INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN:2320-2831

IJPAR |Vol.6 | Issue 2 | April - June -2017

Journal Home page: www.ijpar.com

Research article *Open Access*

Stress degradation studies and development of validated stability indicating assay method by RP-HPLC for estimation of Apixaban in presence of degradation products as per ICH guidelines

M.Madan Mohan Reddy[1,2]*, D.Gowri Sankar[2], JVLN Seshagiri Rao[2]

[1]*Director, Quality & Regulatory Affairs ,Eisai Pharmaceuticals India Pvt. Ltd, Ramky Pharma City(SEZ), Parawada, Visakhapatnam, Andhra Pradesh, India. Pin code: 531019*

[2]*Department of Pharmaceutical Analysis, College of Pharmaceutical Sciences, Visakhapatnam, Andhra University, Andhra Pradesh, India. Pin Code: 530003*

***Corresponding Author: M.Madan Mohan Reddy**

ABSTRACT

A simple, selective, reproducible, economic, stability indicating method has been developed and validated as per ICH guidelines for the estimation of Apixaban (APB) both in bulk drug and in solid dosage forms in the presence of degradation products. Reversed-phase chromatography was performed on a Inertsil ODS (4.6 x 150mm, 5µm) with mobile phase Octo sulphonic acid adjusted with Orthophosphoric acid to pH 3.0 with 300 ml (30%) and 700 ml of Acetonitrile HPLC (70%).at a flow rate of 1.0 ml min^{-1}. Detection was performed at 226 nm and a sharp peak was obtained for APB at a retention time of 2.235 minutes. Linear regression analysis data for the calibration plot showed there was a good linear relationship between response and concentration in the range 10-50 ppm; the regression coefficient was 0.9997 and the Limit of detection (LOD) was 0.33 µg/mL with S/N ratio of 2.99 and limits of quantification (LOQ) was 30 µg/mL with S/N ratio of 9.97 respectively. In order to determine whether the analytical method and assay were stability-indicating, APB 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 it did not interfere with excepients 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 imply the method is suitable for routine quantification of APB with high precision and accuracy.

Keywords: Apixaban, stability Indicating, Forced Degradation studies, RP-HPLC-PDA, Method validation.

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 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-HPLC 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-HPLC method for the determination of process and degradation related impurities of Apixaban. The developed method was successfully validated according to the USP 1225 Validation of Compendial Procedures and ICH guidelines [9-13].

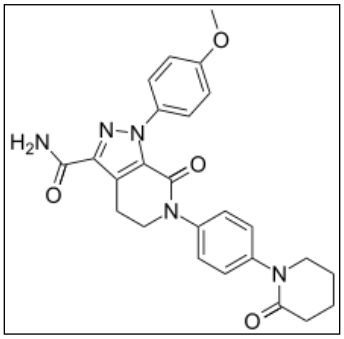


Figure 1: Structure of Apixaban

MATERIALS AND METHODS

Experimental

Materials and methods

Pure sample of Apixaban (APB) was obtained from Eisai Pharmaceuticals and other reagents such as Acetonitrile, Methanol, Ortho phosphoric acid, KH_2PO_4 and water used were of HPLC and milli-Q grade water. 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) High Performance liquid chromatography Waters auto sampler–PDA detector by using, Empower-software version-2, analytical balance (MettlerToledo) UV/Visible-Detector (Standard cell) and data handling system (Autochrome-3000), pH meter (lab India) and Sonicator. The column used is Inertsil ODS (4.6 x 150mm, 5µm) with the flow rate 1 ml/min (isocratic).

Preparation of 0.1% Octa sulphonic acid (pH 3.0)

Accurately weighed 1 grams of Octa sulphonic acid was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.0 with Orthophosphoric acid.

Diluent (Mobile phase) Preparation

Accurately measured 300 ml (30%) of above buffer and 700 ml of Acetonitrile HPLC (70%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Preparation of blank solution

The Mobile phase was used as the diluent. This prepared solution was used as mobile phase. This solution was also used for specificity blank solution.

Preparation of Placebo Solution

The placebo Solution was prepared by Dissolving the Specified amount Excipients in diluent(in house made).

Preparation of Standard solution

Accurately weigh and transfer 10mg of Apixaban working standard into a 50ml clean dry volumetric flask add about 30ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. (30ppm of Apixaban)

Preparation of Test solution

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10mg Apixaban (marketed formulation=240.4mg of tablet Powder) sample into a 50ml clean dry volumetric flask add about 30 ml of Diluent and sonicate it up to 30 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.44 micron Injection filter. (Stock solution)

Further pipette 1.5 ml of Apixaban from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. (30ppm of Apixaban)

Procedure

Inject 10 µL of the standard, sample into the chromatographic system and measure the areas for Apixaban peaks and calculate the %Assay by using the formulae.

Optimization of HPLC Method

The HPLC method was optimized and developed method for APB. The mixed standard solution was injected in HPLC by the following chromatographic conditions. The chromatographic separation was achieved on is Inertsil ODS (4.6 x 150mm, 5µm) with the flow rate 1 ml/min Isocratic mode and the Mobile phase consists of Octo sulphonic acid adjusted with Orthophosphoric acid to pH 3.0 with 300 ml (30%) and 700 ml of Acetonitrile HPLC (70%) was used throughout the analysis and the flow rate of mobile phase was 1ml/min, Run time was 10 min. the column temperature was maintained at Ambient temperature ,volume of injection loop was 10µl.Detection was monitored at 226 nm.(Table 1).

Method validation

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

Linearity and range

Linearity of the method was studied by the injecting the mixed standard solutions with the concentration ranges from linear over the concentration range of 10ppm-50 ppm drug respectively and the correlation coefficient range was found drug levels of target concentrations were prepared and injected six times into the HPLC system keeping the constant injection volume. The peak areas were plotted against the concentrations to obtain the linearity graphs.

Precision

The precision of the optimized method was evaluated by carrying out six independent assays of test sample. %RSD of six assay values was calculated. Intermediate precision was carried out the samples by using another instrument and with different analyst.

Limit of Detection and Quantification

The LOD and LOQ procedures were performed on samples contain very lower concentrations of analytes under the ICH guidelines. By applying the visual evaluation method, LOD was expressed by establishing the lowest concentration at which the analyte can be detected. LOQ was considered as the lowest concentration of analytes that can be detected and quantified, with acceptable accuracy and precision.

Robustness

Robustness was studied by evaluating the effect of small variations in the chromatographic conditions. The conditions studied were flow rate altered by ±0.1ml/min, mobile phase composition with methanol ±5ml. These chromatographic variations are evaluated for Apixaban (APB).

System suitability

The system suitability parameters with respect of tailing factor, theoretical plates, repeatability and resolution between Apixaban (APB).peaks were defined.

Specificity

The specificity of the analytical method is the ability of the method to estimate the analyte response in the presence of additional components such as impurities, degradation products and matrix. The peak purity of Apixaban (APB).were assessed by comparing the Retention time of standard Apixaban (APB). good correlation was obtained between the Retention time of standard and sample of Apixaban(APB).The specificity method was also evaluated to ensure that there were no interference products resulting from forced degradation studies.

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).

The Stability indicating study of Apixaban (APB).undergoes acid, alkali and oxidation degradation, photolysis and heat condition. Placebo Interference: The placebo (in the present of excipients in tablet) sample were prepared as per the test method and analyzed in the HPLC. It expressed there is no additional peaks at the retention time of Apixaban (APB).in the chromatograph it indicates that there is no placebo interference.

Acid Degradation

Sample was treated with 3ml of 1N hydrochloric acid and kept for 10hrs. After 10hrs the solution was neutralized with 3ml of 1N sodium hydroxide, made the volume upto the mark with mobile phase and analyzed using HPLC.

Alkali Degradation

Sample was treated with 3ml of 1N sodium hydroxide and kept for 10hr. After 10hr the solution was neutralized with 3ml of 1N hydrochloric acid, made the volume up to the mark with mobile phase and analyzed using HPLC.

Oxidative Degradation

APB were mixed with 3mL of 30%v/v aqueous hydrogen peroxide solution and kept for 10hrs. After 10hrs made the volume up to the mark with mobile phase and analyzed using HPLC.

Photolytic & Thermal Degradation

The samples were kept under UV light for different time intervals (15minutes – 7days) and made the volume upto the mark with mobile phase and analyzed using HPLC. Thermal Degradation Samples were heated at 800 C for 15minutes - 60minutes and 2000 C for 2-5minutes and analyzed.

Accuracy

Accuracy was carried out by applying the method to drug sample APB of tablet to which known amounts of APB was added. Standard powder corresponding to 50,100 and 150% of label claim was added, mixed and the powder was extracted and determined by the system in optimized mobile phase. The experiment was performed in triplicate and percentage recovery, % RSD was calculated.

Analysis of marketed formulation

The marketed formulation was assayed by above description. The peak areas were monitored at 226 nm and determination of sample concentrations were using by multilevel calibration

developed on the same HPLC system under the same conditions using linear regression analyzed for APB in the same way as described above.

RESULTS AND DISCUSSIONS

The estimation of APB was done by RP-HPLC and in the optimized method the mobile phase consists of Octo sulphonic acid adjusted with Orthophosphoric acid to pH 3.0 with 300 ml (30%) and 700 ml of Acetonitrile HPLC (70%).Then finally filtered using 0.45µ membrane filter paper and degassed in sonicator for 15minutes. The detection is carried out using PDA detector at 226 nm.The solutions are flowing at the constant flow rate of 1.0 ml/min.The retention time for APB was 2.235 minutes respectively. The method was validated in terms of linearity, precision, accuracy, and specificity, limit of detection and limit of quantitation. The method calibration curves were found to be linear over the concentration range of 10-50 µg/ml for APB respectively and the correlation coefficient range was found to be (r^2 = 0.9997). The percentage recovery 100.97 to 99.60 % of APB respectively from solid dosage form .Limit of detection (LOD) was 0.33 µg/mL with S/N ratio of 2.99 and limits of quantification (LOQ) was 30 µg/mL with S/N ratio of 9.97. All the parameters value of RSD is less than 2.0% indicating the accuracy and precision of the method.

