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DEVELOPMENT AND VALIDATION OF A HPLC METHOD SUITABLE FOR ASSAY AND DISSOLUTION TESTING IN APIXABAN TABLETS FINAL DOSAGE FORM

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Abstract

Background: Apixaban is an anticoagulant agent that inhibits coagulation factor Xa, commercially available as coated tablets at the dosages of 2.5 and 5 mg. There is no official monograph of the formulation in the current international pharmacopoeias. **Objective:** This paper presents a simple, specific and precise high-performance liquid chromatographic method for determination of Apixaban in solid dosage form. **Method:** The mobile phase consists of Acetonitrile – Buffer (320 + 680, v/v). A column containing octadecylsilane chemically bonded to porous silica particles (Chromosil® 100 ODS 150 x 4.6 mm, 5 µm) was used as stationary phase. Detection was performed using a variable wavelength ultraviolet-visible detector set at 230 nm for Apixaban. Solutions were injected into the chromatograph under isocratic condition at a constant flow rate of 1.0 mL/min. **Results:** The method demonstrates acceptable accuracy and precision and a wide linearity range. Linearity was observed in the range 25-150 µg /mL for Apixaban (r2 =0.999) for drug estimated by the proposed method was in good agreement with the label claim. The accuracy of the methods was assessed by recovery studies at three different levels. Specificity experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD of less than 2. **Conclusion:** The HPLC method for Assay and dissolution for Apixaban was developed and validated as per guideline and it is found to be precise, specific, accurate and robust.

Highlights: All statistical data proves validity of the methods and can be used for routine analysis of quality control and stability studies in pharmaceutical dosage form.

INTRODUCTION

Apixaban is chemically 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. Physically, it is characterized as white to pale-yellow powder with melting point of 326.53°C. It has good solubility nature in water and dimethyl sulfoxide. It is a new generation of oral anticoagulant drug that selectively inhibits coagulation factor Xa (1). It is used for thromboprophylaxis in patients following total knee replacement surgery, exhibits a high desirable efficiency and safety profile (2). Apixaban is currently sold under the trade name of Eliquis (Bristol-Myers and Pfizer, USA) as coated tablets at dosages of 2.5 and 5 mg. Apixaban has an acidic pKa of 13.12 and a basic of -1.60, therefore it does not ionize at physiological pH and remains in its neutral form (3-4). According to the Biopharmaceutical Classification System, Apixaban is a class III molecule with high solubility and low permeability (5-6).

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During the development of any drug substance or product in the pharmaceutical industry, the development of an accurate and efficient analytical method to determine the quality of the product is a crucial step (7). Analytical method validation ensures that various HPLC analytical techniques give reliable and repeatable results; it is a critical step in developing new dosage forms as it provides information about accuracy, linearity, precision, detection, and quantitation limits. According to the ICH guideline, “the objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose.” It is now obligatory in the process of drug development to supply the validation data to the responsible authorities. Guidelines for analysis method validation include ICH and USP guidelines (8-10).

The dissolution test is a substantial resource both for development and monitoring of formulations and for quality control and characterization of in vitro-in vivo correlations in bioequivalence studies (11). The dissolution test aims to study the drug release process into a solvent over time. The test aims to reproduce as closely as possible what will happen with the pharmaceutical form when administered. Therefore, it is important to assess several factors that may influence the process in vivo by selecting appropriate parameters and methodology for the in vitro dissolution test (12, 13).

The present paper describes a simple, selective, precise, and accurate HPLC method for the determination of Apixaban content with shorter runtime which is used in stability studies and quality control applications associated with this drug (19). The method can also be used to determine dissolution profile studies. Several method performance parameters were evaluated to observe their effect on the chromatography. The method was validated and found to be suitable for the analysis of Apixaban in the final dosage form.

