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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING UPLC METHOD FOR ESTIMATION OF APIXABAN AND ITS PROCESS RELATED IMPURITIES IN TABLET DOSAGE FORM

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ABSTRACT

A validated Ultra Performance Liquid Chromatography (UPLC) stability indicating method has been developed for determination of Apixaban (APB) and its process related impurities as per ICH guidelines in bulk and solid oral dosage forms in the presence of degradation products. Reversed-phase chromatography was performed on a UPLC BEH Waters C_{18} (50 x 2.1 mm; 1.7 µm) with mobile phase consists of phosphate buffer (pH 3.0), Acetonitrile in a gradient proportion of 30:70 v/v respectively at a flow rate of 0.3 mL min^{-1}. Detection was performed at 220 nm and a sharp peak was obtained for Imp-A, Imp-B and APB at a retention time of 0.862, 0.953, 1.325 minutes respectively. Linear regression analysis data for the calibration plot showed there was a good linear relationship between response and concentration in the range 10-60 µg/mL for APB with regression coefficient 0.9999 and for Imp-A and Imp-B is 0.625-3.75 µg/mL with the regression coefficients 0.9994 and 0.9999 respectively. The LOD values for APB and Imp-A & B were found to be 0.12 µg/mL, 0.06 µg/mL, 0.05 µg/mL & the LOQ values 0.40 µg/mL, 0.20 µg/mL, 0.16 µg/mL respectively. In order to determine whether the analytical method and assay were stability-indicating, APB and Impurities was stressed under various conditions to conduct forced degradation studies. Stability indicating forced degradation established studies showed results that there is no interference of any degraded products and there is no intererence from the excipients in the formulation. The detection of APB and the performed assay is thus specific stability- indicating. The wide linearity range, sensitivity, accuracy,

short retention time, and simple mobile phase composition imply the method is suitable for routine quantification of APB with high precision and accuracy.

KEYWORDS: Apixaban; Impurity A & B, Stability Indicating, UPLC.

INTRODUCTION

Apixaban is an specific novel anticoagulant drug chemically known as l-(4-methoxyphenyl)7-oxo-6-[4-(2-oxopiperidin-l-yl)phenyl]-4,5,6,7-tetrahydro-lH-pyrazolo[3,4-c]pyridine-3 carboxamide [Fig.1] and its process related impurities 1-(4-methoxyphenyl)-6-[4-(2-methyl-6-oxopiperidin-1-yl)phenyl]-7-oxo-1H,4H,5H,6H,7H-pyrazolo[3,4-c]pyridine-3carboxamide. [Fig.2] And Isopropyl 1-(4-methoxyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylate;4,5,6,7-Tetrahydro-1 (methoxyphenyl)-7-oxo-6-[4-(2-oxo-1-piperidinyl) phenyl]-1H-pyrazolo[3,4-c]pyridine-3-carboxylic Acid [Fig.3] and sold under the brand name "Eliquis" to treat the people with atrial fibrillation to lower the risk of stroke caused by a blood clot disorders. It was invented by Aderis pharmaceuticals and was developed jointly by Pfizer and Bristol-Myers Squibb.

Apixaban is a selective, reversible, direct inhibitor of factor Xa indicated to reduce the risk of stroke and systemic embolism in patients with non-valvular atrial fibrillation. "Eliquis" was approved both in US and Europe on Dec 2012 and Jan 2010 respectively. "Eliquis" is also used after hip or knee replacement surgery to prevent a type of blood clot called deep vein thrombosis (DVT), which can lead to blood clots in the lungs (pulmonary embolism).[1-3]

Several analytical methods reported in the literature describe the investigation of pharmacokinetics of Apixaban wherein the content of Apixaban and/or its metabolites were studied in human plasma by liquid chromatography-mass spectrometry method[4-6] but failed to provide the details of process-related impurities and degradation related impurities formed under the stress conditions employed. One of the articles reported on stability indicating HPLC method for Apixaban bulk drug sample has been found to be non-selective at our end.[7-8]

Further, Apixaban is not yet official in any of the pharmacopoeia. Hence, we felt the need for the development of a selective, fast and stability-indicating RP-UPLC method. To the best of our knowledge, no proved stability indicating method has been reported for the determination of Apixaban and drug substance and drug product for regular analysis and stability studies in

quality control laboratory. The core-objective of this research work was to develop a fast, precise, sensitive and stability-indicating RP-UPLC method for the determination of process related impurities of Apixaban and its degradation behaviour process was studied at each level for understanding its degradation nature in all angles.[9-13]

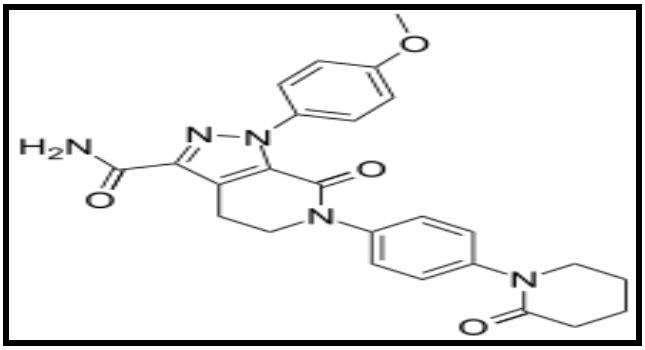


Fig. 1: Chemical structure of Apixaban.

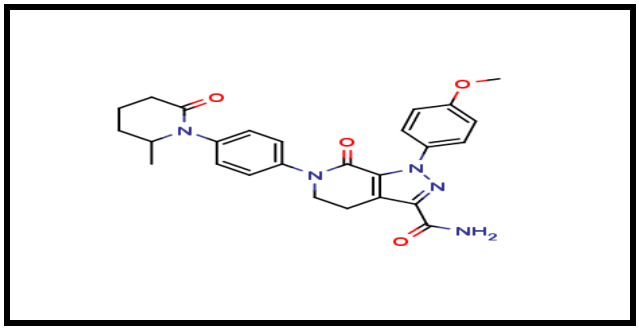


Fig. 2: Chemical structure of Apixaban Impurity-A.

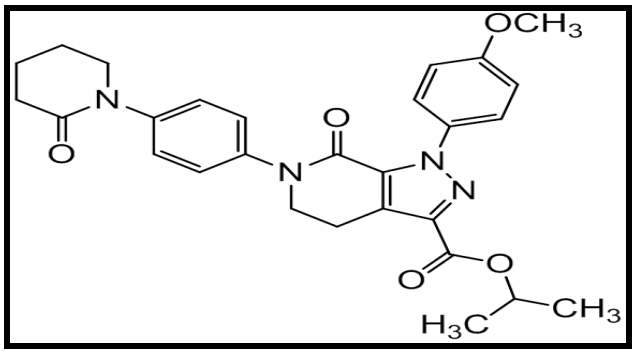


Fig. 3: Structure of Apixaban Impurity-B.

MATERIALS AND METHODS

Experimental

Materials and methods

Pure sample of Apixaban (APB) and two impurities A and B were obtained from Eisai Pharmaceuticals. Acetonitrile, Methanol, Ortho phosphoric acid, and water used were of

HPLC grade. All other chemicals used were of AR grade. Apixaban (APB) Tablets were purchased from local pharmacy.

Instrumentation

The analysis was performed using waters-2695(Model alliance) Ultra Performance liquid chromatography Waters auto sampler–PDA detector by using, Empower-software version-2, analytical balance (Mettler Toledo) UV/Visible-Detector (Standard cell) and data handling system (Autochrome-3000), pH meter (lab India) and Sonicator. The column used is UPLC BEH WATERS C_{18} (50 x 2.1 mm; 1.7 µm) with the flow rate 0.3 ml/min.

