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Development And Validation Of A Stability Indicating Reverse Phase Hplc-Pda Method For Determination Of Apixaban In Bulk And Pharmaceutical Dosage Form

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Abstract

A simple, authentic and stability indicating high performance liquid chromatographic method for determination of Apixaban in bulk and pharmaceutical formulations is develop and validate as per ICH Q2 R1 Guidelines. The separation was performed on Agilent Eclipse Plus C18 column with Acetonitrile and water as mobile phase in gradient mode. A flow rate of 0.7 mL/min with an injection volume of 20 µL was selected for this study and the proposed method was validated with different parameters such as Linearity, Precision, Accuracy, Robustness, Limit of Detection (LOD) and Limit of Quantification (LOQ). The separation was achieved at a temperature of 30ºC and the eluents were observed by photo diode array detector set at 279 nm. A linear range of 1-12 µg/mL with a correlation coefficient of 0.999 unfolds good linear relationship between area and concentration in calibration curve. The retention time obtained was at 3.9 ± 0.05 min. The LOD and LOQ were found to be 0.09 µg/mL and 0.27 µg/mL respectively. A recovery of Apixaban in tablet formulation was observed as 98.89 % ± 0.34. Percentage assay of Apixaban tablets (Eliquis®) was found to be 99.97 % ± 0.63. Stability studies indicate that the drug was stable to oxidation, thermal and photo degradation. The drug gives 3 different degradation products on exposure to acidic condition and 3 degradation products on alkaline condition. The method was applied without any interference from excipients, for determination of drug in coated tablets. It is suggested that the proposed HPLC methods could be used routine quality control and dosage form assay of Apixaban.

Key Words: HPLC, Apixaban, Degradant, Validation

INTRODUCTION

Apixaban, 1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl)phenyl]-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxamide, is indicated to reduce the risk of stroke and systemic embolism in patients with nonvalvular atrial fibrillation. The molecular structure is shown in Fig. 1. The FDA approved Apixaban in December 2012 with an indication of reducing the risk of stroke and dangerous blood clots (systemic embolism) in patients with atrial fibrillation that is not caused by a heart valve problem. Apixaban appears as a white-to-pale yellow, non-hygroscopic crystalline powder, with an aqueous solubility of 0.058 mg/mL at 24°C. Apixaban is a non-ionisable compound and its partition coefficient at 24°C is 44.7 (log Po/w = 1.65) at pH 7.4 (n-octanol / aqueous buffer). The molecule has no chiral centres, therefore, no stereoisomers exist [1]. There are some methods for estimation of Apixaban in human plasma by different liquid chromatographic techniques [2], [3], [4] and [5]. Some stability-indicating chromatographic methods [6], [7] and [8] and stability-indicating spectrophotometric method [9] for its determination were also reported. The aim and objective of the present work was to develop and validate a stability indicating simple, precise, sensitive high performance layer chromatography method for Apixaban in its bulk and tablet doses form and validated as per International Conference of Harmonization (ICH) Q2 (R1) guidelines [10].

MATERIALS AND METHODS

Working standard of Apixaban was kindly provided as a gift sample from Glenmark Generics Limited, Pune. All solvents and chemicals used were of HPLC and analytical grade, purchased from Merck Specialities Pvt. Ltd., India.

Pharmaceutical dosage form: Eliquis®, labeled to contain 5 mg Apixaban per tablet, manufactured by Bristol-Myers Squibb-Pfizer and obtained from local market.

Preparation of Standard solution:

Stock STD solution: About 100.0 mg of Apixaban standard sample was accurately weighed and transferred to a 10.0 mL volumetric flask, dissolve and diluted to volume with methanol to obtain a concentration of 10 mg/mL.

Working STD solution: Transfer accurately 1 mL of stock STD solution to a 10.0 mL volumetric flask and diluted to volume with acetonitrile to obtain a concentration of 1 mg/mL. From this solution, 0.2 mL of aliquot was further transferred to a 10.0 mL volumetric flask and diluted up to the mark with acetonitrile to prepare a final concentration of 20 µg/mL.

Preparation of sample solution for force degradation studies

To access the stability indicating property of the developed HPTLC method, stress studies were carried out under ICH recommended conditions. Force degradation of Apixaban was carried by exposing the bulk sample to acidic, alkaline, oxidative, photolytic, dry heat and neutral conditions. The aim was to study the ability of the proposed method to measure the analyte response in presence of its degradation products.

Acid hydrolysis

Aliquot of 1 mL of stock standard solution was transferred to a round bottom flask and it was mix with 9 mL of 1 N hydrochloric acid (1mg/mL solution). The prepared solution was subjected to reflux for 3 h in a boiling water bath. The sample were cooled to room temperature (25^{o}), neutralized with an amount of alkali equivalent to that of the previously added. Resulting solution was diluted appropriately with acetonitrile to obtained a solution of 20 µg/mL.