Method Development and Optimization

The HPLC 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. It was found that mobile phase consists of Octo sulphonic acid adjusted with Orthophosphoric acid to pH 3.0 with 300 ml (30%) and 700 ml of Acetonitrile HPLC (70%) and given acceptable retention time 2.235 minutes for APB , the theoretical plates, and good resolution for APB at the flow rate of 1.0ml/min **(Table.1; Fig. 2 & 3).**

Table 1: Optimized Chromatographic Conditions

Parameters	*Method*
Stationary phase (column)	Inertsil ODS (4.6 x 150mm, 5µm)
Mobile Phase	30% buffer 70% Acetonitrile(30:70 v/v)
pH	3.0
Flow rate (ml/min)	1ml/min
Run time (minutes)	10 minutes
Column temperature (°C)	Ambient
Volume of injection loop (µl)	10µl
Detection wavelength (nm)	226 nm
Drug RT (min)	2.235 minutes

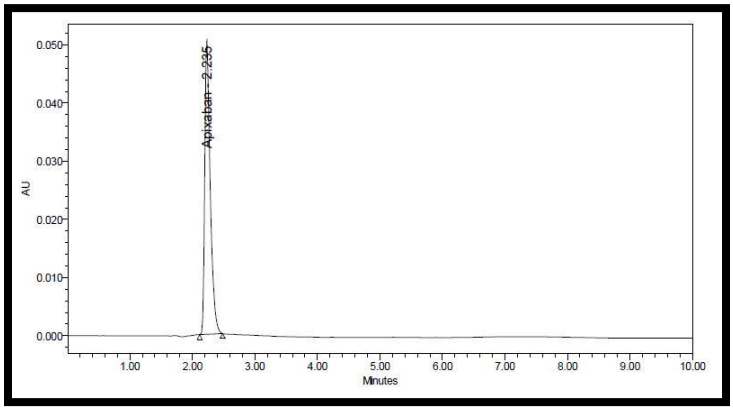


Fig. 2: Chromatogram of APB at 226 nm from bulk drug

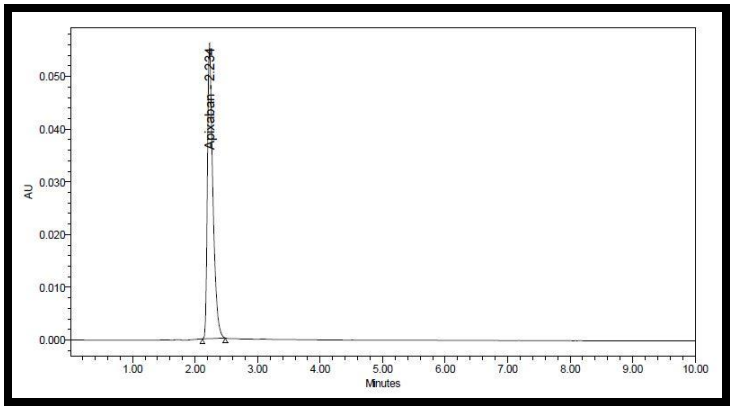
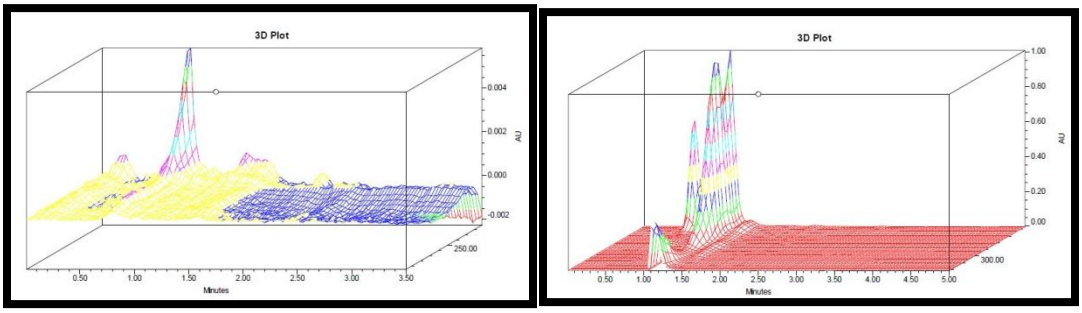
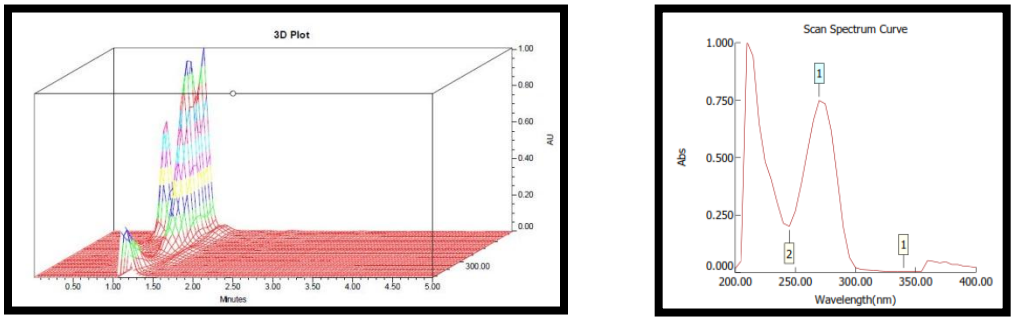


Fig. 3: Chromatogram of APB at 226 nm from pharmaceutical tablet formulation



1. Blank

2. Standard APB



3. Sample APB

4. UV Graph of APB

Fig. 3.1: 3D Chromatogram plots for APB by PDA detector

VALIDATION OF DEVELOPED METHOD

Linearity

The linearity five levels of concentrations with correlation regression curves are obtained and the conc. range of 10-50 µg/mL for APB. The reports of drug were found to be linear in prepared concn range & a correlation regression equation of APB was y =11612X+1000 with correlation coefficient 0.999 (Figure 4) Where X was the conc of the drug in µg/ml & Y was area of the peak in the absorbance unit. The chromatograms were obtained during the linearity were shown in the Figure 6-13 & Table 2.

Table 2: Linearity study of APB

Linearity level	**APB**	
	Conc. (µg/ml)	***Mean Area***
1	10	117116
2	20	234231
3	30	351347
4	40	458463
5	50	585578
Correlation co-efficient		0.999
Slope		11612
Intercept		1000