Preparation of Potassium dihydrogen phosphate buffer (pH 3.0)

Accurately weighed 6.8 grams of KH_2PO_4 was taken in a 1000 ml volumetric flask, dissolved and diluted to 1000 ml with HPLC water and the volume was adjusted to pH 3.0 with orthophosphoric acid.

Preparation of Mobile phase

700 mL of acetonitrile (Phase A) and 300 mL of phosphate buffer (pH 3.0) (Phase B) were mixed and degassed in an ultrasonic water bath for 10 min and then filtered through 0.45 µ filter.

Preparation of Placebo Solution

The placebo solution was prepared by dissolving the specified amount excipients in mobile phase.

Preparation of Standard solution

10 mg of apixaban was accurately weighed and transferred in to a 10 mL clean dry volumetric flask and about 40 mL of diluent was added and sonicated to dissolve it completely. The volume was made up to the mark with diluent (Stock solution).

3 mL of the above stock solution was pipette out into a 10 mL volumetric flask and the contents diluted up to the mark with the diluent. This solution makes the working standard (300 µg/mL of Apixaban).

Impurity stock solution preparation

25 mg each of Impurity –A and Impurity-B was accurately weighed and transferred into a 100 ml of volumetric flasks separately and about 30 ml of diluent was added was added to

each flask and sonicated to dissolve it completely. The volume was made up to the mark with the diluent, then it was filtered through 0.44 micron Injection filter. This contains 250 µg/ml of each impurity A and impurity B.

Impurity standard solution

1 ml each from the above standard solution of impurity A and B was pipetted out in to 10 ml volumetric flask separately and the contents diluted up to the mark with the diluent. This solution makes the impurity standard (25 µg/ml of each impurity A and B).

Preparation of binary mixture of apixaban, impurity A and impurity B

1 mL of standard solution (300 µg/mL of APB) and 1 mL each of impurity standard solution (25 µg/mL of each Impurity standard A and B) into a 10 ml volumetric flask and make up with the diluent. This solution gives apixaban 30 µg/mL and 2.5 µg/mL of each Impurity A and B.

Preparation of sample solution

Ten tablets of each containing 2.5 mg apixaban were weighed and ground to a fine powder. From this an amount equivalent to about 10 mg Apixaban was transferred into a 50 mL clean dry volumetric flask. About 30 mL of diluent was added and sonicated it up to 30 mins to dissolve it completely and volume made up to the mark with the same. Then it is filtered through a 0.45 µm injection filter. Further, 1.5 mL of Apixaban from the above solution was pipetted into a 10 mL volumetric flask and to it 1 mL of each Impurity A and Impurity B added from their stock solutions (25 µg/ml). The content in the flask was mixed well and diluted up to the mark with the diluent to get a concentration of 30 µg/mL of Apixaban, 2.5 µg/mL of Impurity A and B respectively.

Method validation

The method validation was done according to the ICH guidelines. The following validation parameters i.e accuracy, precision, linearity, and specificity, LOD, LOQ and robustness and Ruggedness were studied.

Forced degradation study

Forced degradation or Stress testing of a drug substance will help to identify the degradation products, which can help to establish the intrinsic stability of the molecule. All stress decomposition studies were performed at an initial drug concentration for Apixaban (APB)

along with impurity A and B. For the stability indicating study the Apixaban (APB) undergoes acid, alkali and oxidation degradation, photolysis and heat condition.

RESULTS AND DISCUSSIONS

Method Development and Optimization

The UPLC procedure was optimized with a view to develop a suitable LC method for the analysis of APB in fixed dose for bulk and combined dosage form and its impurities A and B. It was found that mobile phase consists of potassium dihydrogen phosphate with buffer (pH 3.0), Acetonitrile in a gradient in proportion 30: 70 v/v and given acceptable retention time for APB and impurity A and B, the theoretical plates, and good resolution for APB along with impurity A and B at the flow rate of 0.3 ml/min (Table.1; Fig. 4-6).

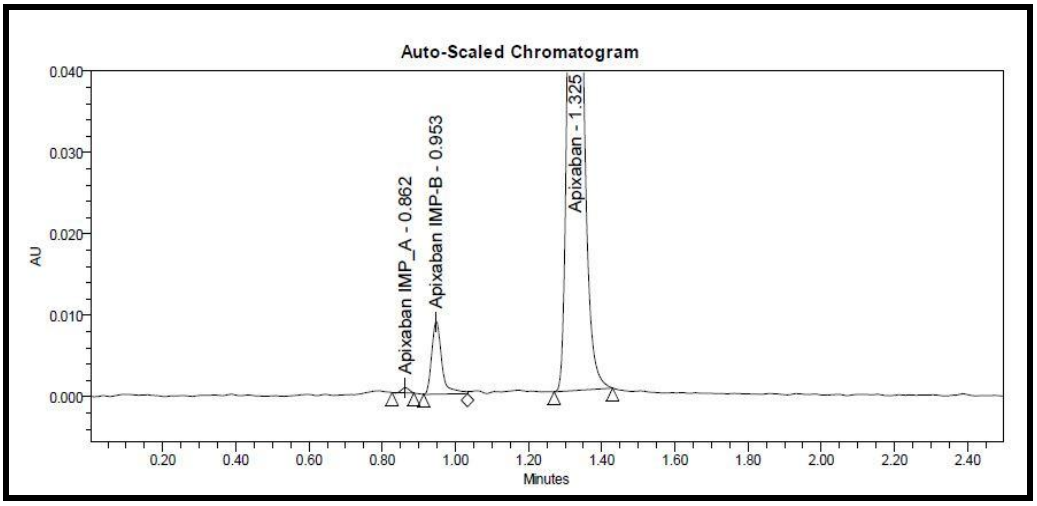


Fig. 4: Chromatogram of APB at 220 nm from bulk drug spiked with Imp-A &B.

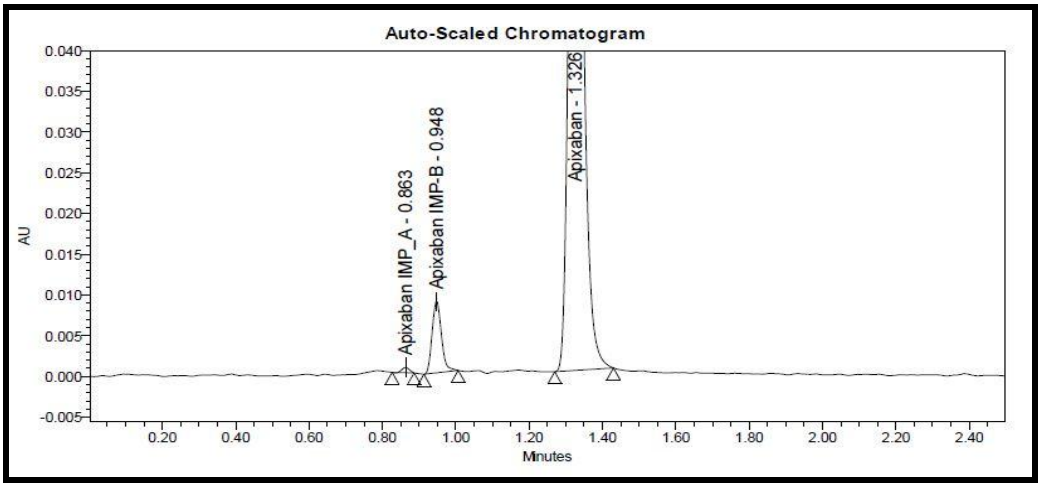
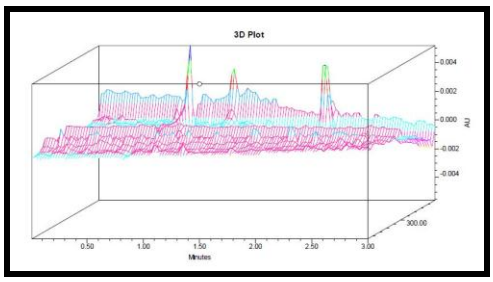
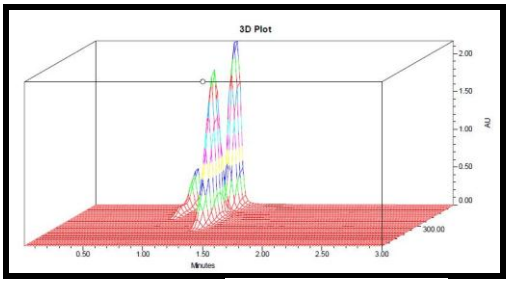