Alkali hydrolysis

Aliquot of 1 mL of stock standard solution was transferred to a round bottom flask and it was mix with 9 mL of 1 N sodium hydroxide solution (1mg/mL solution). The prepared solution was subjected to reflux for 1 h in a boiling water bath. The sample were cooled to room temperature (25^{o}), neutralized with an amount of acid equivalent to that of the previously added. Resulting solution was diluted appropriately with acetonitrile to obtained a solution of 20 µg/mL.

Neutral hydrolysis

Aliquot of 1 mL of stock standard solution was transferred to a round bottom flask and it was mix with 9 mL of water (1mg/mL solution). The prepared solution was subjected to reflux for 5h in a boiling water bath. The sample were cooled to room temperature (25^{o}). Resulting solution was diluted appropriately with acetonitrile to obtained a solution of 20 µg/mL.

Oxidation

Aliquot of 1 mL of stock standard solution was transferred to a 25 mL amber colored volumetric flask and it was mix with 9 mL of 30% hydrogen peroxide solution, the resulting solution was allowed to proceed in dark at room temperature for 24 h with intermittent shaking. Resulting solution was diluted appropriately with acetonitrile to obtained a solution of 20 µg/mL.

Photo and Thermal Degradation

For photo degradation Apixaban bulk sample was evenly spread in a thin layer in a covered petri dish and exposed to sunlight for 10 days. The same amount of sample was placed in a petri dish for thermal degradation at 120 °C for 15 days. The sample was dissolve separately in a 10 mL of acetonitrile to get the concentration of 1 mg/mL. Resulting solution was diluted appropriately with acetonitrile to obtained a solution of 20 µg/mL.

Chromatography

Chromatographic measurements were made on Shimadzu LC-20AB prominence model which consisted of Shimadzu SPD-20A prominence PDA detector, Shimadzu DGU-20A3 prominence degasser and Rheodyne injector 7725 I with 20 µL loop. The system was controlled through personal computer using chromatographic software (LC Solution).

Chromatographic separations were performed on Agilent Eclipse Plus C18 column having 100 x 4.6 mm i.d and 3.5 µm particle size. The flow rate was 0.7 mL/min and quantitation was achieved with UV detection at 279 nm. The injection volume of the sample was 20 µL and the total run time was 10 min. The HPLC system was used in an air conditioned laboratory atmosphere (30 ± 2^{o}C)

Initially, pure drugs solution was chromatographed using single solvents to ascertain the movement of the drug. Use of mobile phase containing acetonitrile and water (60:40 v/v) gives well separated, sharp, symmetrical peaks of drugs and but degradants are not resolved from each other. So in order to achieve the separation of degradation products gradient program was applied using the acetonitrile and water as mobile phase.

Retention time of Apixaban was 3.9 ± 0.05 min. Typical HPLC chromatogram (obtained at 279 nm) and PDA spectra obtained from standard solution is shown in Figure 1 A and B.

Assay/Precision:

Standard solution: About 3.0 mg of Apixaban standard sample was accurately weighed and transferred to a 10.0 mL volumetric flask, dissolve and diluted to volume with acetonitrile. Transfer accurately 1 mL of this solution to a 10.0 mL volumetric flask and diluted to volume with the same. From this solution 2 mL of aliquot was further transferred to a 10.0 mL volumetric flask and diluted up to the mark with acetonitrile to prepare a final concentration of 6 µg/mL.

Preparation of sample solution: Twenty tablets of Eliquis® 5 mg was accurately weighed and finely powdered. Accurately weighed portions of the powdered tablets

equivalent to 3 mg of Apixaban were transferred to 100.0 mL volumetric flask. About 60 mL of acetonitrile was added and sonicated for 20 min and diluted to the mark with acetonitrile. The mixture was filtered through 0.2 µm nylon syringe filter. From this solution 2 mL of clear aliquot was further transferred to a 10.0 mL volumetric flask and diluted up to the mark with the same to prepare a final concentration of 6 µg/mL.

Method precision was demonstrated by preparing six assay sample solutions at a concentration of 6 µg/mL and Intermediate precision was demonstrated by preparing three assay sample solutions at a concentration of 6 µg/mL on a different day by different analyst.