EXPERIMENTAL

Instrumentation

a. HPLC – HPLC analysis was performed on integrated system LC-2030C (Shimadzu Corporation, Kyoto, Japan) consisted of a 4-liquid gradient system, high-speed autosampler, column oven, and UV-visible (UV-Vis) detector. Chromatograms were recorded and integrated with LC solution (Shimadzu) chromatographic PC software.

b. Dissolution apparatus – A dissolution apparatus was used (Electro Lab., India), consisted of 12 glass vessels.

c. Milli-Q water purification system – A Milli-Q integral 3 system mode was used (Millipore, Billerica, MA).

d. Glassware - All glassware used in analysis purchased from Pyrex (Germany).

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Standards, Reagents and Chemicals

a. Apixaban – Standard (Hetero Laboratories, India).
b. Acetonitrile – HPLC grade (Fisher scientific, USA).
c. Orthophosphoric acid – Analytical grade (Panreac Applichem, USA).
d. Sodium hydroxide – Analytical grade (Panreac Applichem, USA).
e. Monobasic potassium phosphate - Analytical grade (Panreac Applichem, USA).
f. Sodium dihydrogen phosphate – Analytical grade (Panreac Applichem, USA).
g. Sodium Lauryl sulphate – Analytical grade (Scharlau, Spain).
h. Water – Purified in-house using the Milli-Q integral 3 system.
i. Sample – Obtained from Jamjoom Pharmaceuticals Co. Ltd, Jeddah, Saudi Arabia

Chromatographic conditions

The isocratic mobile phase consisted of acetonitrile and buffer (1.4 g of monobasic potassium phosphate was transferred into 1000 mL of water and mixed. Adjusted the pH 4.5 ±0.05 with diluted Ortho-phosphoric acid) in the ratio of (32: 68, v/v). A Chromosil® 100 ODS 150 x 4.6 mm, stainless steel analytical column packed with octadecylsilane chemically bonded to porous 5 mm silica particles (Waters Corp., Milford, MA) was used as the stationary phase. A constant flow rate of 1.0 mL/min was used throughout the analysis. The variable UV-Vis detector was set at 230 nm. All analyses were performed at ambient temperature, and the volume of solution injected onto the column was 20 µL.

Dissolution test conditions

USP apparatus 1 (basket) and 2 (paddle) were available for the dissolution study. USP Apparatus 2 (Paddle) was chosen because it is the apparatus most used for the evaluation of tablets. Filters with a pore size of 45 µm full flow filter were used.75 rpm rotation speed was applied to the paddle and sample specimen was collected from each vessel after 30 minutes. 900 mL volume was selected for dissolution medium and maintained temperature at 37ºC throughout the experiment.

Solution preparation (For Assay and Dissolution test)

a. Diluent for Assay

   Acetonitrile and water in the ratio of (60: 40, v/v)

b. Media preparation for Dissolution test

   The release medium is phosphate buffer pH 6.8 + 0.5% sodium lauryl sulphate prepared by dissolving about 60 g of Sodium dihydrogen phosphate anhydrous in 10000 mL of water. Adjust the pH to 6.8 ± 0.05 with 1N sodium hydroxide solution. Add 5 g of sodium lauryl sulfate, heat the dissolution medium at 37° C to dissolve. Mix well and degas.

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c. Standard stock solution

26.50 mg of Apixaban working standard was weighed and transferred into a 100 mL volumetric flask. 70 mL of diluent was added and sonicated to dissolve followed by dilution to volume with diluent.

d. Standard solution for Assay

5 mL of Apixaban stock standard was pipetted into 25 mL volumetric flask and diluted to volume with diluent and mixed well. This solution contained about 0.05 mg/mL of Apixaban.

e. Standard solution for Dissolution

4 mL of Apixaban stock standard was pipetted into 200 mL volumetric flask and diluted to volume with dissolution medium and mixed well. This solution contained about 0.0055 mg/mL of Apixaban.