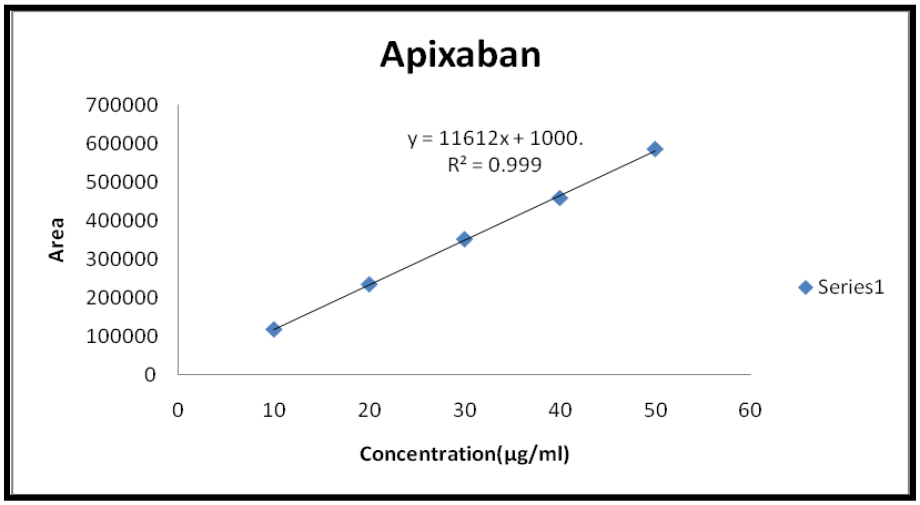


Figure 4: Linearity curve for standard APB

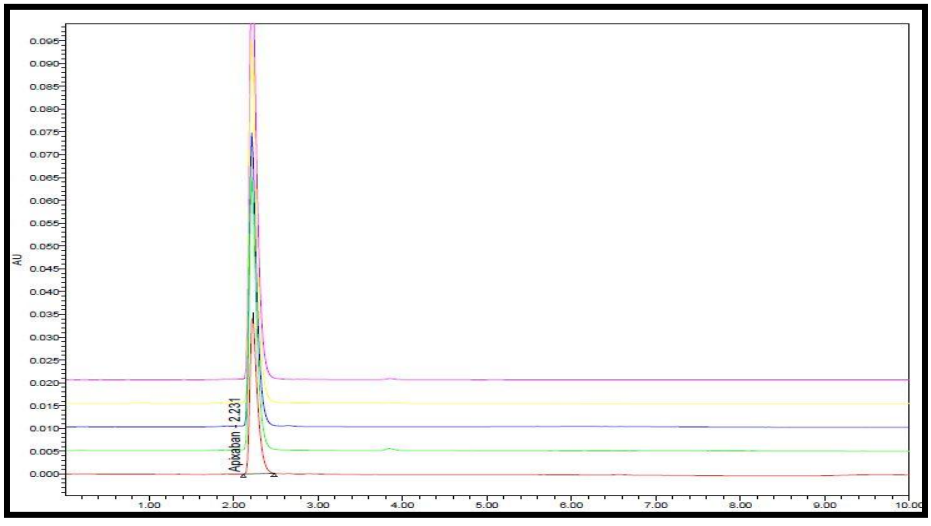


Figure 5: Overlay linearity Chromatogram for APB

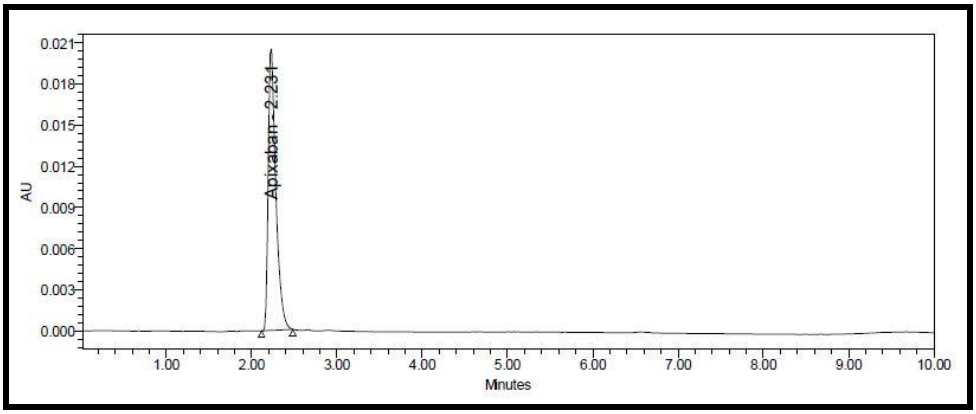


Figure 6: Linearity chromatogram for level-1 APB

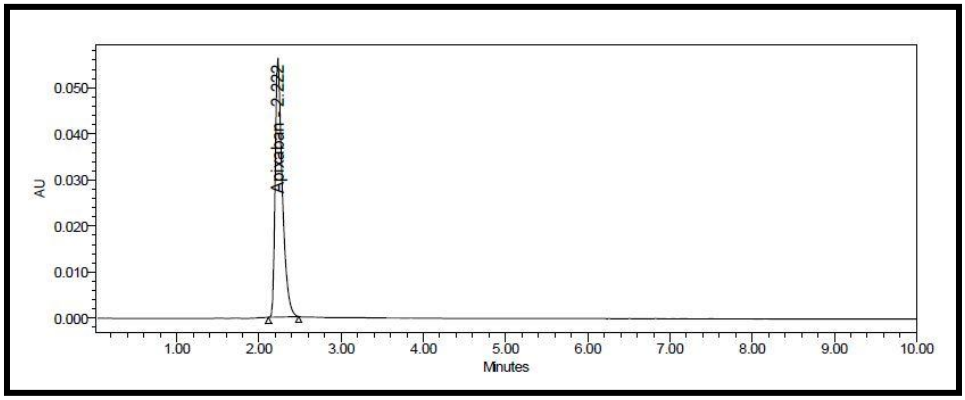


Figure 7: Linearity chromatogram for level-2 APB

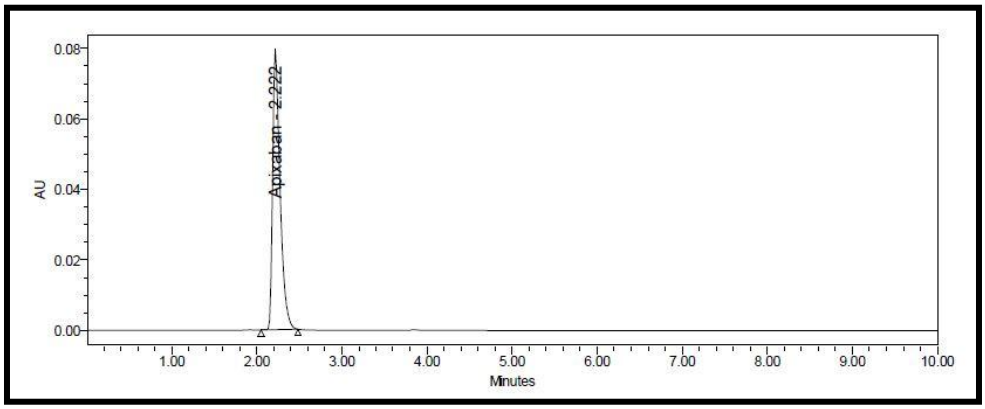


Figure 8: Linearity chromatogram for level-3 APB

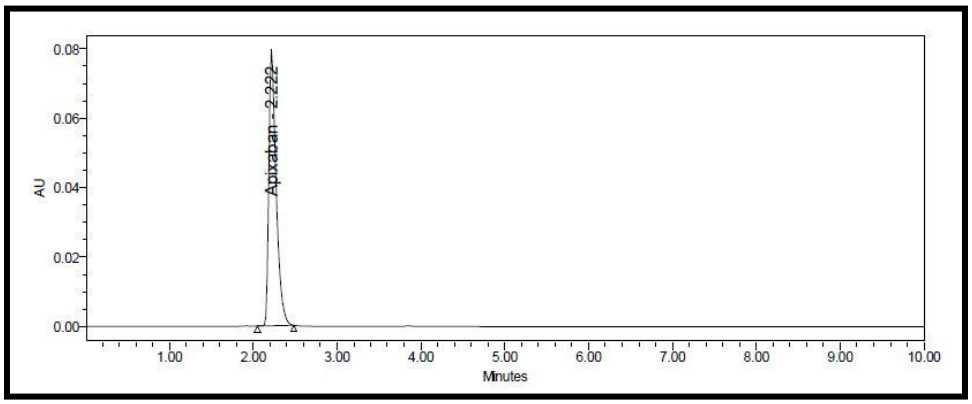


Figure 9: Linearity chromatogram for level-4 APB

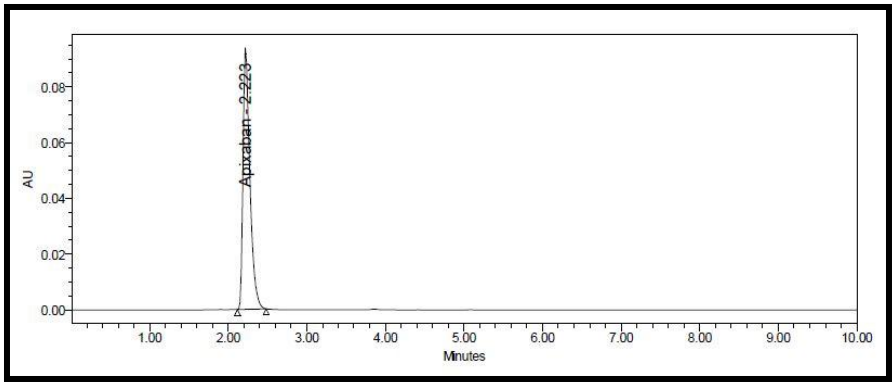


Figure 10: Linearity chromatogram for level-5 APB

Precision

Precision of this analysis, as the intraday precision was evaluated by performing five individual test samples prepared & calculated the % RSD. Interday precision of this method was analyzed by performing same the procedure with the various days by the person with the same developed environment. The % RSD values of the intra-day precision & interday precision study was < 2.0% for APB. This is confirmed that method was precise and overlain chromatogram (Figure 11) and Resulting data of precision was given in the (Table 3).

Table 3: Precision study of APB

Replicate	***Area of APB***	
	Intra-day precision	***Inter-day precision***
1	347358	349537
2	345898	342874
3	349624	348593
4	351347	345487
5	345567	340784
6	349045	345292
Mean	341839.8	345427.8
St. dev.	2261.2	3317.0
% RSD	0.6	1.0

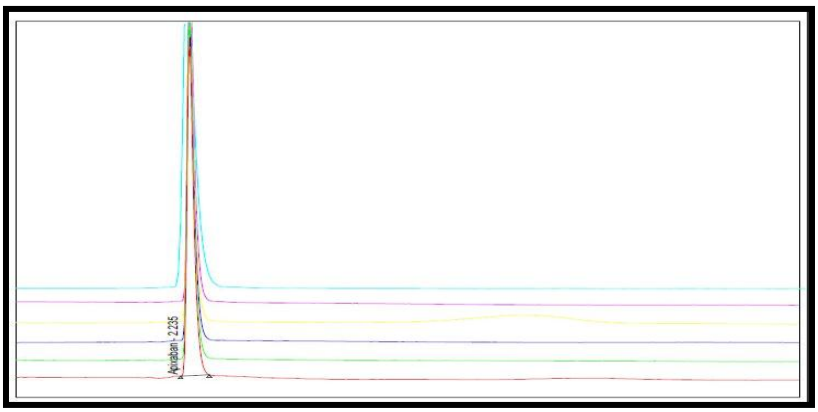


Figure 11: Overlay precision Chromatogram for APB

LOD and LOQ

Limit of detection (LOD) & the limit of quantifications (LOQ) are evaluated by the serial dilutions of APB 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 were found to be 0.10 µg /mL & the LOQ value 0.33 µg/mL respectively. The chromatogram of the LOD and LOQ were shown in the (Figure 12-13).

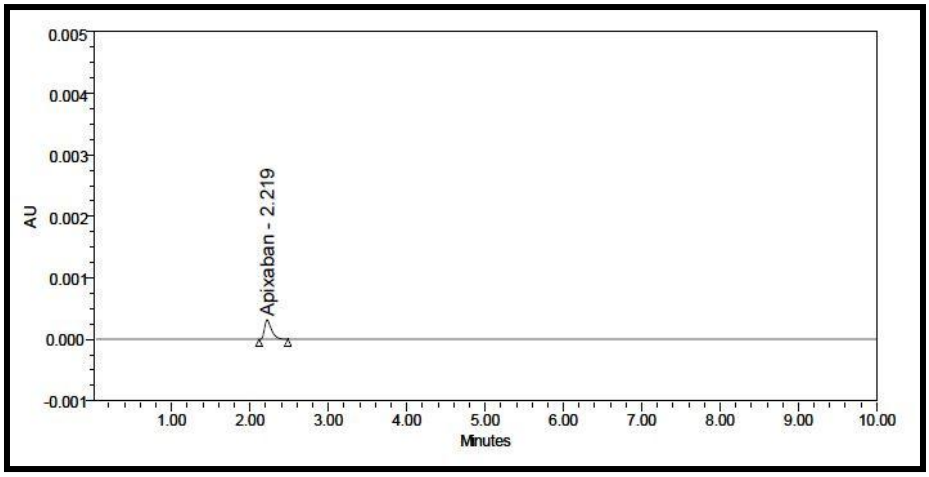


Figure 12: Chromatogram of LOD study of APB

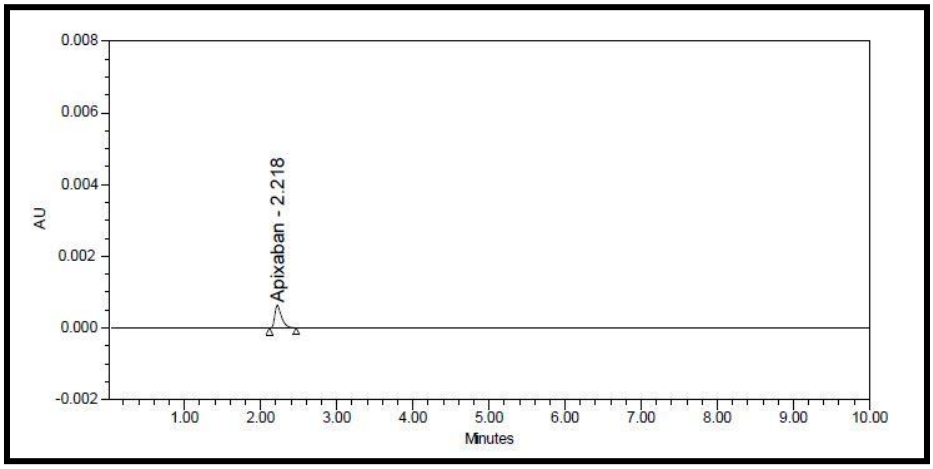


Figure 13: Chromatogram of LOQ study of APB

Specificity

The specificity is a method for drug establishing by the verifying for the interferences with drug quantification from degradation products are formed during forced degradation study and peak purity for APB was found better under the various conditions. There were no other interferences of any other peaks degradated product with the drug peaks.