RESULTS AND DISCUSSION

Method development:

Apixaban and its degradation products were optimally resolved on a Agilent Eclipse Plus C18 column having 100 x 4.6 mm i.d and 3.5 µm particle size at ambient temperature (30 °C) with Acetonitrile (mobile phase A) and water (mobile phase B) flowing at a rate of 0.7 mL/min in gradient mode (0- 4 min, A:B; changes from 75:25% to 78:22 % → 4-9 min, A:B from 78:22 % to 75:25→ 9-10 min) as mobile phase. The injection volume and detection wavelength were fixed at 20 µL and 279 nm, respectively. Retention times of Apixaban was 3.9 ± 0.05. Then samples obtained from forced degradation were then chromatographed with the same mobile phase and it was found that chromatogram obtained after alkaline hydrolysis gave three degradation products at 1.37 ± 0.05 min (Deg. I), 2.25 ± 0.05 min (Deg. II), 2.71 ± 0.05 min (Deg. III) and after acidic hydrolysis gave three degradation products at 1.83 ± 0.05 min (Deg. IV), 2.73 ± 0.05 min (Deg. V), 5.95 ± 0.05 min (Deg. VI), and in oxidative degradation one very small degradation product at 1.82 ± 0.05 (Deg. VII) min [Figure 4]. No degradation products were obtained after neutral hydrolysis, photo and thermal degradation condition. The drug showed stability with 30% H_2O_2 for 24 h at room temperature, in a dry oven at 120 °C for 15 days and on exposure to UV light for 10 days. Apixaban was liable to stress hydrolytic degradation and degrade up to 18-20 % in strongly acidic condition (1 N HCl, reflux for 3 h) and 20-22 % in alkaline degradation condition (1 N NaOH, reflux for 1 h).

Validation:

The proposed method was validated for linearity, precision, accuracy, specificity, limits of detection and quantification and robustness. Linearity was established by

least-squares linear regression analysis of the calibration data. Calibration plots were linear over the concentration range 1-12 µg/mL by area. Peak areas were plotted against the respective concentrations and linear regression analysis performed on the resulting curves. Equation for the calibration plots of Apixaban was Y= 45756X + 86470 and correlation coefficient was 0.999 for peak area [Fig. 3]. The limits of detection (LOD) and quantification (LOQ) were calculated from the standard deviation of the response and the slope of calibration plot. LOD and LOQ were established, in accordance with ICH definitions, by use of the equations LOD = 3.3σ/S and LOQ = 10σ/S, where σ is the standard deviation of the regression line and S is the slope of the calibration plot. The LOQ for which precision and accuracy were satisfactory was 0.27 µg/mL for peak area and LOD was 0.09 µg/mL for peak area.

Method, system and intermediate precision data are summarized in Table 1. Method precision was investigated by injecting extracts from six tablet samples (n = 6) in triplicate. Intermediate precision (inter-day and intra-day) was investigated by injecting three samples (n = 3) in triplicate.

Accuracy: The accuracy of the method was determined on the basis of recovery studies performed by standard addition at different levels (80-120%) of the label claim. A known amount of powder standard was added to samples of tablet powder, which was then mixed, extracted, and subsequently diluted to volume with HPLC grade acetonitrile, to yield the required concentration of drug.

Standard solution: Prepared same as given under precision.

Sample solution: Sample solution was prepared by accurately weighing quantities of pre-analyzed tablet powder equivalent to 2 mg of Apixaban, which then transferred 10 mL volumetric flasks and accurately known amount of standard Apixaban for 80-120% levels were added. Extraction and dilutions were performed with acetonitrile. About 5 mL of acetonitrile was added and sonicated for 10 min. Volumes was made up to the mark with acetonitrile. The drug content in different flasks represents 80-120 % of labeled claim with constant amount of excipients. The solution was mixed, filtered and one milliliter of clear filtrate diluted to 10 mL. Two milliliter resulting solution was again transferred to 10 mL volumetric flask and diluted upto mark with acetonitrile. The accuracy was determined and expressed as percentage recovery depicted in Table 2.

Specificity studies were conducted by attempting deliberate degradation of tablet samples by exposure for 24 h to the stress conditions acidic (0.1N HCl), alkaline hydrolysis (0.1 N NaOH) and oxidation (30 % H_2O_2) at room temperature, oven at 120^oC and UV exposure in sun light. The results showed in Table 3. Robustness was studied by varying the mobile phase composition gradient programme and flow rate. The results are listed in Table 4.

Conclusion

The method enables simple, precise, and accurate analysis of Apixaban and its degradation products in the bulk drug and pharmaceutical preparations. It was validated for linearity, LOD, LOQ, precision, accuracy, specificity and robustness. The method can therefore be used for routine quality-control analysis of Apixaban in dosage forms.