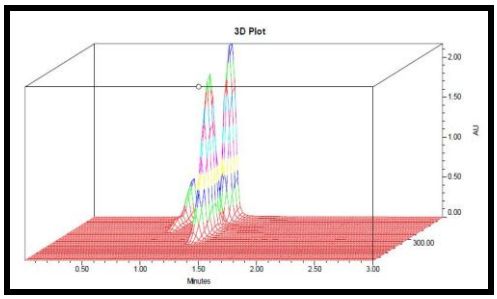
Fig. 5: Chromatogram of APB at 220 nm from pharmaceutical tablet formulation spiked with Imp-A &B.



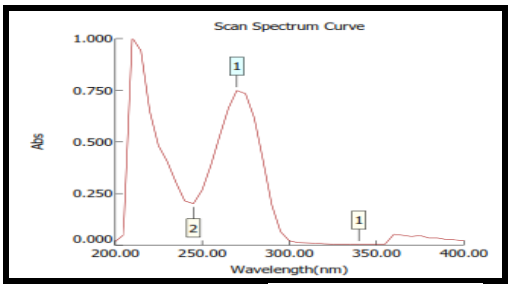
1. Blank.



2. Standard APB and Imp-A &B.



3. Sample APB.



4. UV Graph of APB and Imp-A &B.

Fig. 6: 3D Chromatogram plots for APB and Imp-A &B by PDA detector.

Table 1: Optimized Chromatographic Conditions.

Column	**UPLC BEH WATERS C18 (50x 2.1mm;1.7 µm)**
Mobile Phase	Acetonitrile –Phosphate Buffer (pH 3.0)-Gradient programing
Flow rate (mL/min)	0.3 mL/min
Run time (minutes)	3 min
Column temperature (°C)	30 °C
Volume of injection loop (µl)	4 µL
Detection wavelength (nm)	220 nm
Retention time of Impurity A	0.862 min
Retention time of Impurity B	0.953 min
Retention time of Apixaban	1.325 min

Validation of Developed Method

Linearity

For linearity study, standard apixaban solution with impurities A and B were taken at six concentration levels and analysed. A linear plot was constructed between peak area versus concentration. The corresponding regression coefficients were obtained from the plots and the conc. range were found as for APB 10-60 µg/mL, for Imp. A and Imp. B 0.625 -3.75 µg/mL. The reports of drug were found to be linear in prepared concn range & a correlation regression equation of APB was y= 7686.3x-754.33 with correlation coefficient 0.9999(Fig 7), Imp. A was y=1271 x +70.6 with correlation coefficient 0.9994 (Fig.8) and Imp.B was y = 7668 x -31.33 with correlation coefficient 0.9999 (Fig.9). Where x was the conc in µg/mL &

y was area of the peak. The linearity data was shown in Table 2-4. The overlain chromatogram obtained during the linearity is shown in the Fig.10.

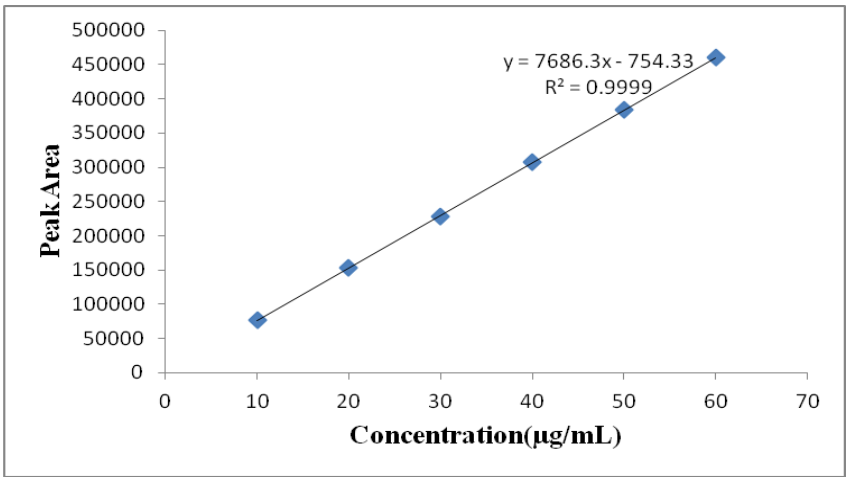


Fig. 7: Linearity curve of standard Apixaban.

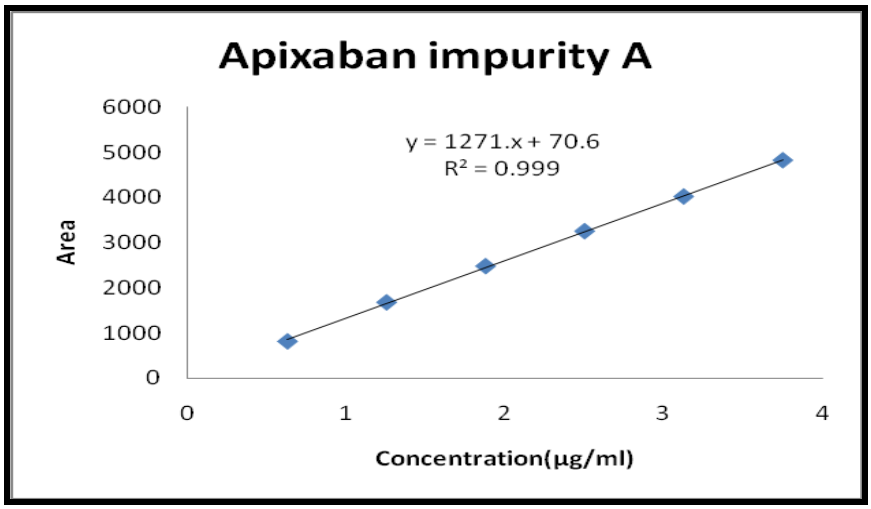


Fig. 8: Linearity curve of standard Imp-A.

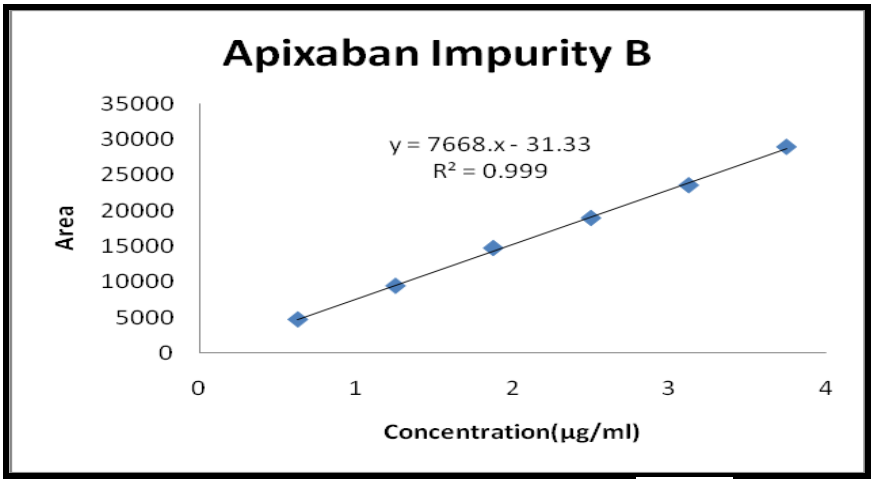


Fig. 9: Linearity curve for standard Imp-B.