Table 4: System suitability parameters for APB

System suitability parameters	APB
Retention time (min)	1.788
Repeatability of retention time; %R.S.D (n=5)	0.01
Repeatability of peak area;	0.7

%R.S.D= (S.D./Mean)×100	
Resolution (Rs)	-
Tailing factor (asymmetric factor)	1.53
USP plate count	3248.37
LOD (µg/mL)	0.33
LOQ (µg/mL)	30

Robustness

The robustness is studied by the evaluating effects of small but the deliberate differences in method condition. The condition is Flow rate (± 0.1/min) and MP composition (altered by ± 10 % organic solvent using 40:60 and 20:80 v/v buffers: methanol). The results of robustness for developed methods were started in the (Table 5). The results are shown during all the different conditions of the test solution wasn't affective & in the accordance with an actual one. The suitability also found better; hence this method was conformed as robust. The chromatograms were obtained during the robustness were shown in the (Figure 14-17).

Table 5: Evaluation data of Robustness study of APB

Parameters		Adjusted to	USP Plate Count	USP Tailing
APB	**Flow Rate As per method 1.0ml/min**	**0.8 ml/min**	3639.37	1.55
		***1.0 ml/min**	3248.37	1.53
		1.1 ml/min	3386.38	1.54
	Mobile Phase (30:70) (Buffer:Acetonitrile)	**10% Less**	3674.67	1.55
		****30:70**	3248.37	1.53
		10% More	3465.33	1.53

***** Results for actual flow (1.0ml/min) have been considered from Assay standard.

****** Results for actual Mobile phase composition Buffer (30%) and Acetonitrile (70%) have been considered from Accuracy standard.

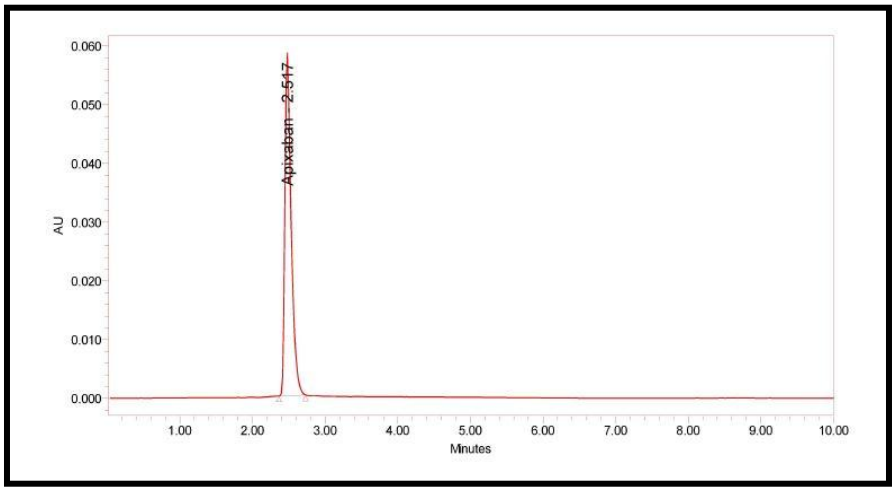


Figure 14: Chromatogram of APB (0.8 ml/min flow rate)

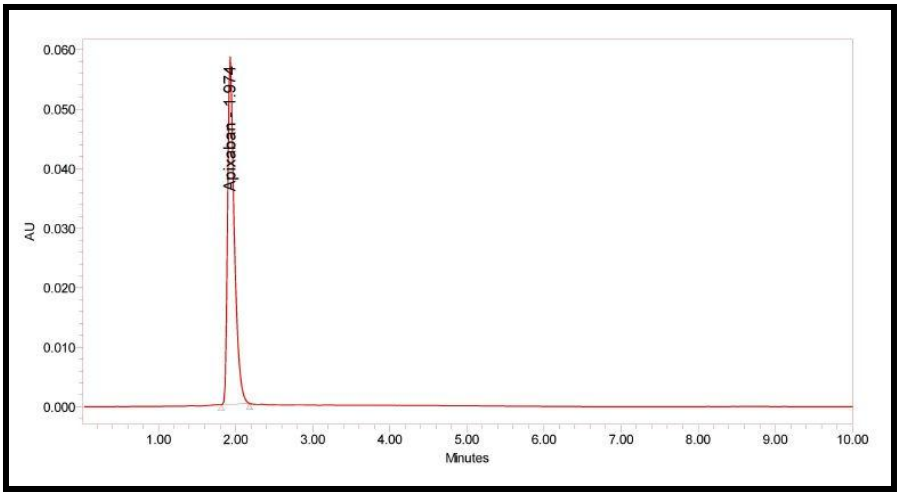


Figure 15: Chromatogram of APB (1.1 ml/min flow rate)

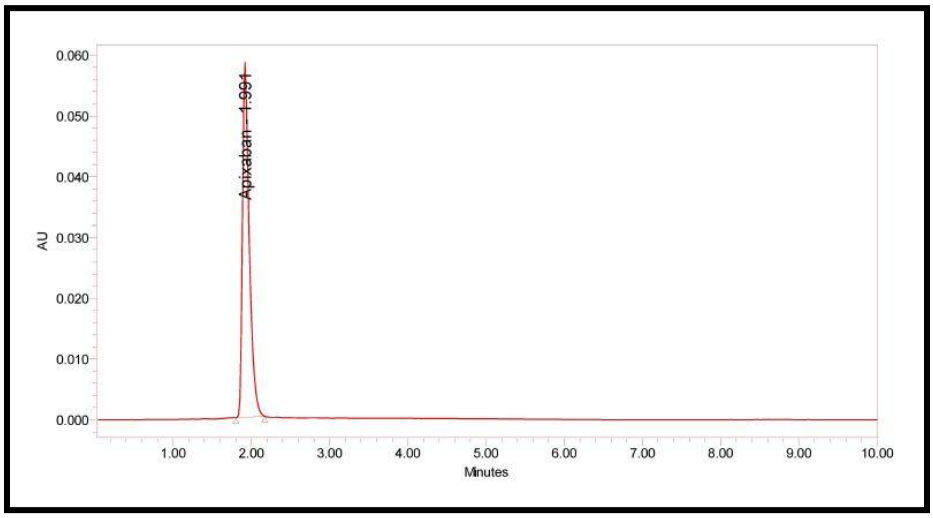


Figure 16: Chromatogram of APB [Buffer: Acetonitrile (40:60v/v)

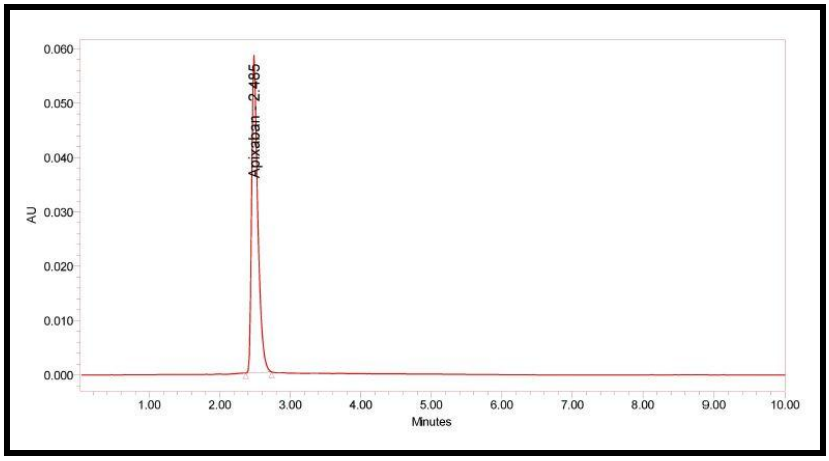


Figure 17: Chromatogram of APB [Buffer: Acetonitrile (20:80v/v)

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. Chromatograms are obtained during ruggedness was shown in the (Figure 18-23)

Table 6: Evaluation data of Ruggedness study of APB

ID Precisions	No. of Injections	APB	
		Peak Area	**RT**
	1	349537	2.234
ID Precision - 1			
	2	342874	2.235
	3	348593	2.235
	1	345487	2.236
ID Precision - 2			
	2	340784	2.238
	3	345292	2.239
MEAN		345427.8	
STDEV		3317.0	
% RSD		1.0	

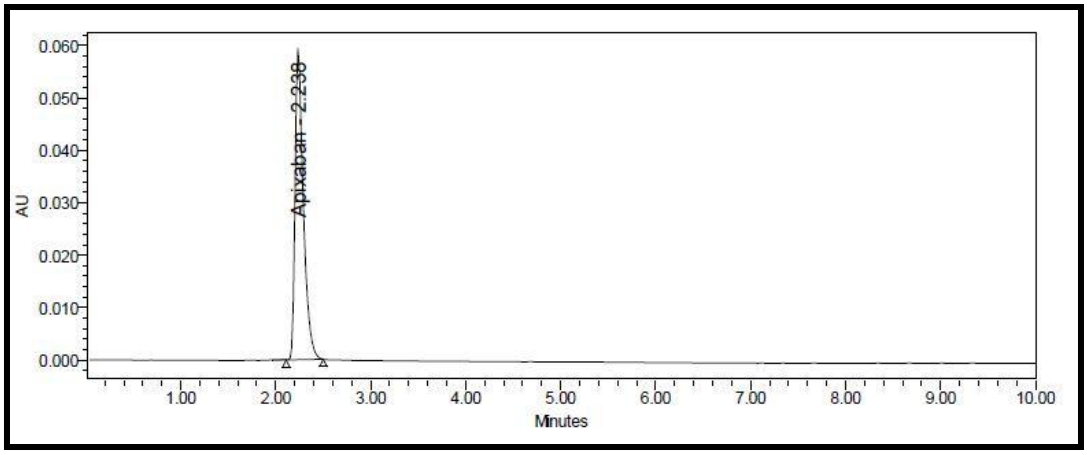


Figure 18: Chromatogram of APB [ID Precision-1 (Injection-1)

