103	23372929
Avg	3.103	23341270
		100.04
amt added(mg)		20.00
amt Recoverd(mg)		19.99
%Recovery		99.96
120% accuracy		
Accuracy-120-1	3.12	28178075
Accuracy-120-2	3.117	28117262
Accuracy-120-3	3.123	28195873
Avg	3.120	28163737
		120.71
amt added(mg)		24.00
amt Recoverd(mg)		24.24
%Recovery		101.01

Accuracy: In the prepared test solution mixture, apixaban standard solution was added at 80%, 100% and 120% concentration level. 20µl of each solution were injected three times and were chromatographed.

When the method was used for analysis of apixaban from previously analysed laboratory mixture solution after spiking of 80, 100 and 120% apixaban standard, the recovery was found 99.96-101.01%. Table 5 presents the results of accuracy determination.

Robustness: The experimental conditions of the method were deliberately changed to determine the robustness. The wavelength ($259\text{nm} \pm 2\%$) and mobile phase flow rate ($1.0 \pm 0.1\text{mL/min.}$) were varied. In each case, and %RSD of area of apixaban peak and retention time was calculated. Comparison of the retention time and area of apixaban peak were done with that obtained under the optimized method, also. It was found that method was robust regarding any minor deviation in the column temperature. Table 6 shows the results of the robustness.

Ruggedness: A 20 μl aliquot of fixation 10 $\mu\text{g/ml}$ was infused to consider the unpleasantness of apixaban by two particular sensible logical specialists (Analyst-1 and

Analyst-2) and the results were recorded and are in the good reach for apixaban. The results (Table 7) exhibited the % RSD was less than 2% separately.

Table 6. Robustness studies

S No	Parameter	RT	Area
1	Standard	3.17	23518790
2	Robustness-Flow-1	3.883	29190915
3	Robustness-Flow-2	2.620	19483082
4	Robustness-nm-1	3.110	22787010
5	Robustness-nm-2	3.117	23990725

Table 7. Ruggedness Study

S No	Ruggedness-Day-1 Name	RT	Area
1	Ruggedness-Day-1-1	3.100	23604447
2	Ruggedness-Day-1-2	3.143	23504066
3	Ruggedness-Day-1-3	3.127	23566316
4	Ruggedness-Day-1-4	3.110	23532889
5	Ruggedness-Day-1-5	3.140	23535350
6	Ruggedness-Day-1-6	3.100	23502240
Avg		3.120	23540885
Std Dev		0.019	39065.36
RSD		0.621	0.166
S No	Ruggedness-Day-2 Name	RT	Area
1	Ruggedness-Day-2-1	3.127	23591751
2	Ruggedness-Day-2-2	3.117	23589267
3	Ruggedness-Day-2-3	3.113	23614793
4	Ruggedness-Day-2-4	3.113	23623894
5	Ruggedness-Day-2-5	3.143	23604438
6	Ruggedness-Day-2-6	3.12	23588335
Avg		3.122	23602080
Std Dev		0.011	14851.38
RSD		0.367	0.063

Table 8. Assay Calculation for Apixaban

23508743	20.1	1	50	10	99.8	120.5	mg/tab	% Of Assay
23534632	50	10	480.3	1	100		5.03	100.54

Application To Pharmaceutical Preparations: There is no chromatographic interference found for tablet dosage forms with internal standard, formulation components. The results given in Table 8 indicated good accuracy of apixaban for pharmaceutical preparations.

CONCLUSION

The present work described the development of a validated stability-indicating assay HPLC approach to determine apixaban for pharmaceutical preparations using UV detection mode. The chromatographic conditions were finally optimized on Thermo BDS, C8, 150 X 4.6, 5 μ , and buffer (pH 4.5) and Acetonitrile at 60:40%v/v ratio as the mobile phase, at a stream pace of 1.0 ml min⁻¹ and UV detection at 259nm. The developed method was found to be selective, sensitive, precise, linear, accurate, and reproducible and stability indicative for determining the apixaban in pharmaceutical preparations.

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