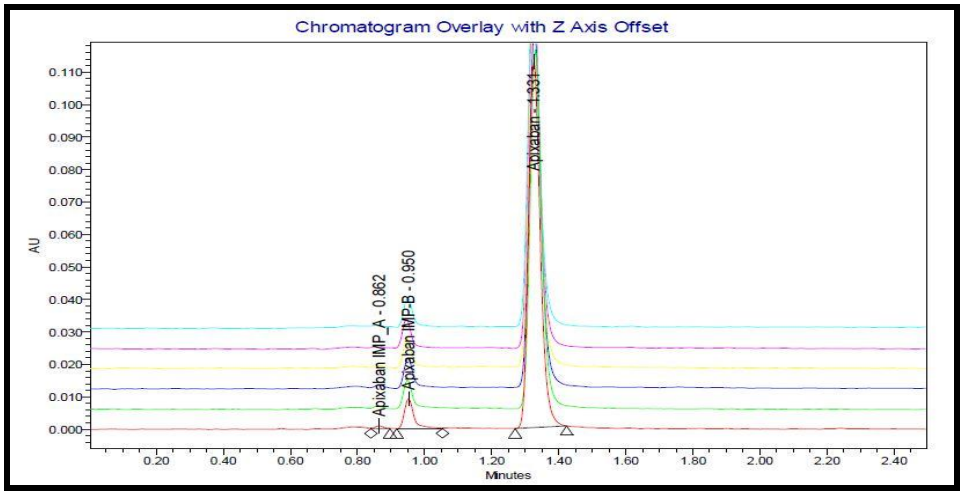


Fig. 10: Overlay linearity Chromatogram for Apixaban, Imp-A & B.

Table 2: Linearity data of Apixaban.

Linearity level		
	Conc. (µg/mL)	Mean Peak Area
I	10	76737
II	20	153276
III	30	227712
IV	40	307545
V	50	383875
VI	60	460452
Correlation co-efficient		0.9999
Slope		7686.3
Intercept		-754.33

Table 3: Linearity data of Imp-A.

S. No	Linearity Level	Concentration(µg/mL)	Mean Peak Area
1	I	0.625	824
2	II	1.25	1686
3	III	1.875	2486
4	IV	2.5	3260
5	V	3.125	4026
6	VI	3.75	4827
Correlation Coefficient			0.999

Table 4: Linearity data of Imp-B.

S. No	Linearity Level	Concentration(µg/mL)	Mean Peak Area
1	I	0.625	4735
2	II	1.25	9452
3	III	1.875	14754
4	IV	2.5	18976
5	V	3.125	23578
6	VI	3.75	28964
Correlation Coefficient			0.999

Precision

The precision was evaluated by injecting six times (n=6) of the working standard solution APB (30 µg/ml) spiked with working standard solution of impurity A (2.5 µg/mL) and impurity B (2.5 µg/mL) and the percent RSD was calculated. The % RSD values of the precision study were < 2.0 % for APB, Imp A and Imp B. This confirms that the method is precise, The overlain chromatogram is shown in Fig. 11 and the results of precision study are given in the Table 5.

Table 5: Precision data of APB and Imp-A, B.

	Imp.A	Imp. B	APB
No. of Injections	**Peak Areas**		
Injection-1	3278	18975	223541
Injection-2	3260	18939	228557
Injection-3	3264	18928	228943
Injection-4	3208	18964	229034
Injection-5	3279	18976	229639
Injection-6	3274	18957.8	228476
Average	3260.5	18956.5	228031.7
Standard Deviation	26.83	19.48	2238.67
%RSD	0.82	0.10	0.98

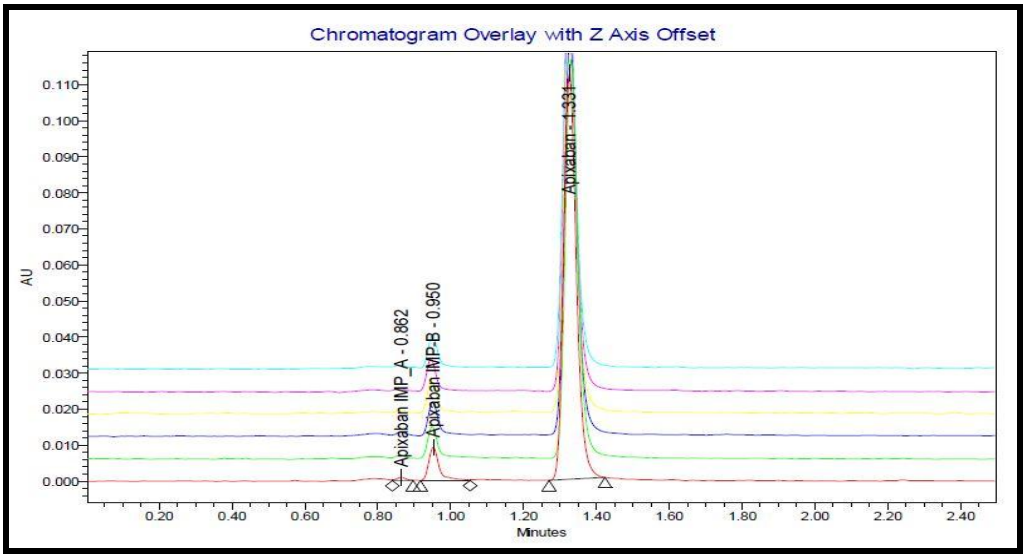


Fig. 11: Overlay precision Chromatogram for Apixaban, Imp-A & B.

LOD and LOQ

Limit of detection (LOD) & the limit of quantifications (LOQ) are evaluated by the serial dilutions of APB and Imp-A&B stock solutions in the ordered to be obtaining the signal to the noise ratio 3:1 for the LOD & 10:1 for the LOQ. Then the LOD value for APB and Imp-A & B were found to be 0.12 µg/mL, 0.06 µg/mL, 0.05 µg/mL & the LOQ value 0.40 µg/mL

0.20 µg/mL, 0.16 µg/mL respectively. The chromatogram of the LOD and LOQ were shown in the (Fig. 12 & 13).

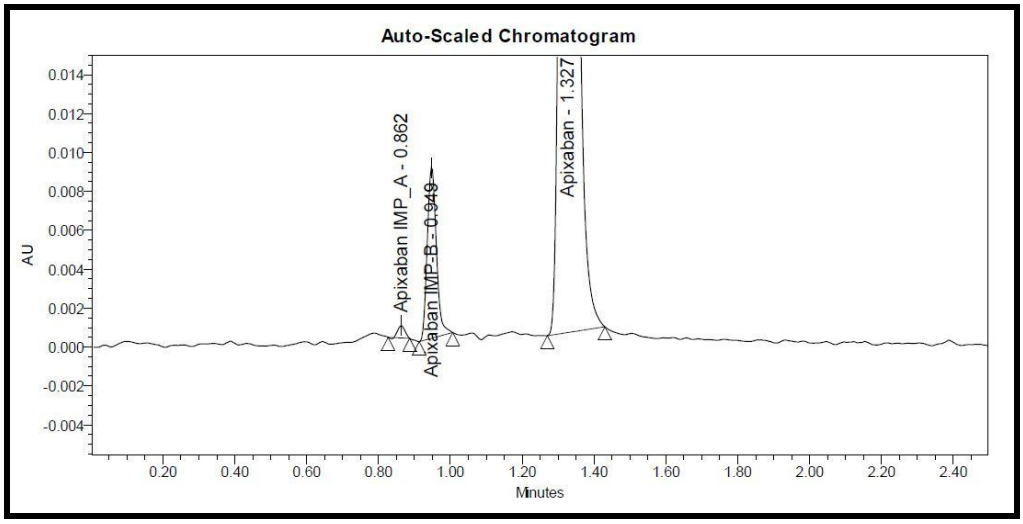


Fig. 12: Chromatogram of LOD study of APB, Imp-A & B.