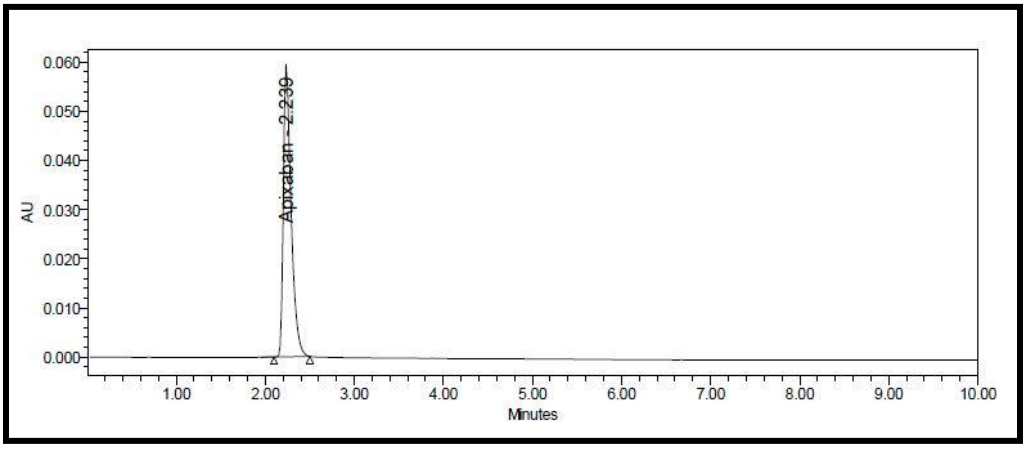


Figure 19: Chromatogram of APB [ID Precision-1 (Injection-2)

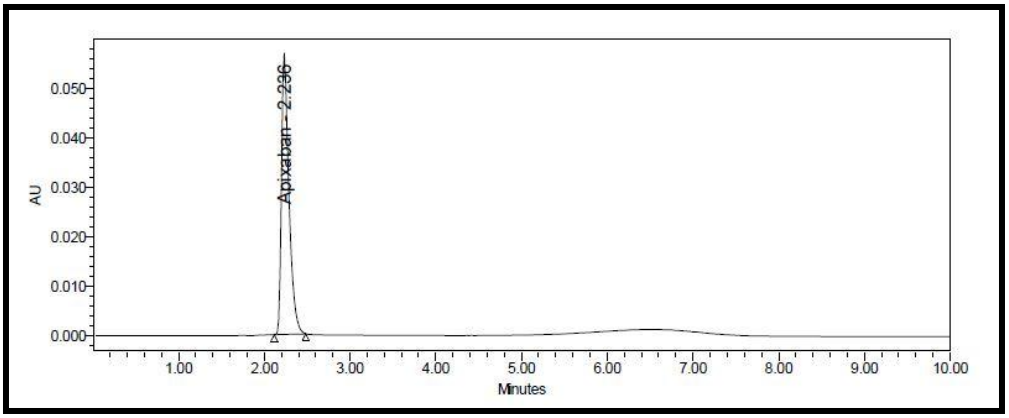


Figure 20: Chromatogram of APB [ID Precision-1 (Injection-3)

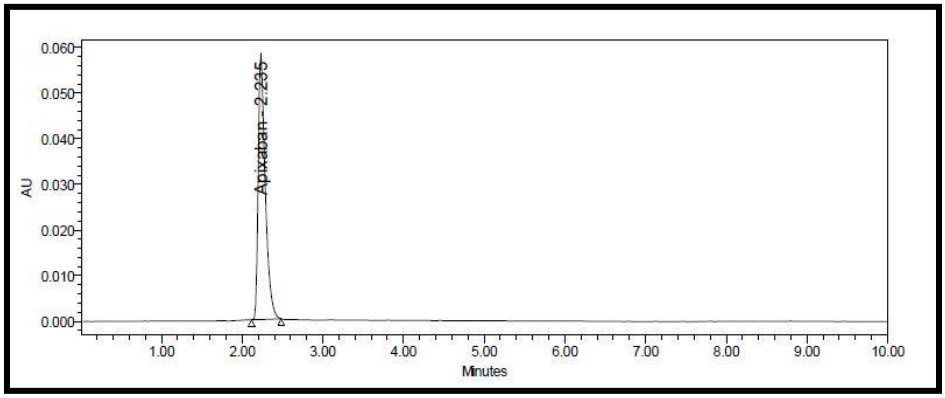


Figure 21: Chromatogram of APB [ID Precision-2 (Injection-1)]

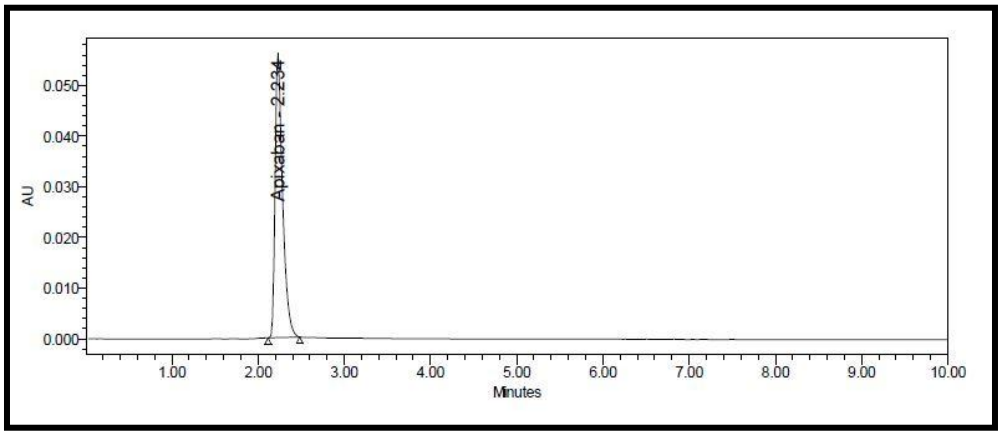


Figure 22: Chromatogram of APB [ID Precision-2 (Injection-2)]

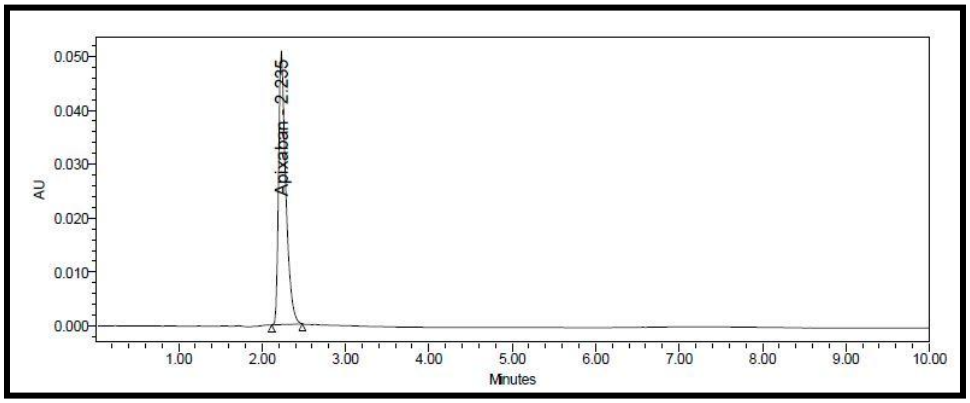


Figure 23: Chromatogram of APB [ID Precision-2 (Injection-3)]

Solution stability study

Sample Stability was evaluated by shorting at the ambient temp & analysis was done in initial time, after 3hrs, 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). (Table 7- 8) shows results are obtained the stability of solution study at various intervals for a test preparations and it was conformed that the test solutions were stable upto the 24hrs at the ambient temp, because difference in the measured & the original values were < 2.0 %

Table 7: Evaluation of solution stability for APB

Apixaban(APB)					
S. No	**Standard Area (Mean*3)**	**Standard area after 24hrs (Mean*3)**	**Sample area after 24hrs (Mean*3)**	**% Variation**	**% Assay**
1	346744	342122	343722	1.3 & 0.9	100.31

Recovery Studies (Accuracy)

The recovery of APB was determined by the 3 various conc. levels. % recovery was found to be % (Table 9). The results are indicating that this method was accurate. Chromatograms obtained during the study of accuracy were shown in (Figure 22-25).