f. Sample solution for Assay

10 tablets were transferred into 200 mL volumetric flask. 20 mL of water was added and sonicated for 5 minutes. 140 mL of diluent was added and further sonicated for 15 minutes with intermittent shaking and diluted to volume with diluent. The resulted solution was centrifuged at 4000 rpm for 10 minutes. 5 mL of clear supernatant was pipetted into 25 mL volumetric flask and diluted to volume with diluent and mixed well.

g. Sample solution for Dissolution

Transfer one tablet in each of six dissolution vessels containing 900 mL of dissolution medium, which has been previously equilibrated to a temperature of 37°C ± 0.5°C. After 30 minutes, a specimen was withdrawn from a zone midway between the surface of the dissolution medium and the top of the rotating blade, not less than 1 cm from the vessel wall. Filtered through 45 µm Agilent full flow filter by discarding first few mL of the filtrate. Use the filtrate as test solution.

RESULTS AND DISCUSSION

Mobile phase composition containing Acetonitrile and buffer (320 + 680, v/v) on a Chromosil® 100 ODS 150 x 4.6 mm, stainless steel column at ambient temperature was optimized to obtain good peak symmetrical shape. This mobile phase ratio supported low back pressure and shorter retention time. A detection wavelength of 230 nm provided maximum absorption of Apixaban obtained by PDA detector. Choice of the extraction solvent is a key issue in an analytical procedure. Samples diluted in Acetonitrile and water solution in the ratio of (60: 40, v/v) with an injection volume of 20 µL was used for the analysis which showed good solubility of Apixaban. For dissolution test, several conditions were evaluated until reaching the one that proved to be most suitable for this purpose. The preferred method was established under the following conditions: USP apparatus II (Paddle), 75 rpm, pH 6.8 phosphate buffer with 0.5% sodium lauryl sulphate, 900 mL used as released medium at 37 °C, total experimental time of 30 min and quantification by HPLC.

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Method validation

The method was validated to include requirements of the International Conference on Harmonization (ICH) guidelines and united state pharmacopeia requirements (14-16). Parameters like specificity, linearity, accuracy, precision, robustness, and system suitability were examined and found to be acceptable. In the method validation, 100% target level for Apixaban correspond to amounts present in the formulation (17-18).

a) System suitability

System suitability tests were performed on chromatograms obtained from standard solution to check parameters such as %RSD between 6 replicate injections, tailing factor and theoretical plate count of HPLC column and found satisfactory results. Values obtained from standard solution are provided in (Table-1).

b) Specificity

Specificity is the ability to assess unequivocally the analyte in presence of the components, which may be present such as impurities, degradation products and other matrix components. Specificity study carried out to determine the retention time of principal peaks and demonstrating that the determination is unaffected by the presence of diluent and placebo peaks. No interferences were observed due to the presence of excipients in the formulation (Figure-2).

c) Precision

The precision of method is degree of agreement between the results. Precision of the method was studied for system precision, method precision and intermediate precision.

i. Precision of system

Precision of the system is to determine reproducibility of results obtained by injecting of six replicates of single preparation of standard solution. The relative standard deviation (RSD) for six determination was found to be 0.11%.

ii. Precision of method

Precision of a method is to determine by analyzing six preparation of the same concentration and evaluate the % RSD between these preparations. Six separate test sample solutions were prepared at the specification level for measuring the method precision. The calculated RSD of results was found to be 1.02% (Table-2). For dissolution method precision, calculated the %drug dissolved for each vessel after 30 minutes of interval and evaluated the RSD between the obtained results which was found 0.80%. (Table-2).

iii. Intermediate precision

The similar procedure of method precision was carried out by a different analyst, using different mobile phase and diluent preparations and instrument on a different

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day with different lot of same brand column for intermediate precision study. The %RSD of results for intermediate precision study was calculated and compared with the method precision results. RSDs obtained from 12 assay results by 2 analysts were 0.85% for Assay method (Table-3). Similarly, intermediate for dissolution test was carried out and the RSD obtained from results by 2 analysts were 0.86% (Table-3).