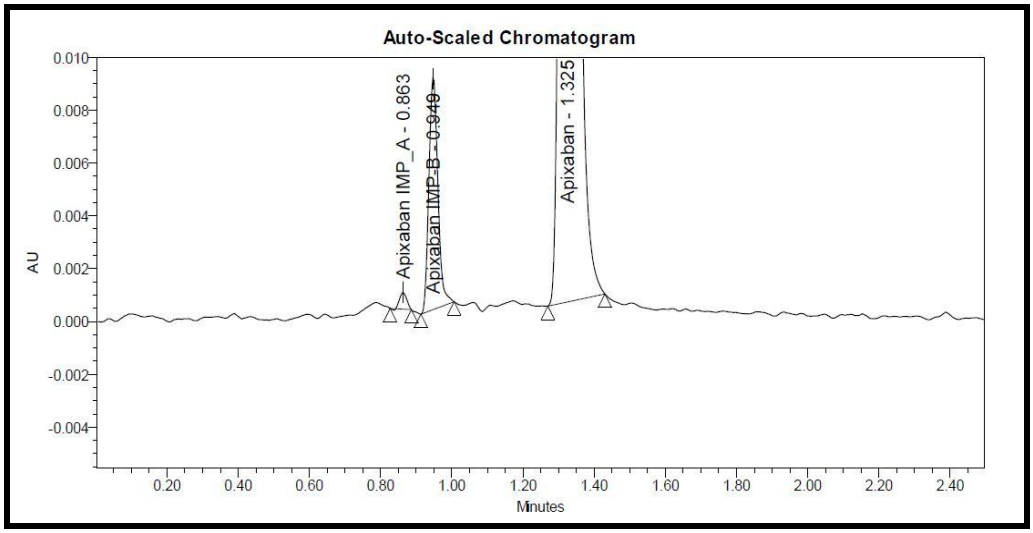


Fig. 13: Chromatogram of LOQ study of APB, Imp-A&B.

Specificity

A study to establish the interference of Blank and Placebo was conducted. Chromatograms of Blank and Placebo solutions show no peaks at the retention times of APB and its related impurities A and B. This indicates that the excipients used in the formulation do not interfere in the estimation of APB in the tablets.

The corresponding chromatogram of blank, and standard APB solution and standard APB solution spiked with impurities A and B are shown in Fig.14 to 16.

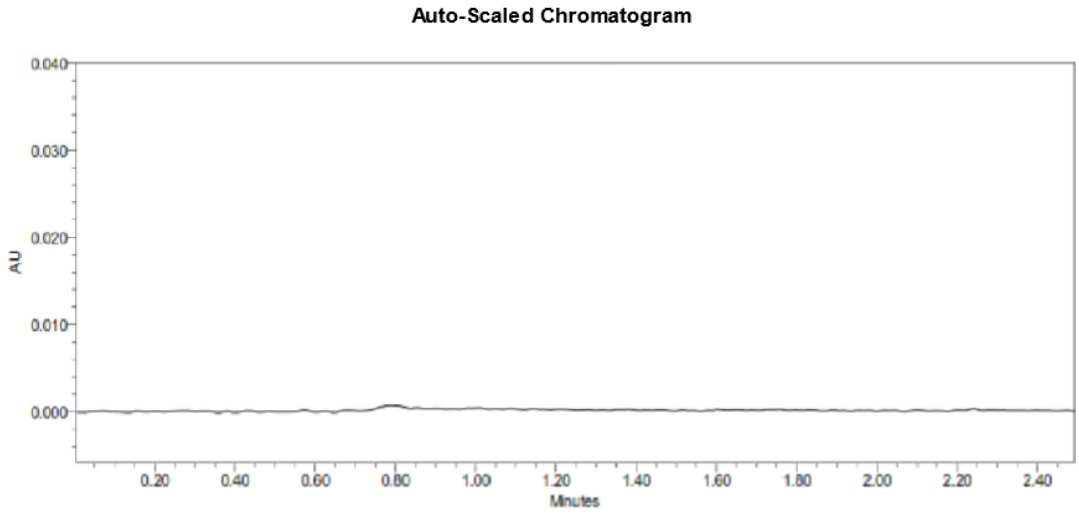


Fig.14: Chromatogram of Blank.

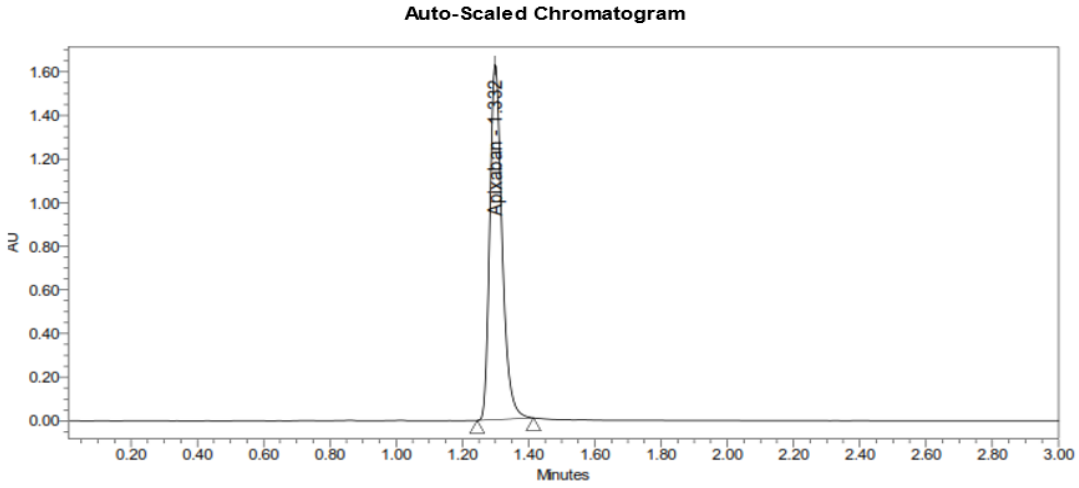


Fig. 15: Chromatogram of standard APB.

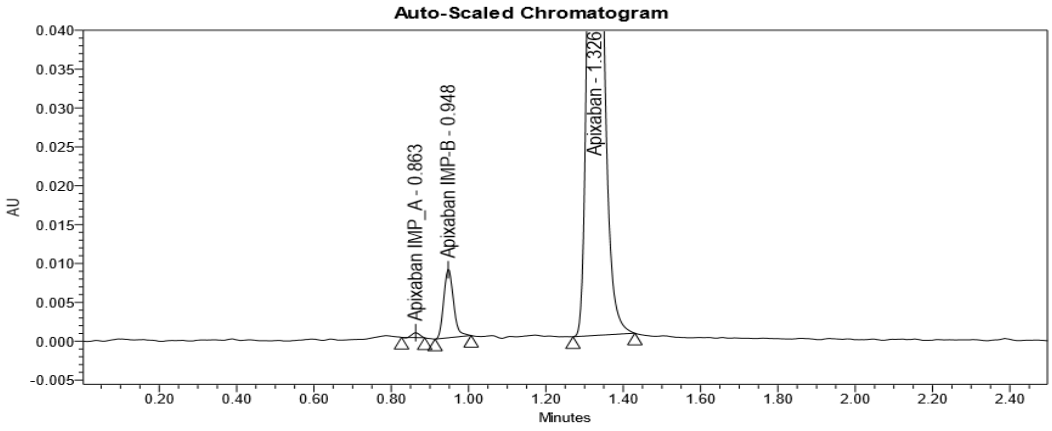


Fig. 16: Chromatogram of standard solution of APB spiked with Imp A and Imp B.