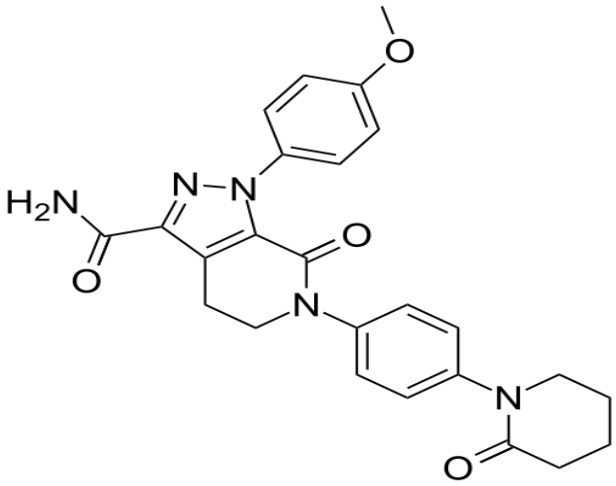


Fig. 1: Chemical Structure of Apixaban

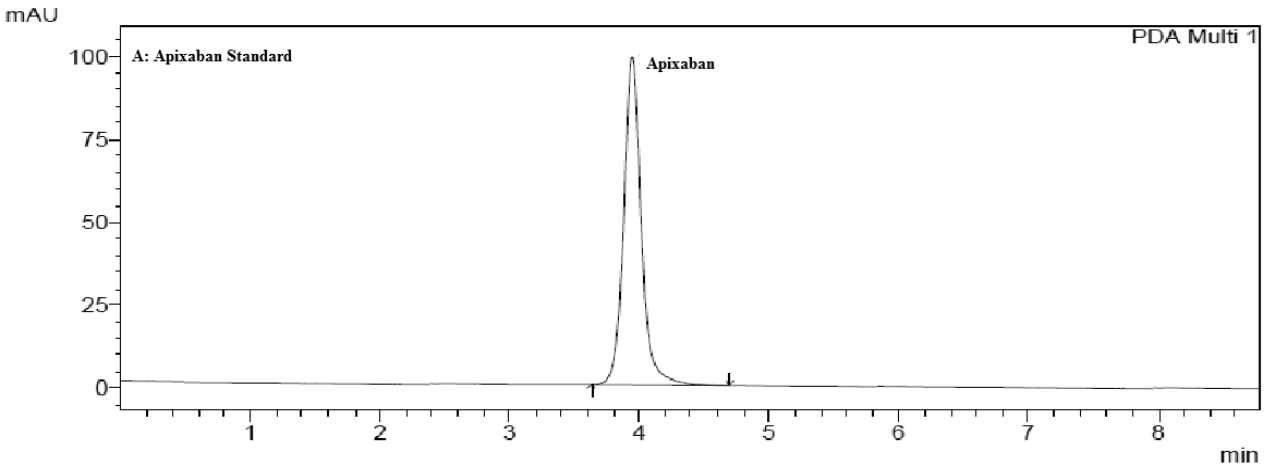


Fig. 2-A: Typical HPLC Chromatogram of Apixaban

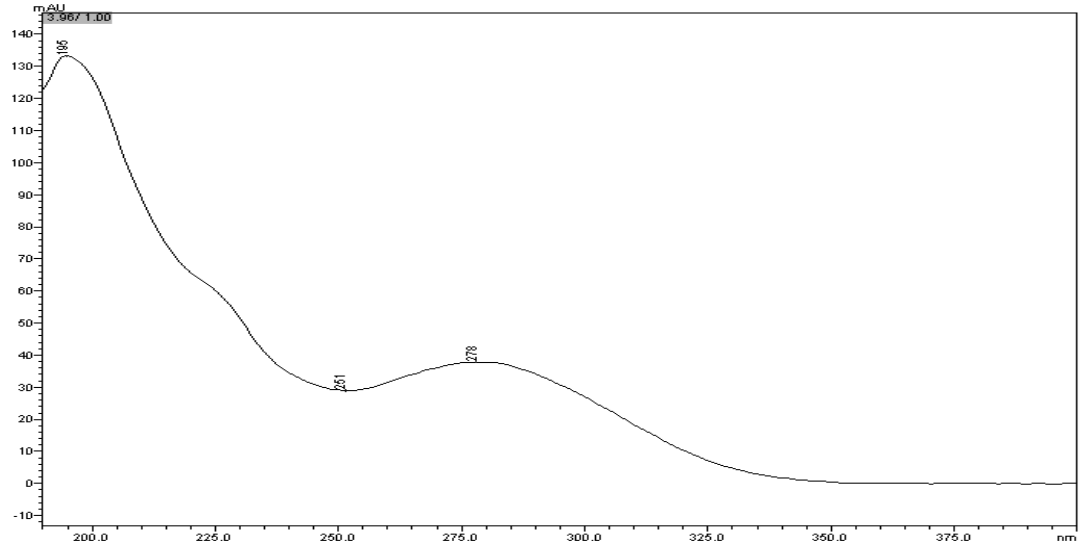


Fig. 2-B: Typical PDA spectra of Apixaban

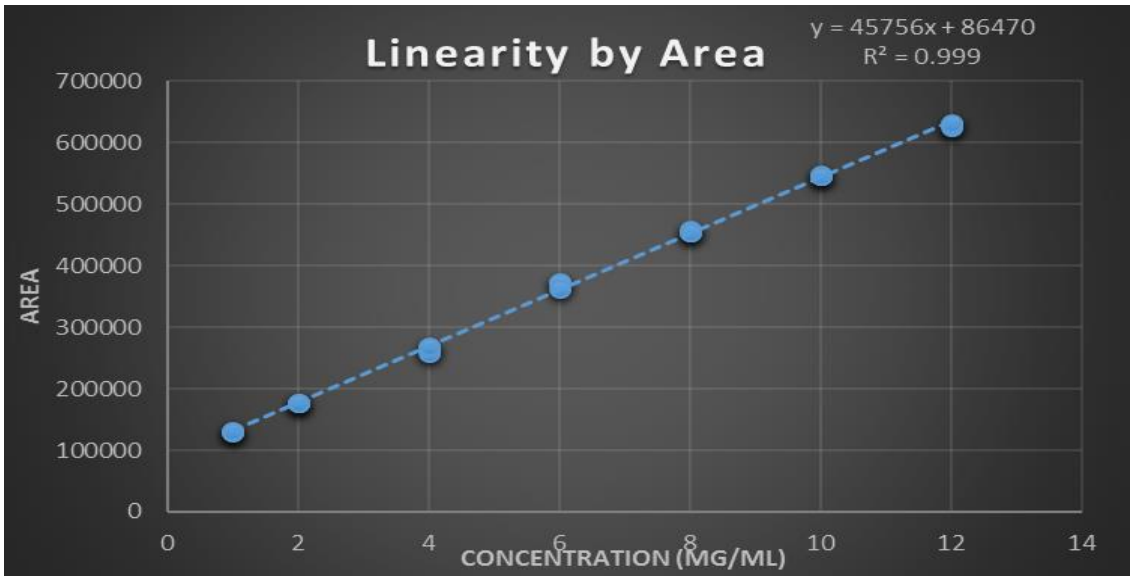


Fig. 3 (A): Linearity by Area

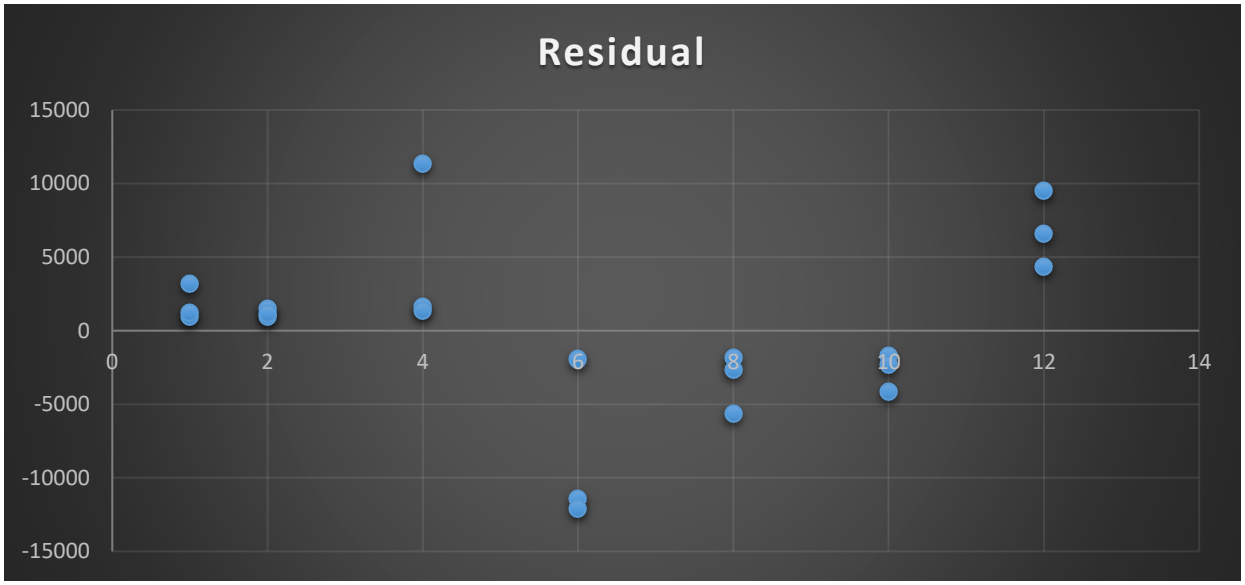


Fig. 3 (B): Residul plot by area

Table 1: System, method, and intermediate precision data

Validation Parameters		**Eliquis®**		
		Mean	**SD[±]**	**RSD [%]**
System Precision [a)]		99.552	0.659	0.662
Method Precision [a)]		99.975	0.629	0.629
Intermediate precision	**Interday** [b)]	99.128	0.498	0.503
	Intraday [b)]	98.541	0.753	0.764
	Different Analyst [b)]	99.321	0.573	0.577

a) Mean from six analyses (n =6)
b) Mean from 3 analyses (n =3)

n = Number of samples, SD = standard deviation; RSD = relative standard deviation