d) Linearity

Linearity of the analytical method is its ability to elicit test results that are directly proportional to the concentration of the drug substance taken for test, within a given range. Peak areas (average of 2 replicate injections) vs concentrations, in µg/mL, were plotted for Apixaban in the concentration range of 80–120% (80, 90, 100, 110 and 120) of the target level for Assay test and the range of 25–150% (25, 50, 75, 100, 125 and 150) of the target level for dissolution test. Individual calibration graphs showing lower and higher concentration ranges, linear regression equations, and linearity correlation coefficient (r-value) are provided in (Table 4 and 5). The r-value for all compounds was >0.999, suggesting that the method has a broad linear dynamic range. Linearity graph for assay and dissolution concentration ranges are provided in (Figure-2 and Figure-3).

e) Accuracy

Accuracy, a measure of the exactness of the analytical method, is often expressed as the percent recovery by the assay of known, added amounts of standards / test of interest. For Assay method, equal quantities of blank tablet powder matrix were weighed and transferred into separate 100 mL volumetric flasks. Portion of Apixaban was weighed and spiked into each flask at 80, 100 and 120% of the target level in the tablets. Each flask was treated according to the test solution preparation and injected in duplicate. All level samples were prepared in triplicate. Mean recovery obtained from 9 samples ranges from 98.33 to 100.39%. Accuracy data for Assay test are provided in (Table-6). For dissolution method, equal quantities of blank tablet powder matrix were weighed and transferred into separate dissolution vessels contained 900 mL dissolution medium. Portion of Apixaban was weighed and spiked into each dissolution vessels at 25, 100 and 150% of the target level in the tablets. Each vessel was treated according to the test procedure for dissolution and injected in duplicate. Mean recovery obtained from 9 samples ranges from 99.57 to 101.42%. Accuracy data for dissolution test are provided in (Table-7).

f) Robustness

Robustness 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 of the method was studied by deliberate changes in the method like alteration in flow rate from 1.0 mL /minute to 0.9 mL/minute and column oven temperature from ambient to 30°C. It was observed that there were no marked changes in the test results as provided in (Table-8).

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g) Filter suitability

Filter suitability study carried out to determine the suitable filter for the dissolution of Apixaban in film coated tablets. Preparation of standard and sample solution for filter suitability study as per dissolution test method. A few portions of standard and sample solution were centrifuged and filtered through different types of filters. (Centrifuged, PVDF filter, PTEF and Nylon filter) & sample solutions (Centrifuged, PVDF filter, PTEF and Nylon filter). The similarity factor of standard solution for Centrifuged, PVDF and Nylon filter was 1.00 for all three types of filters. The similarity factor for sample solution for Centrifuged, PVDF and Nylon was 1.00 for all three types of filters. The compliance of similarity factor of standard solution and sample solutions reveals the suitability of the Centrifuged, PVDF and Nylon filters. Results are shown in (Table-9).

ADVANTAGES AND APPLICATIONS

The analysis of commercial formulation sample and bulk drug sample indicated that the method is specific and selective for determination of Assay and Dissolution test in the formulation and bulk drug samples. The developed method is capable for quantitative analysis of Apixaban in the bulk drug and in a pharmaceutical dosage form. The described method is rapid, with a run time of less than 10 min and selective for Apixaban.

CONCLUSION

The present study aimed to develop and validate Assay and Dissolution test method for determination of Apixaban in film coated tablets. Several conditions were evaluated until reaching the one that proved to be most suitable for this purpose. The validation was carried out as recommended in the official guidelines and covered the parameters of specificity, linearity, precision, accuracy, and robustness, with all results found in accordance with the recommended validation parameters. All the objectives presented in this study were achieved as the proposed method demonstrated to be adequate, feasible, and reliable, presenting a profile of gradual dissolution that would favor the detection of potential production process problems in the dissolution performance of the product. This study contributes to the future monograph of Apixaban immediate release coated tablets as well as the development of new products containing this drug. Finally, this method can be used for routine analysis, quality control and stability studies of pharmaceutical preparations containing this compound.