Robustness

The robustness is studied by the evaluating effects of small but the deliberate differences in method condition. The condition is variation in flow rate (± 0.03/min) and wavelength (altered by ± 3 wavelength). During all the different conditions of the test solution results wasn't effected & in the accordance with an actual one. The system suitability also found better; hence this method was conformed as robust. The chromatograms were obtained during the robustness were shown in the (Fig. 17-20).

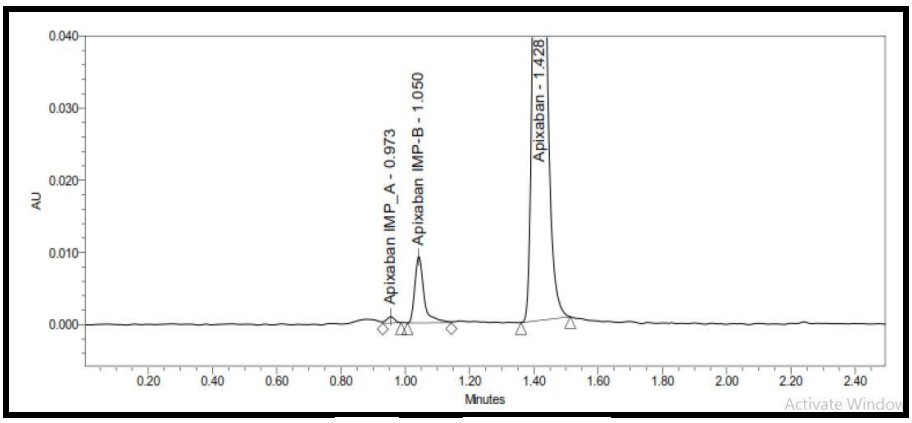


Fig. 17: Chromatogram of APB and Imp-A&B (0.27 mL/min flow rate).

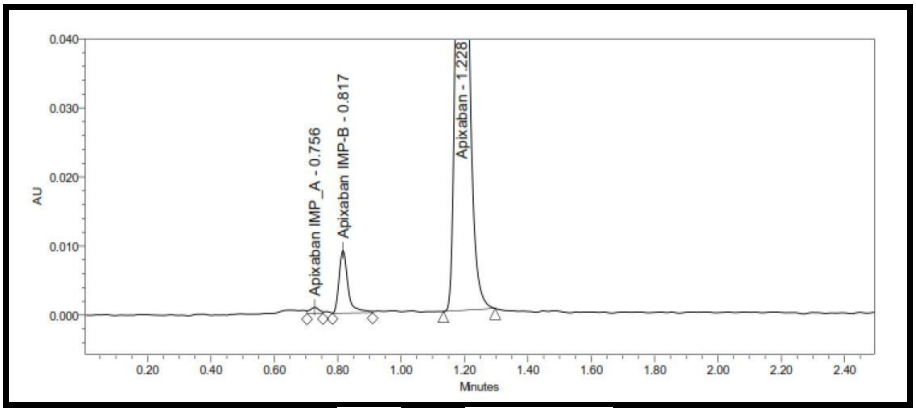


Fig. 18: Chromatogram of APB and Imp-A&B (0.33 mL/min flow rate).

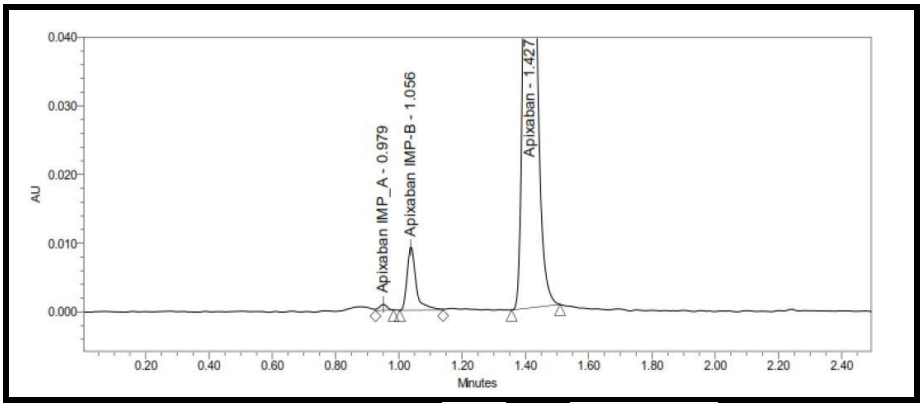


Fig. 19: Chromatogram of APB and Imp-A&B (217 nm).

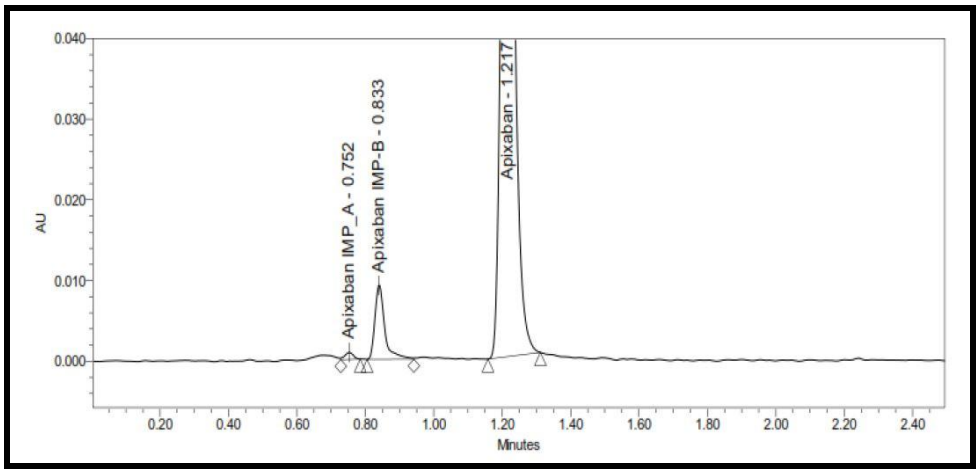


Fig. 20: Chromatogram of APB and Imp-A&B (223 nm).

Ruggedness

The ruggedness was studied by evaluating by different analysts but in the same chromatographic conditions. The results of ruggedness of developed method are started in the (Table 6). The results are shown during by different analysts but in the same chromatographic condition of the test solution wasn't affected & in the accordance with the actual. The suitability parameters are also been found good; hence this method was concluded as rugged.

Table 6: Results of Ruggedness data for APB and Imp- A, B.

		Imp. A	Imp. B	APB
	No. of Injections	Peak Areas		
Analyst-II	Injection-1	3268	18703	228674
	Injection-2	3296	18967	229834
	Injection-3	3257	18856	228436
	Injection-4	3286	18964	229463
	Injection-5	3296	18907	229867
	Injection-6	3260	18356	229765
	Average	3277.2	18792.17	229339
	Standard Deviation	17.73	234.75	629.03
	%RSD	0.54	1.24	0.27

Solution stability study

Sample Stability was evaluated at the ambient temp & analysis was done in initial time, after 3 hrs, 6 hrs, 12 hrs and 24 hrs. The analysis of the reports from all aged solutions was compared with those of from the freshly prepared solution (initial solution). The test solutions were stable upto the 24 hrs at the ambient temp, because difference in the measured & the original values were < 2.0 %.

Recovery Studies (Accuracy)

The recovery of APB and Imp-A & B was determined at three conc. levels. The % recoveries were shown in Table 7-9. The mean recoveries of APB, Imp A and Imp B from spiked samples were found to be in the range of 99.60-100.85 % for APB, 99.86-101.40 % for Imp A and 98.96-99.80 % for Imp B. The percentage recoveries were found to be within the limit indicates the accuracy of the method.

Table 7: Accuracy study Results of Apixaban.