Table 2: Accuracy data

Range	Wt. Tablet Powder	Wt. of STD added	T. D. Estimated	Amt. of pure drug cont. by tab powder	Recovery	Mean % recovery
80	84.5	0	1.9482	2.01	97.12	98.76
			1.9867		99.04	

			2.0082		100.12	
			2.5081		99.44	
			2.5088		99.57	
100	84.7	0.5	2.4914	2.01	96.09	98.37
			2.9912		98.74	
			2.9883		98.46	
120	84.4	1	3.0006	2.00	99.68	98.96
					Mean	98.89
					± SD	0.340
					%RSD	0.344

Table 3: Specificity data

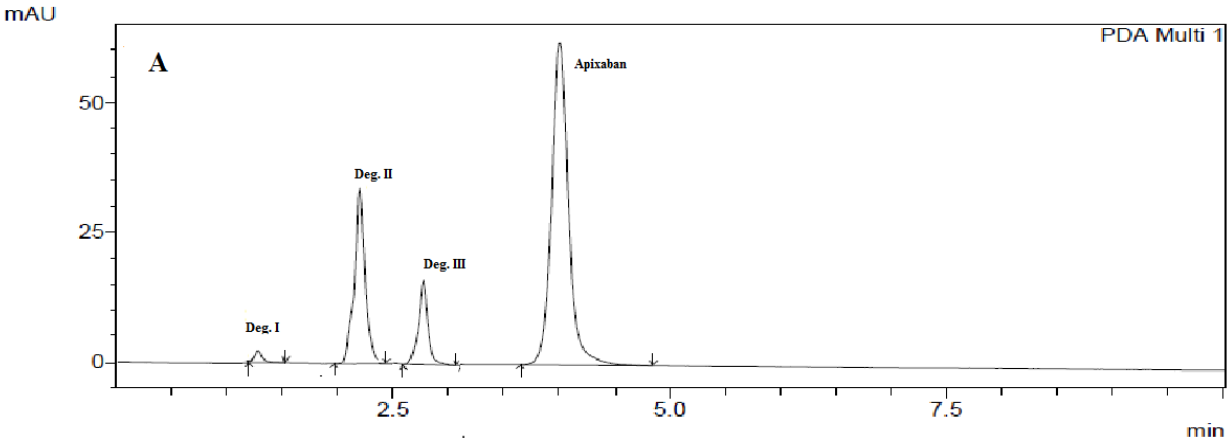
Formulation	Normal	Acid	Alkali	Neutral	Oxide	Thermal	Photo
Eliquis®	99.94	94.21	95.15	99.24	98.12	99.91	100.22

Table 4: Robustness

Conditions		**Eliquis®**	
		By peak area*	
		Amount estimated [%] ± SD	**RSD [%]**
Change in mobile phase composition gradient program (Acetonitrile as mobile phase A & Water as mobile phase B)	0- 4 min, A:B; changes from 70:30% to 78:22 % → 4-9 min, A:B from 78:22 % to 70:30→ 9-10 min	98.74 ± 0.984	0.986
	Acetonitrile: Water 0- 4 min, A:B; changes from 80:20% to 78:22 % → 4-9 min, A:B from 78:22	99.48 ± 0.452	0.454

	% to 80:20→ 9-10 min		
Change in flow rate (± 0.1 mL/min)	**F.R. 0.6 mL/min**	99.81 ± 0.90	0.902
	F.R. 0.8 mL/min	99.50 ± 1.461	1.468