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Conflict of interest

The authors declared that they have no conflict of interest.

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Tables

Tables 1: Results of system suitability

Injection No.	Apixaban		
	Area (uAU)	Tailing factor	Theoretical plate count
1	2913178	1.09	25214
2	2913436	1.09	25033
3	2912799	1.10	25081
4	2913212	1.09	25109
5	2920478	1.09	24946
6	2917715	1.10	25184
Average (n=6)	2915136.33	1.09	25094.50
% RSD	0.11		

Tables 2: Results of method precision (For Assay and Dissolution)

Preparation No.	%Assay	%Dissolution
1	99.91	98.50
2	100.30	98.55
3	99.12	97.27
4	98.80	97.30
5	98.80	99.07
6	97.42	98.87
Average (n=6)	99.06	98.26
%RSD	1.02	0.80

Tables 3: Results of intermediate precision (For Assay and Dissolution)

Analyst	Analyst-1	Analyst-2	Analyst-1	Analyst-2
Day	Day-1	Day-2	Day-1	Day-2
Preparation No.	%Assay	%Assay	%Dissolution	%Dissolution
1	99.91	97.98	98.50	100.45
2	100.30	97.94	98.55	100.83
3	99.12	98.08	97.27	100.74
4	98.80	98.12	97.30	101.11
5	98.80	98.48	99.07	101.17
6	97.42	97.89	98.87	99.24
Average (n=12)	98.57		99.42	
%RSD	0.88		1.42	

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Tables 4: Results of Linearity (For assay method)

Linearity (%) Level	Concentration in ppm	Average Area (uAU)
80	40.91	2472122
90	46.03	2809237
100	51.14	3218730
110	56.25	3573496
120	61.37	3811320
Slope		67318.3
Intercept		-265675.3
Correlation Coefficient		0.999

Tables 5: Results of Linearity (For dissolution method)

Linearity (%)	Concentration in ppm	Average Area (uAU)
25	1.38	114585
50	2.75	224253
75	4.13	331963
100	5.50	439036
125	6.88	556031
150	8.25	663343
Slope		80163.5
Intercept		2026.0
Correlation Coefficient		0.9999

Tables 6: Results of Accuracy (For Assay method)

Accuracy Level (%)	Amount Added (ppm)	Amount found (ppm)	Average Area (uAU) of Apixaban	% Recovery (Specification limit)	% Recovery
80-1	39.55	40.15	2403176	78.80	98.51
80-2	39.25	38.78	2407347	79.04	98.80
80-3	39.20	38.65	2384000	78.87	98.59
100- 1	50.55	49.70	3066001	98.33	98.33
100- 2	49.90	49.69	3065090	99.58	99.58
100- 3	49.40	49.26	3057949	99.72	99.72
120- 1	61.00	61.24	3777603	120.47	100.39
120- 2	60.75	60.32	3720650	119.15	99.29
120- 3	61.15	61.24	3777338	120.17	100.14
Confidence Interval (n=9)			**99.26 ± 0.574**		

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Tables 7: Results of Accuracy (For Dissolution method)

Accuracy Level (%)	Amount added (ppm)	Amount found (ppm)	Average Area (uAU) of Apixaban	% Recovery (Specification limit)	% Recovery
25- 1	1.46	1.44	111591	25.36	101.42
25- 2	1.46	1.48	111551	25.36	101.42
25- 3	1.48	1.48	111945	25.05	100.19
100- 1	5.94	5.94	448998	99.92	99.92
100- 2	5.79	5.80	438325	100.24	100.24
100- 3	5.72	5.79	437707	101.10	101.10
150- 1	8.69	8.71	658130	150.30	100.20
150- 2	8.76	8.75	661156	149.95	99.97
150- 3	8.77	8.73	659609	149.36	99.57
Confidence Interval (n=9)			**100.45 ± 0.448**		