Table 9: Accuracy study Results of APB

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%-1	175573	5	5.1	101.07	100.87
50%-2	174599	5	5.03	100.55	
50%-3	175447	5	5.05	100.99	
100%-1	347420	10	10.00	99.99	99.96
100%-2	346696	10	9.98	99.79	
100%-3	347754	10	10.01	100.09	
150%-1	518990	15	14.96	99.74	
150%-2	517750	15	14.90	99.35	99.60
150%-3	519564	15	14.95	99.69	

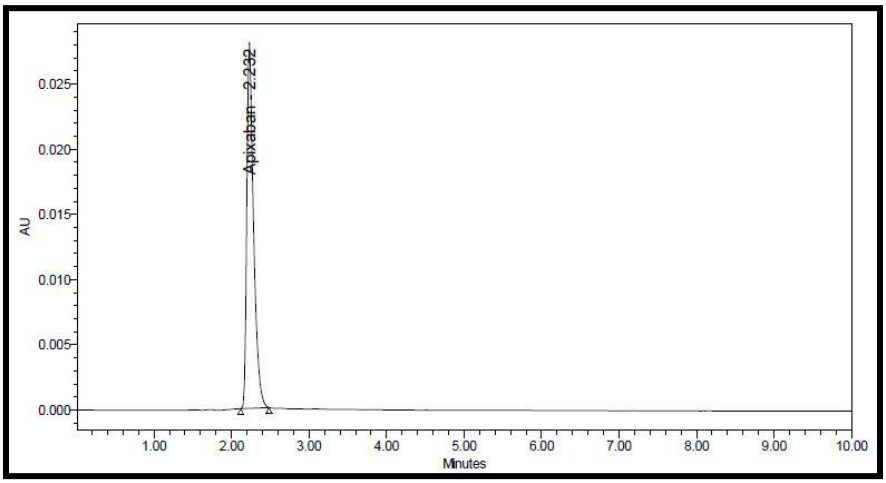


Figure 23: Accuracy chromatogram for APB level-1 (50%)

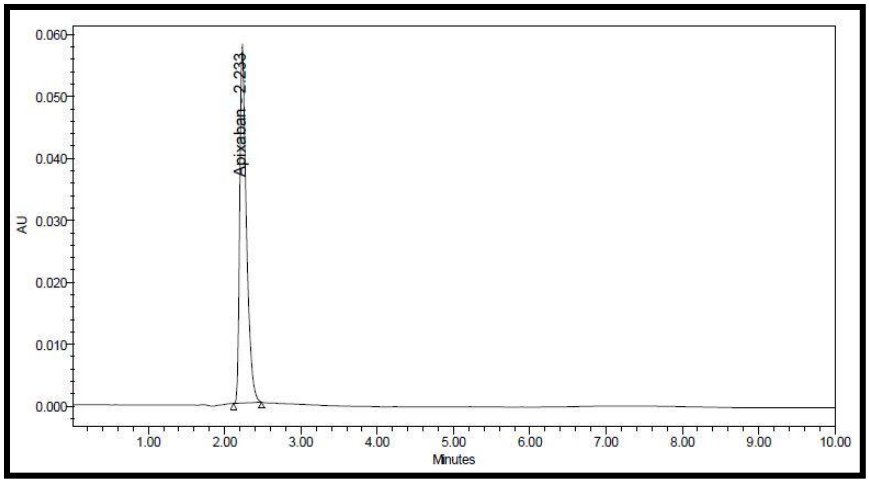


Figure 24: Accuracy chromatogram for APB level-2 (100%)

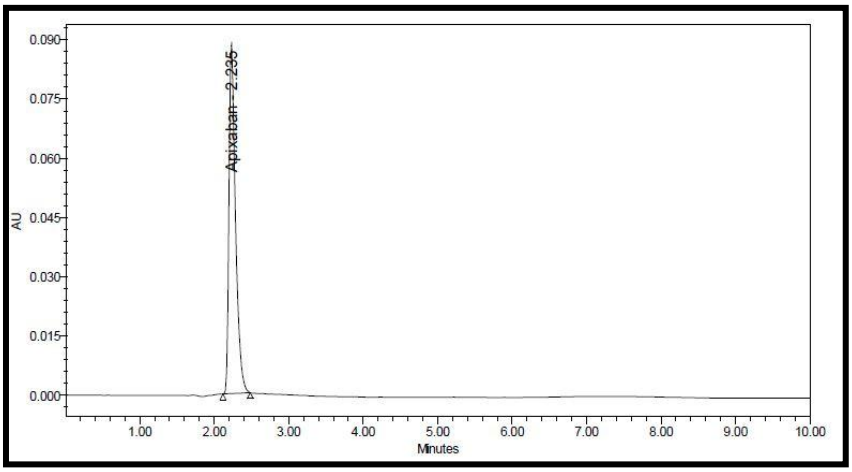


Figure 25: Accuracy chromatogram for APB level-3 (150%)

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.

Forced Degradation study

In a order to the determine whether the analytical methods were stable. APB dosage form have been stress on the different conditions to applied degradation 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.

Preparation of stock

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10mg Apixaban (marketed formulation=240.4mg of tablet Powder) sample into a 50ml clean dry volumetric flask add about 30 ml of Diluent and sonicate it up to 30 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.44 micron Injection filter. (Stock solution).

Acid degradation study

The Acid degradation was done for sample and it was treated with 3ml of 1N hydrochloric acid and kept for 10hrs at 60ºC. After 10hrs the solution was neutralized with 3ml of 1N sodium hydroxide, made the volume up to the mark with mobile phase and analyzed using HPLC. The degrading drug content was found for APB up to 8.62 % in the acidic condition (Figure 26-28 & Table 10, 11).

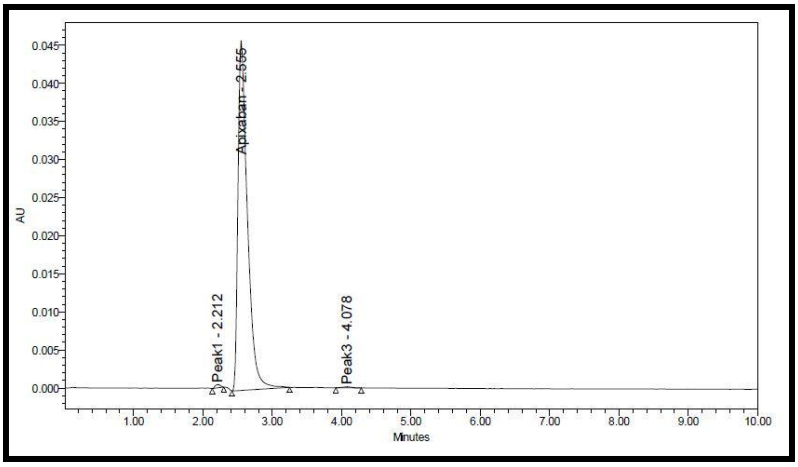


Figure 26: Chromatogram of acidic forced degradation of APB

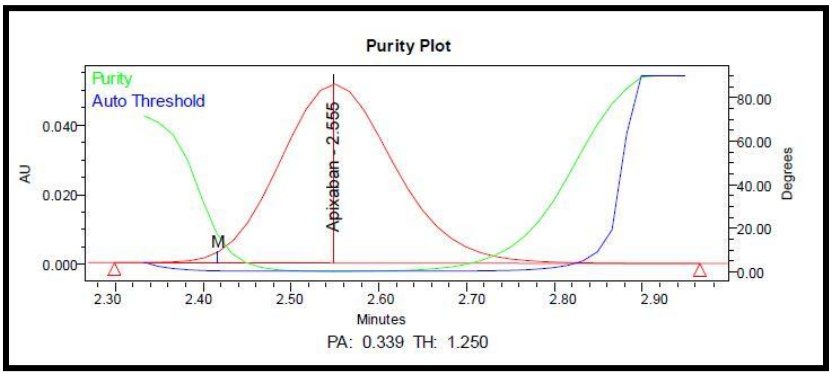


Figure 27: Purity Plot for APB in acidic forced degradation

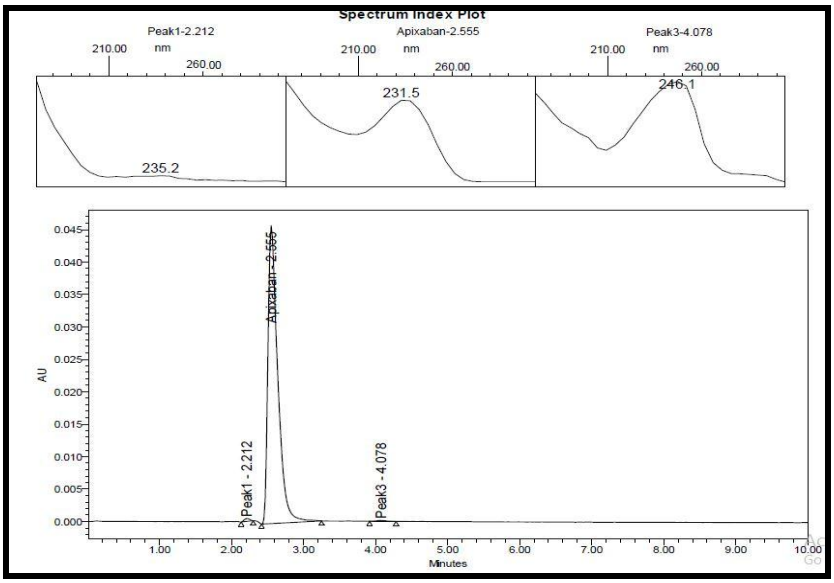


Figure 28: Spectrum index for APB in acidic forced degradation

Alkaline degradation

The Alkaline degradation was done by sample was treated with 3ml of 1N sodium hydroxide and kept the sample for 10hr. After 10hr solution was neutralized to add 3ml of 1N hydrochloric acid, made the volume up to the mark with mobile phase and analyzed using HPLC. In alkali degradation study, it was found for APB to be 2.36% of the degraded drug (Figure 30-32 & Table 10, 11).