* Each value is a mean of three observations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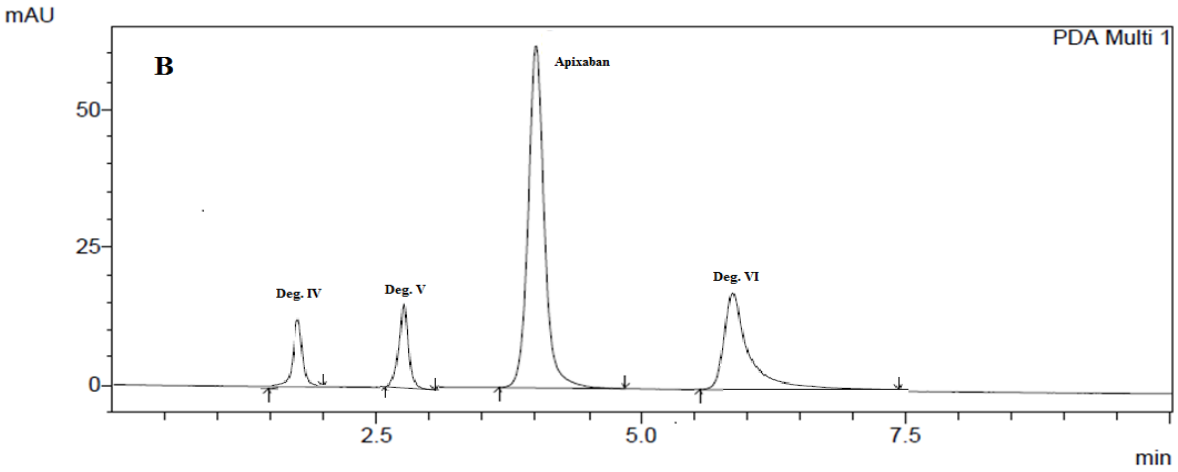
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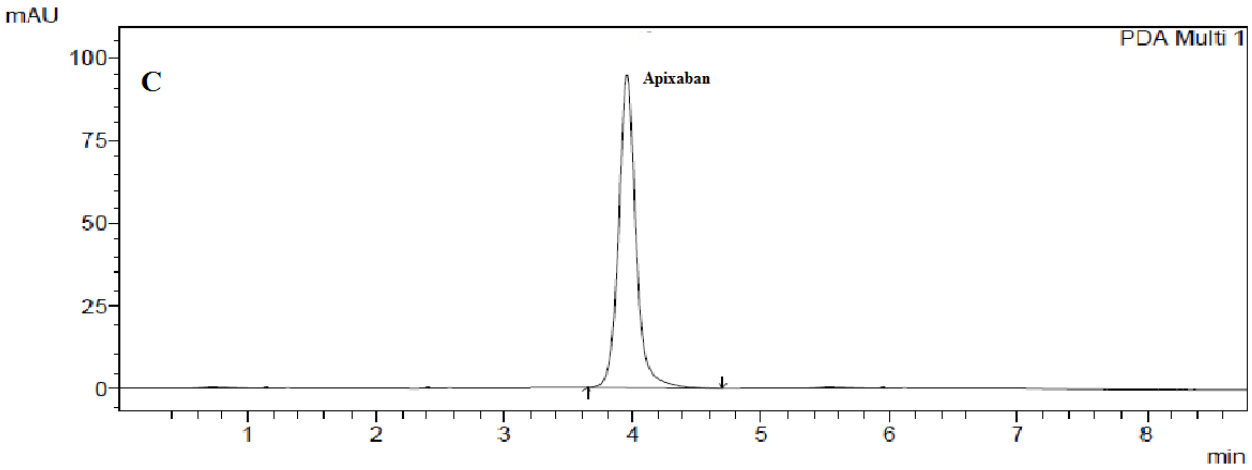
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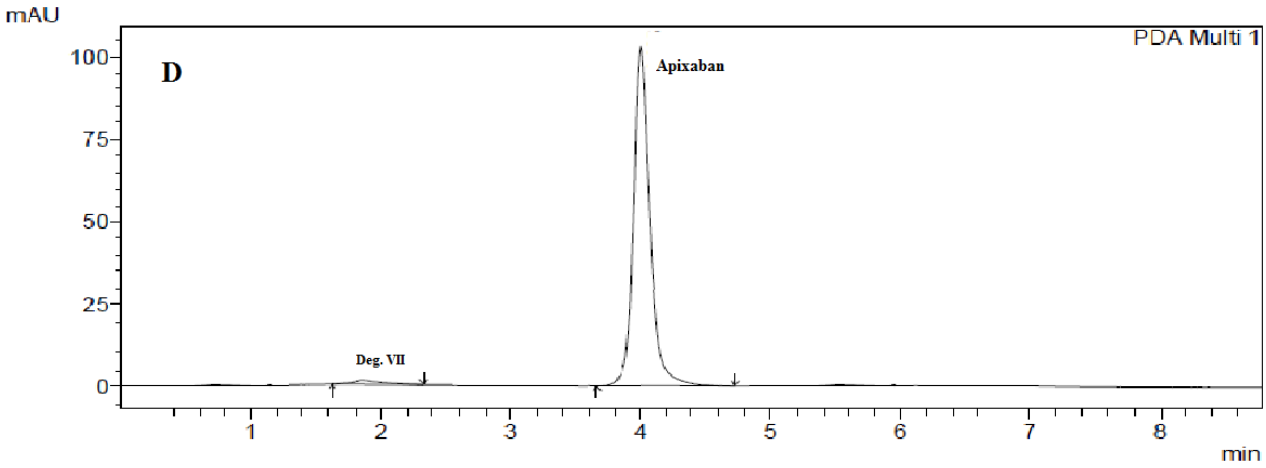