%Concentration (at specification Level)	Peak Area	Amount Added (mg)	Amount Found (mg)	% Recovery	% Mean Recovery
50%-1	115573	15	15.16	101.07	100.85
50%-2	114599	15	15.08	100.55	
50%-3	115447	15	15.14	100.93	
100%-1	227420	30	29.99	99.96	99.98
100%-2	226696	30	29.93	99.76	
100%-3	227754	30	30.07	100.23	
150%-1	348990	45	44.88	99.74	
150%-2	347750	45	44.70	99.35	99.60
150%-3	349564	45	44.86	99.69	

Table 8: Accuracy Study results for Impurity A.

%Concentration (at specification Level)	Peak Area	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	% Mean Recovery
50%-1	1644	1.25	1.258	100.64	101.40%
50%-2	1667	1.25	1.275	102.05	
50%-3	1659	1.25	1.269	101.52	
100%-1	3264	2.5	2.49	99.60	99.86
100%-2	3285	2.5	2.51	100.40	
100%-3	3258	2.5	2.49	99.60	
150%-1	4916	3.75	3.76	100.26	100.88
150%-2	4953	3.75	3.79	101.07	
150%-3	4976	3.75	3.80	101.33	

Table 9: Accuracy Study results for Impurity B.

%Concentration (at specification Level)	Area	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	% Mean Recovery
50%-1	9483	1.25	1.249	99.92	99.53
50%-2	9428	1.25	1.241	99.28	
50%-3	9427	1.25	1.241	99.28	
100%-1	18946	2.5	2.473	98.99	98.96
100%-2	18923	2.5	2.471	98.87	
100%-3	18953	2.5	2.475	99.03	
150%-1	28639	3.75	3.77	100.53	99.80
150%-2	28396	3.75	3.70	98.86	
150%-3	28476	3.75	3.75	100.02	

Analysis of a commercial formulation

Experimentally the results for the amount of APB in tablets, expressed as a percentage of label claims were in good agreement with the label claims thereby suggesting that there is no interaction from the excipients which are commonly present in formulation of tablets. The results are shown in Table 10.

Table 10: Application of method in commercial formulation.

Formulation	Labeled Amount (mg)	Amount* Found (mg)	% Recovery ± SD
Eliquis Tablets	2.5	2.46	99.54 % ± 0.985

Forced Degradation study

For this study, apixaban tablet formulation spiked with impurity A and impurity B was subjected to various stress conditions like acid, alkali, oxidation, photolytic and thermal. The active pharmaceutical ingredient in the tablet formulation is well resolved from degradation products obtained during stress studies. The guidelines are expressed in ICH Q2A, Q3B, Q2B & FDA 21 CFR section of 211 all the required for development & for the validation of stability study.

Acid degradation

For acid degradation 10 tablets were accurately weighed and triturated to a fine powder. From this tablet powder equivalent to 10 mg of apixaban was accurately weighed, mixed with a 8.34 mg impurity A and impurity B and transferred into a 50 mL volumetric flask.

To it 3 mL of 1N hydrochloric acid was added and kept for 10 hrs at 60 ºC. After 10 hrs the solution was neutralized with 3 mL of 1N sodium hydroxide and the volume was made with the mobile phase, filtered and volume was made up with the same. From this 1.5 ml was transferred into 10 ml volumetric flask and diluted with mobile phase. In acid degradation study, it was found that the 9.56 % of the drug and 1.12 %, 2.01 % Imp A and Imp.B were degraded. The acid degradation chromatogram is shown in Fig.21.

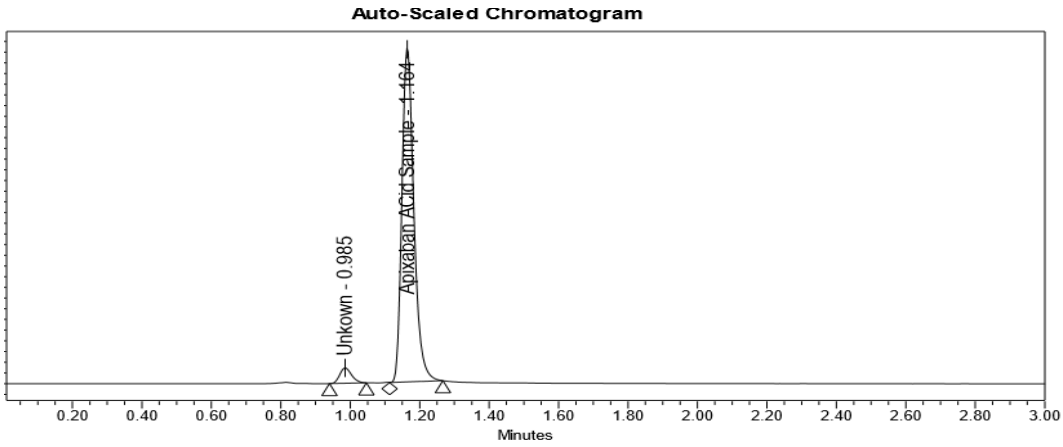


Fig. 21: Chromatogram of acidic forced degradation of APB, Imp-A&B.

Alkaline degradation

For alkaline degradation 10 tablets were accurately weighed and triturated to a fine powder. From this tablet powder equivalent to 10 mg of apixaban was accurately weighed, mixed with a 8.34 mg impurity A and impurity Band transferred into a 50 mL volumetric flask.

To it 3 mL of 1N sodium hydroxide was added and kept for 10 hrs at 60 ºC. After 10 hrs the solution was neutralized with 3 mL of 1N hydrochloric acid and the volume was made with the mobile phase, filtered and volume was made up with the same. From this 1.5 ml was transferred into 10 ml volumetric flask and diluted with mobile phase. In alkaline degradation study, it was found that the 8.04 % of the drug and 0.91 %, 6.17 % Imp A and Imp.B were degraded. The alkaline degradation chromatogram is shown in Fig.22.

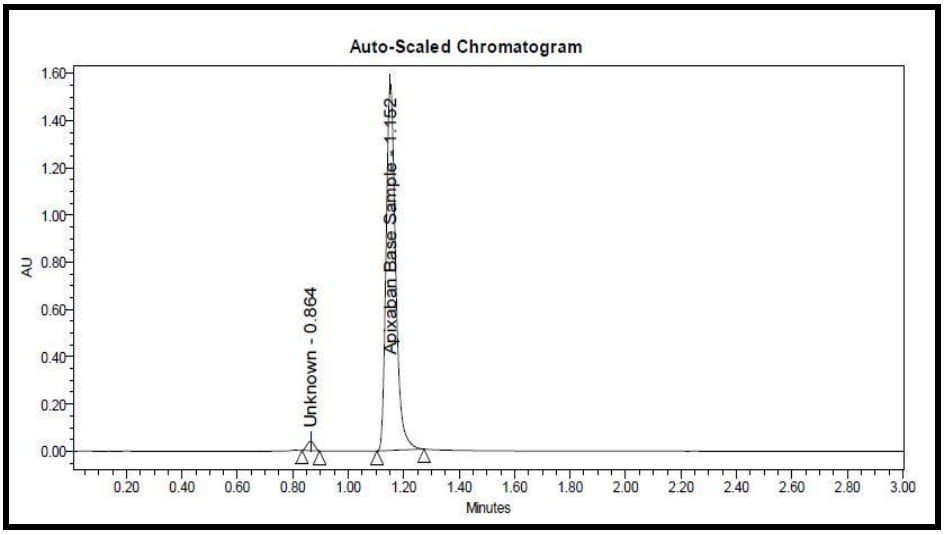


Fig. 22: Chromatogram of alkali forced degradation of APB, Imp-A&B.

Oxidative degradation

For oxidative degradation 10 tablets were accurately weighed and triturated to a fine powder. From this tablet powder equivalent to 10 mg of apixaban was accurately weighed, mixed with a 8.34 mg impurity A and impurity B and transferred into a 50 mL volumetric flask. To it 3 mL of 30 % v/v aqueous hydrogen peroxide was added and kept for 10 hrs. After 10 hrs the solution was filtered and volume was made up with the mark. From this 1.5 ml was transferred into 10 ml volumetric flask and diluted with mobile phase. In oxidative degradation study, it was found that the 2.83 % of the drug and 1.92 %,6.74 % Imp A and Imp.B were degraded. The oxidative degradation chromatogram is shown in Fig 23.