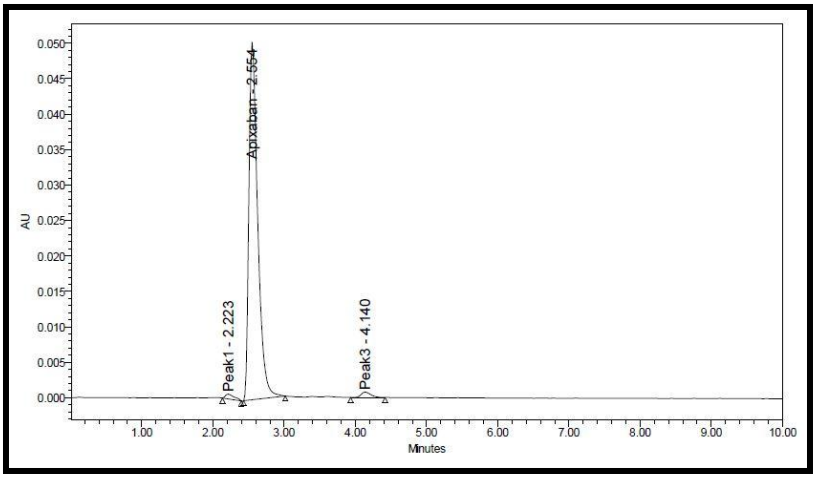


Figure 30: Chromatogram of alkali forced degradation of APB

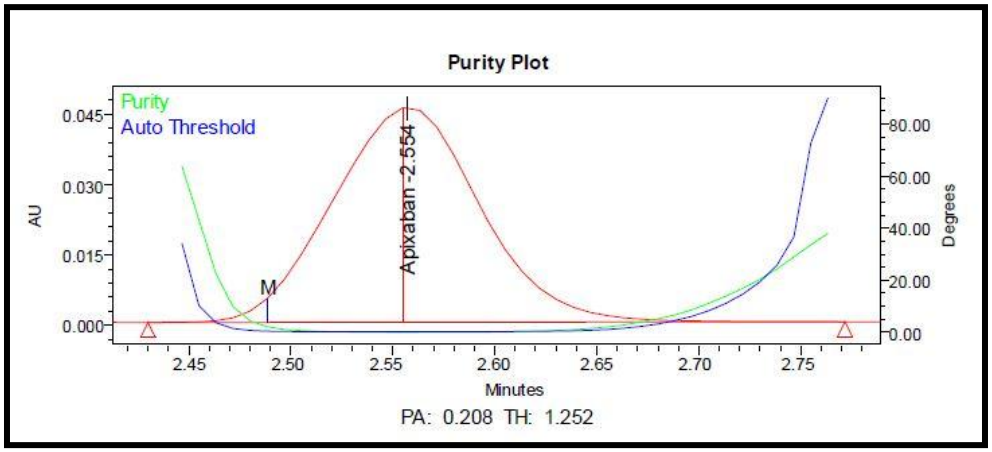


Figure 31: Purity Plot for APB in alkali forced degradation

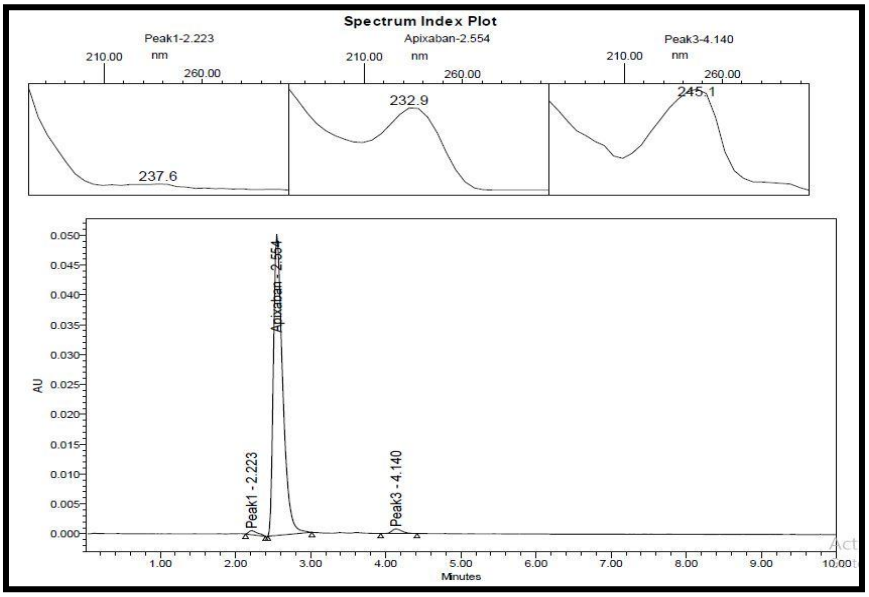


Figure 32: Spectrum index Plot for APB in Base forced degradation

Oxidative degradation

The oxidative degradation was done by sample was mixed with 3mL of 30%v/v aqueous hydrogen peroxide solution and kept for 10hrs. After 10hrs made the volume upto the mark with mobile phase and analyzed using HPLC. In oxidative degradation, it was found for APB to be 6.33% of the degraded drug (Figure 34-36 & Table 10, 11).

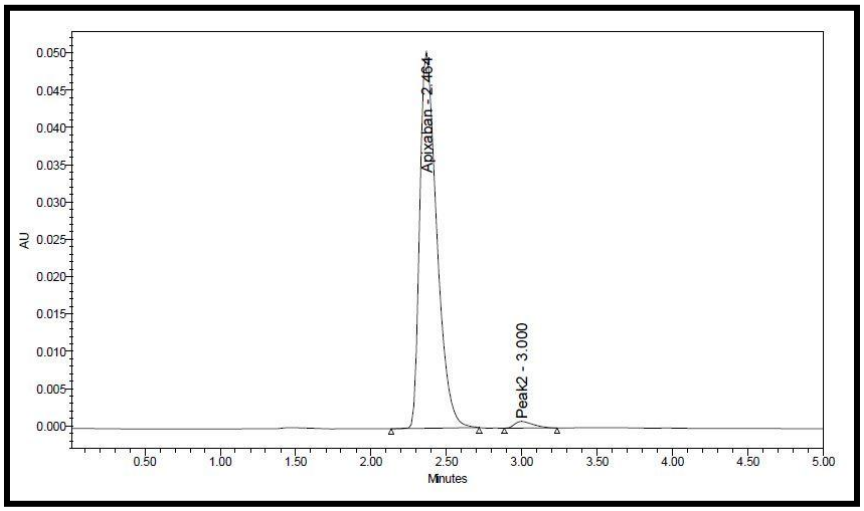


Figure 34: Chromatogram of oxidative forced degradation of APB

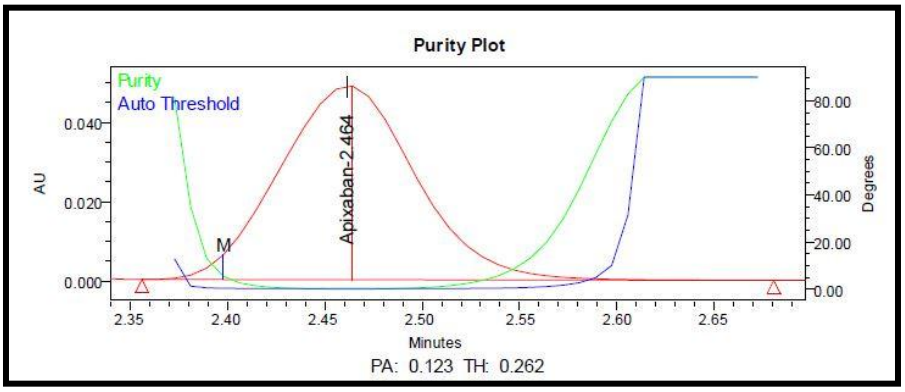


Figure 35: Purity Plot for APB in oxidative forced degradation

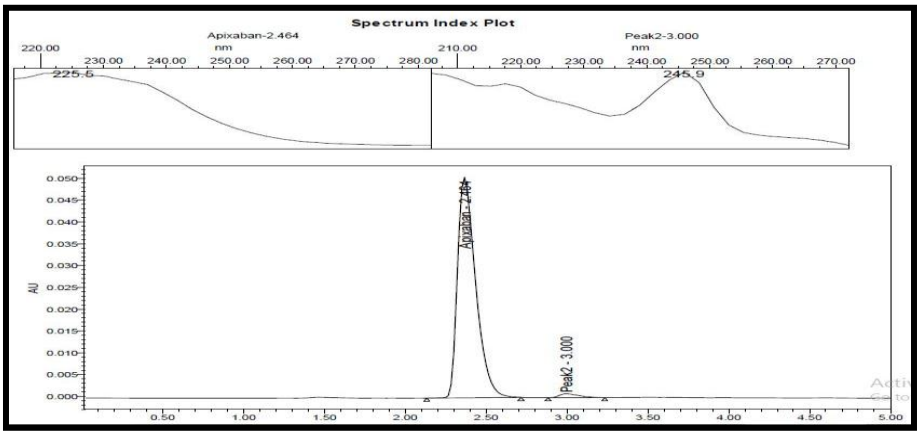


Figure 36: Spectrum index for APB in oxidative forced degradation

Photolytic degradation

The photolytic degradation was done by exposing of drug content under the UV light for 15minutes to 7days. There is for APB to be 3.46 % of the drug degradation observed in the above specific photolytic degradation condition (Figure 37-39 & Table 10, 11).