Tables 8: Results of Robustness

Preparations	Precision data	Robustness - 1 (Change in flow rate)	Robustness - 2 (Change in Column temperature)
1	99.91	102.38	102.83
2	100.30	99.82	100.74
3	99.12	98.24	98.38
4	98.80	97.59	97.83
5	98.80	98.14	98.78
6	97.42	96.60	97.06
Ave. (n=12)	--	98.93	99.16
%RSD (n=12)		1.56	1.61

Tables 9: Results of Filter suitability

Sample Name	Area (uAU) of Apixaban	Similarity Factor
Standard (PTFE)	397105	--
Standard	398496	1.00
Standard (PVDF)	397273	1.00
Standard (Nylon)	398067	1.00
Sample (PTFE)	381640	--
Sample (Centrifuged)	382696	1.00
Sample (PVDF)	381802	1.00
Sample (Nylon)	381823	1.00

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Figures

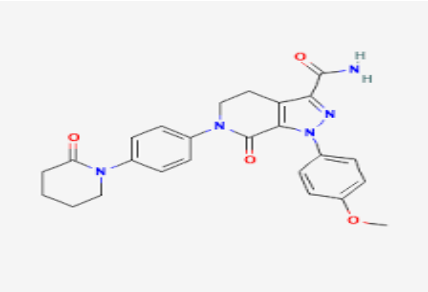

Figure 1: Molecular structure of Apixaban

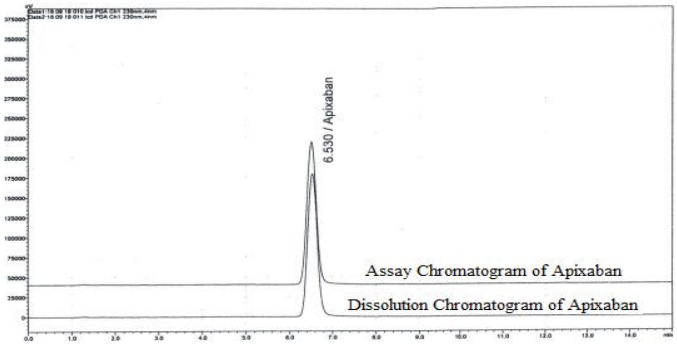

Figure 2: Reference Chromatogram of Assay & Dissolution of Apixaban

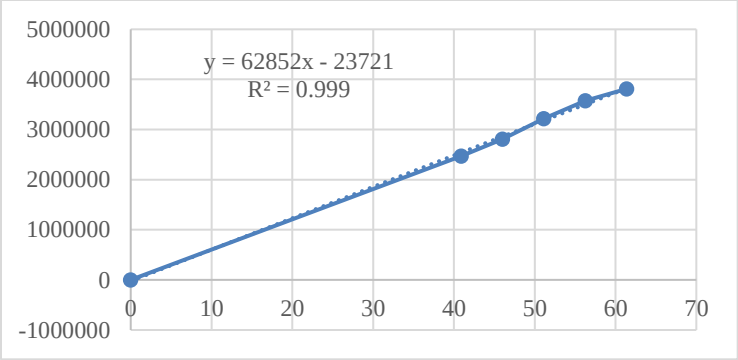

Figure 3: Linearity graph for Assay (X=Concentration vs Y= Peak Area)

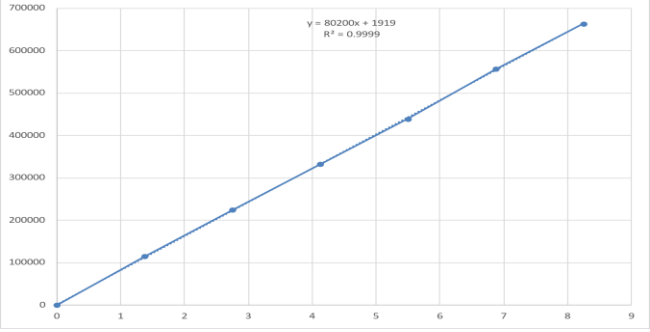

Figure 4: Linearity graph for Dissolution (X=Concentration vs Y= Peak Area)