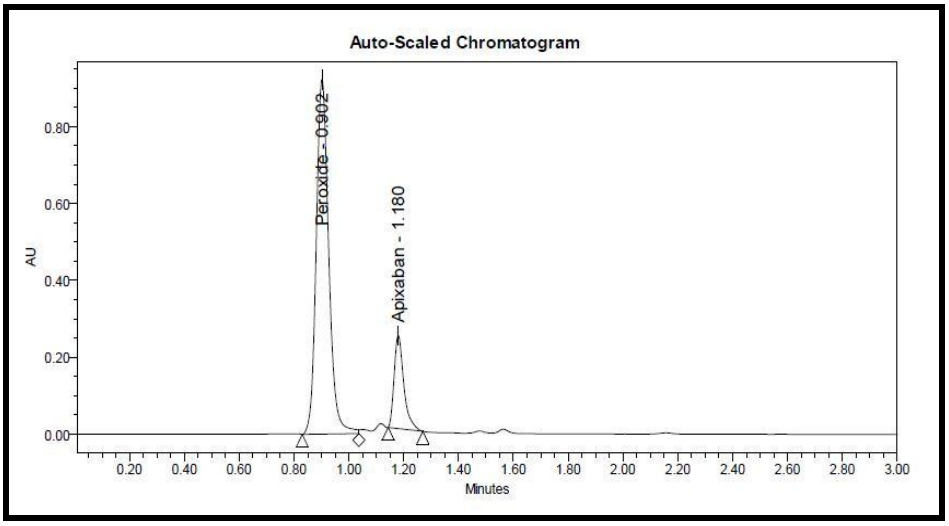


Fig. 23: Chromatogram of oxidative forced degradation of APB, Imp-A&B.

Photolytic degradation

For photolytic degradation 10 tablets were accurately weighed and triturated to a fine powder. From this, tablet powder equivalent to 10 mg apixaban was accurately weighed and transferred into a petri dish and mixed with 8.34 mg each of impurity A and impurity B. The drug was exposed at 1.2 million lux hr for 7 days. The exposed drug was dissolved in mobile phase, filtered and diluted upto the mark. In photolytic degradation study, it was found that the 4.46 % of the drug and 1.60 %, 7.21 % Imp A and Imp.B were degraded. The chromatogram is shown in Fig. 24.

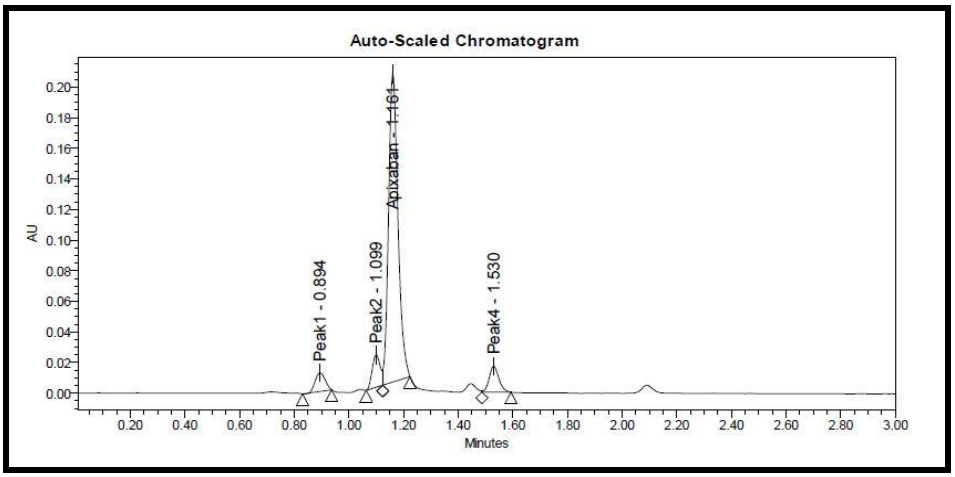


Fig. 24: Chromatogram of UV-light degradation of APB, Imp-A&B.

Thermal degradation

For Thermal degradation 10 tablets were accurately weighed and triturated to a fine powder. From this, tablet powder equivalent to 10 mg apixaban was accurately weighed and transferred into a petri dish and mixed with 8.34 mg each of impurity A and impurity B. The mixture exposed at constant temperature of 80°C for 60 min by keeping it in an oven. The exposed drug was dissolved in mobile phase, filtered and volume made upto the mark with mobile phase. In thermal degradation study, it was found that the 1.05 % of the drug and 2.20 %, 12.41 % Imp A and Imp. B were degraded. The chromatogram is shown in Fig.25.

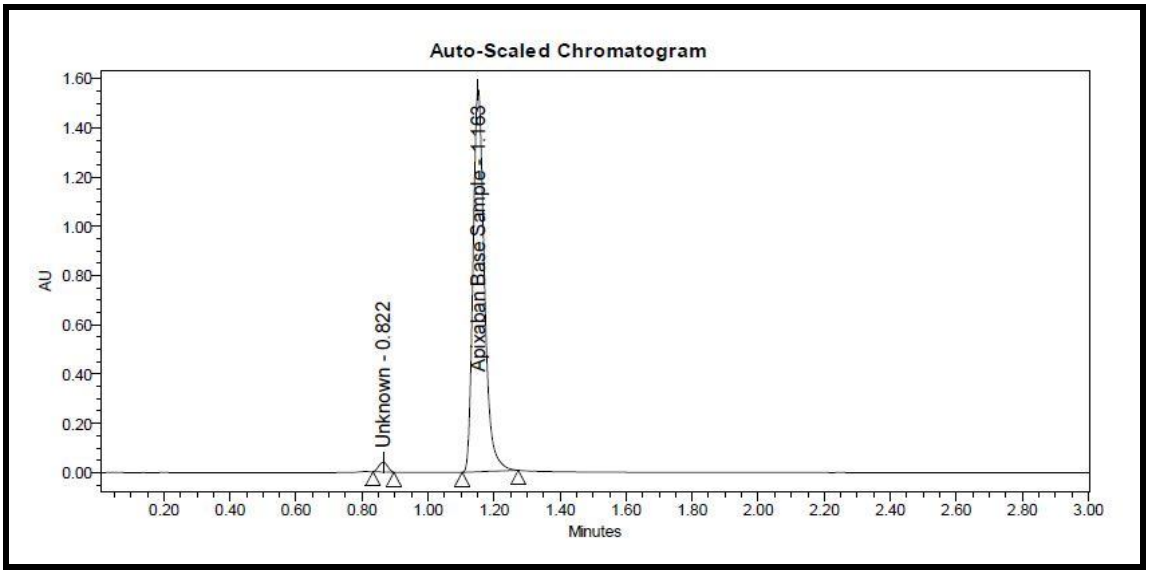


Fig. 25: Chromatogram of thermal degradation of APB along with, Imp-A&B.

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CONCLUSION

A new RP-UPLC stability indicating method for Apixaban and its related substances is developed for analysis in pharmaceutical formulation. Satisfactory results are obtained from validation of the method. The run time (3 min) enabled rapid determination of APB along with RS. This method exhibited an excellent performance in terms of sensitivity and speed. This stability-indicating method can be applied for the routine analysis of production samples and to check the stability of APB along with selected related substances in bulk drug and formulation. Moreover, it can be applied for determination of assay, blend uniformity, content uniformity and in vitro dissolutions of pharmaceutical products, where sample load is higher and high throughput is essential for faster delivery of results.

COMPETING INTERESTS

The author declares that he has no competing interest.

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