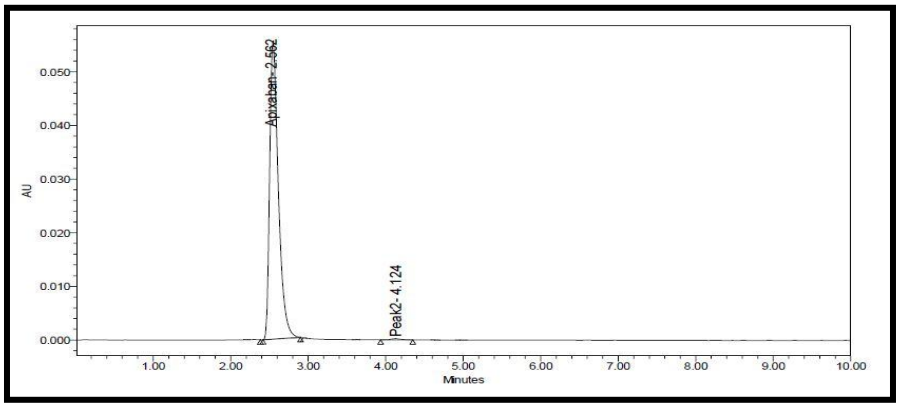


Figure 37: Chromatogram of UV-light degradation of APB

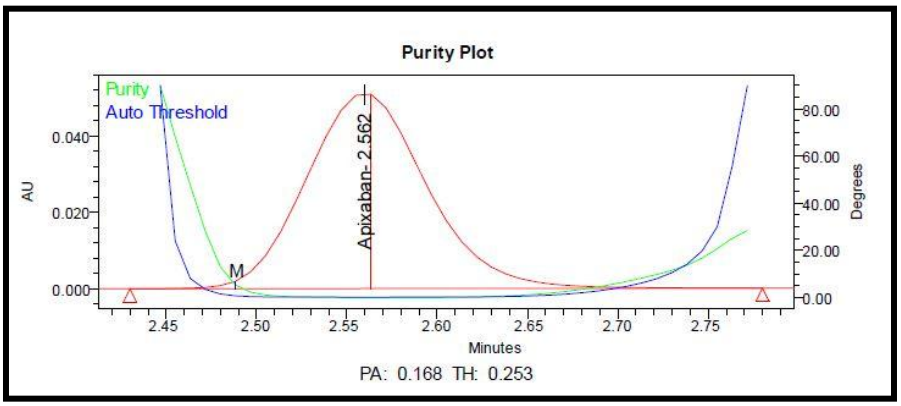


Figure 38: Purity Plot for APB in UV-light degradation

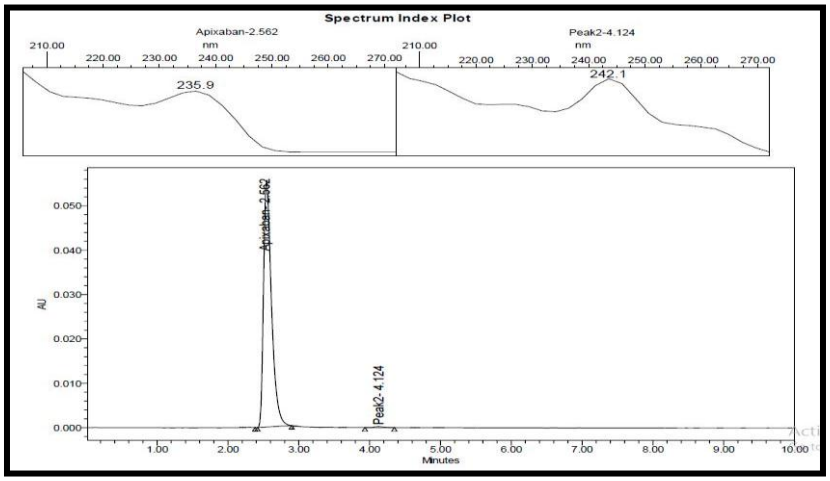


Figure 39: Spectrum index for APB in Photolytic forced degradation

Thermal degradation

The Thermal degradation is to be performing by the exposing the solid drug at the 80°C for 15minutes to 60minutes and at 220°C for 2-5minutes. Resultant chromatogram of thermal degradation study (Figure 40-42 & Table 10, 11) was indicates that the drug was found to be slightly stable under thermal condition. It was only for APB to be 1.67 % of the drug content was degraded.

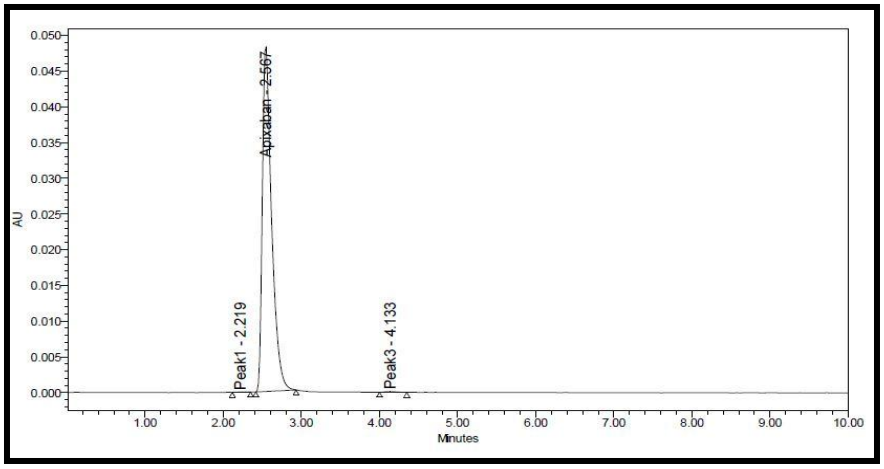


Figure 40: Chromatogram of thermal degradation of APB

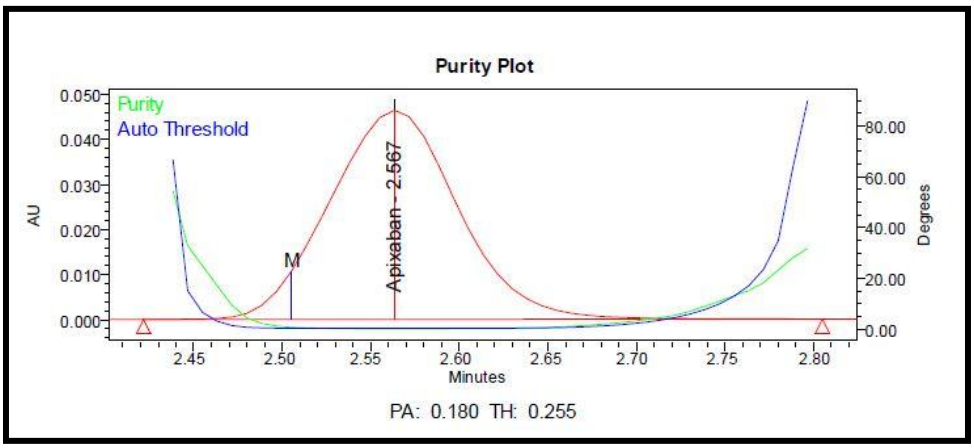


Figure 41: Purity Plot for APB in Thermal degradation

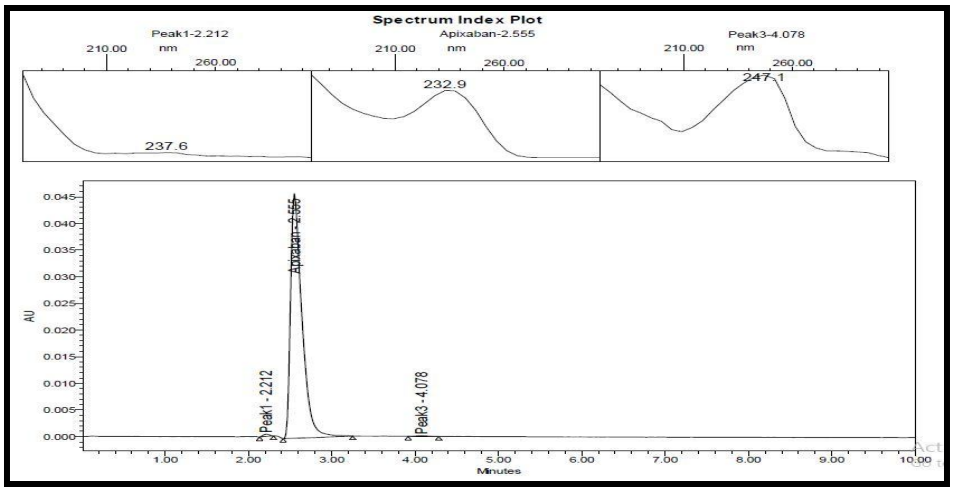


Figure 42: Spectrum index for APB in Thermal forced degradation

Table 10: Peak purity, Thresholds, Purity angle results of APB

Stress Condition	***Purity Angle*** APB	***Purity Threshold*** APB	***Peak Purity*** APB
Acid Degradation	0.339	1.250	Passes
Alkali Degradation	0.208	1.252	Passes

Oxidative Degradation	0.123	0.262	Passes
Thermal Degradation	0.180	0.255	Passes
Photolytic Degradation	0.168	0.253	Passes

Table 11: Percentage of degradation of APB

Drug Name		***Acid***	***Alkali***	***Oxidative***	***Photolytic***	***Thermal***
APB	***Std Area***	346387				
	Sample Area	316528	338212	324461	340602	334402
	% of Degradation	8.62%	2.36%	6.33%	3.46%	1.67%

CONCLUSION

A new RP-HPLC detailed stability indicating method for Apixiban was described in this manuscript which provides a simple, convenient and reproducible approach for estimation and quantification of APB in routine quality control analysis.

Acknowledgement

The Corresponding authors wish to convey his sincere thanks to Dr.D.Gowri Sankar, Dr.JVLN Seshagiri Rao for their valuable help, support and inspiration. I am also thankful and also acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The author is also grateful to authors / editors / publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

Competing Interests

The author declares that he has no competing interest.

REFERENCES

[1]. K.Snyder, L.R., Kirkland, J.W. Dolan, *Jour. of. Modern Liquid Chromatography*, 21, 1997, 129-132.

[2]. M.L.Pujadas, G Escolar G.,E Arellano-Rodrigo, E. and Galan, *Drugs Today* , ,49, 2013, 425-436.

[3]. C.Frost,J.Wang, J.Nepal, *British Journal of Clinical Pharmacology*, 75, 2013, 476-487.

[4]. AG.Turpie,*Jou.of. Vascular Biology*, 27, 2007, 1238-1247.

[5]. M.R.Lassen, B.L.Davidson, A.Gallus, G.Pineo, J.Ansell, D.Deitchman, *Journal of Thrombosis and Haemostasis*, 5, 2007, 2368-2375.

[6]. T.Gous, L.Couchman, J.P.Patel, C.Paradzai, R.Arya, R.J.Flanagan, Therapeutic Drug Monitoring, 36, 2014, 597-605.

[7]. M.R.Lassen, A.Gallus, G.E.Raskob, G.Pineo, D.Chen,L.M. Ramirez, *The New England Journal of Medicine*, 363, 2010, 2487-2498.

[8]. P.V.Solanki, S.B.Uppelli, P.V.Dhokrat, V.T.Mathad, World *Journal of Pharmaceutical Science*, 3, 2015, 663-677.

[9]. S.A.C.Wren, P.Tchelitcheff, *Journal of Chromatography A,* 21, 2006, 140-146.

[10]. X.Delavenne, X., P.Mismetti, P. and T.Basset, T, *Journal of Pharmaceutical and Biomedical Analysis*, 150, 2013, 78-79.

[11]. J.Pursley, J.X.Shen, A.Schuster, O.T.Dang, J.Lehman, M.H.Buonarati, *Jour. of .Bioanalysis*, 6, 2012, 2071-2082.

[12]. T.Gous, L.Couchman, J.P.Patel, C.Paradzai, R.Arya, R.J.Flanagan, *Therapeutic Drug Monitoring*, 36, 2014, 597-605.

[13]. S.S.Prabhune, S.S., R.S.Jaguste, R.S., P.L.Kondalkar, P.L. and N.S.Pradhan, N.S., Jour. of. *Liquid Chromatography*, 21, 2014, 234-239.