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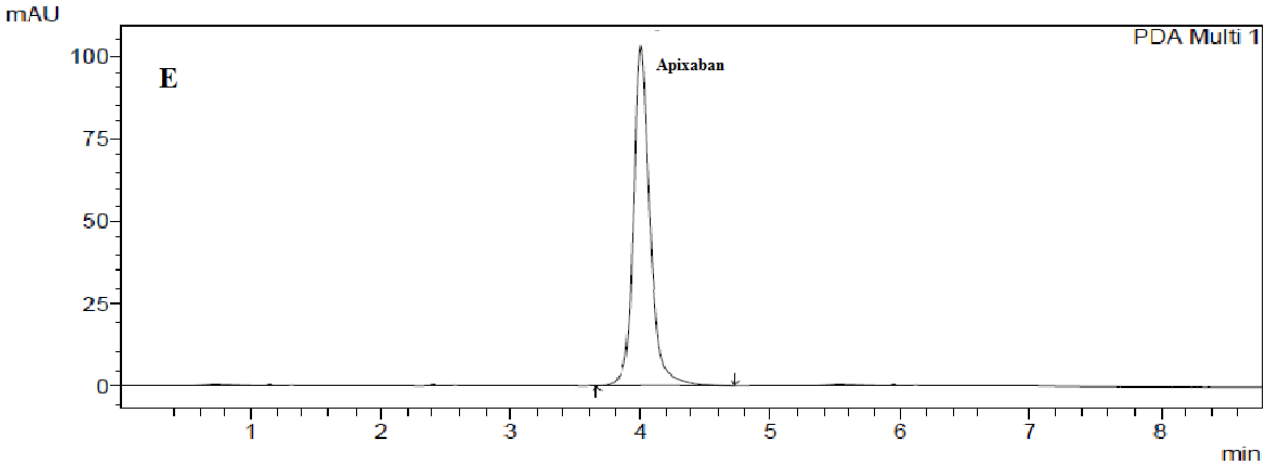
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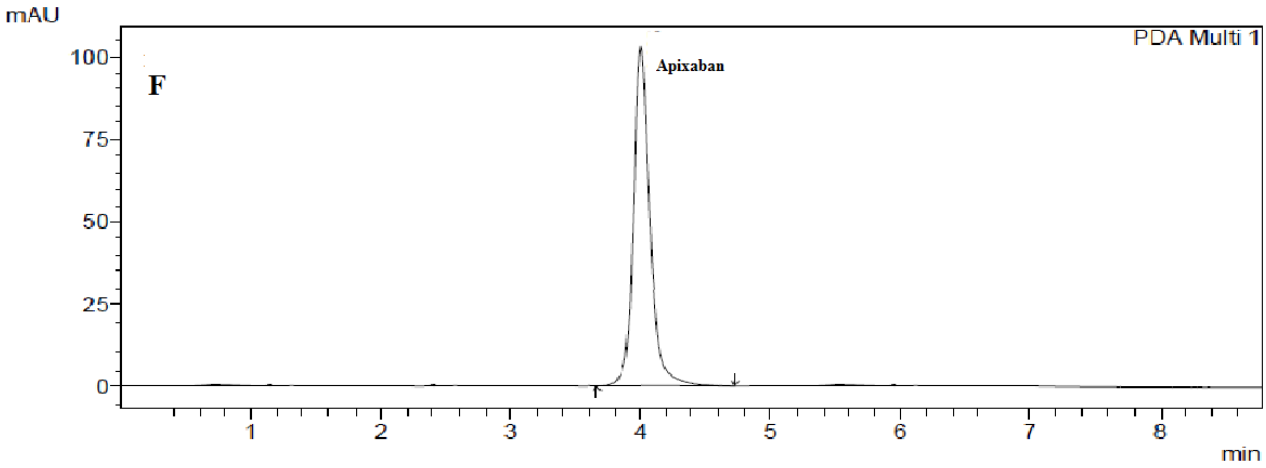

Fig. 4: A typical HPLC chromatogram of (A) Alkaline Hydrolysis (1N NaOH, 1h reflux), (B) Acid Hydrolysis (1 N HCl, 3 h reflux), (C) Neutral Degradation, (D) Oxidative (30% H2O2), (E) Thermal (105° C 15 days), (F) Photo

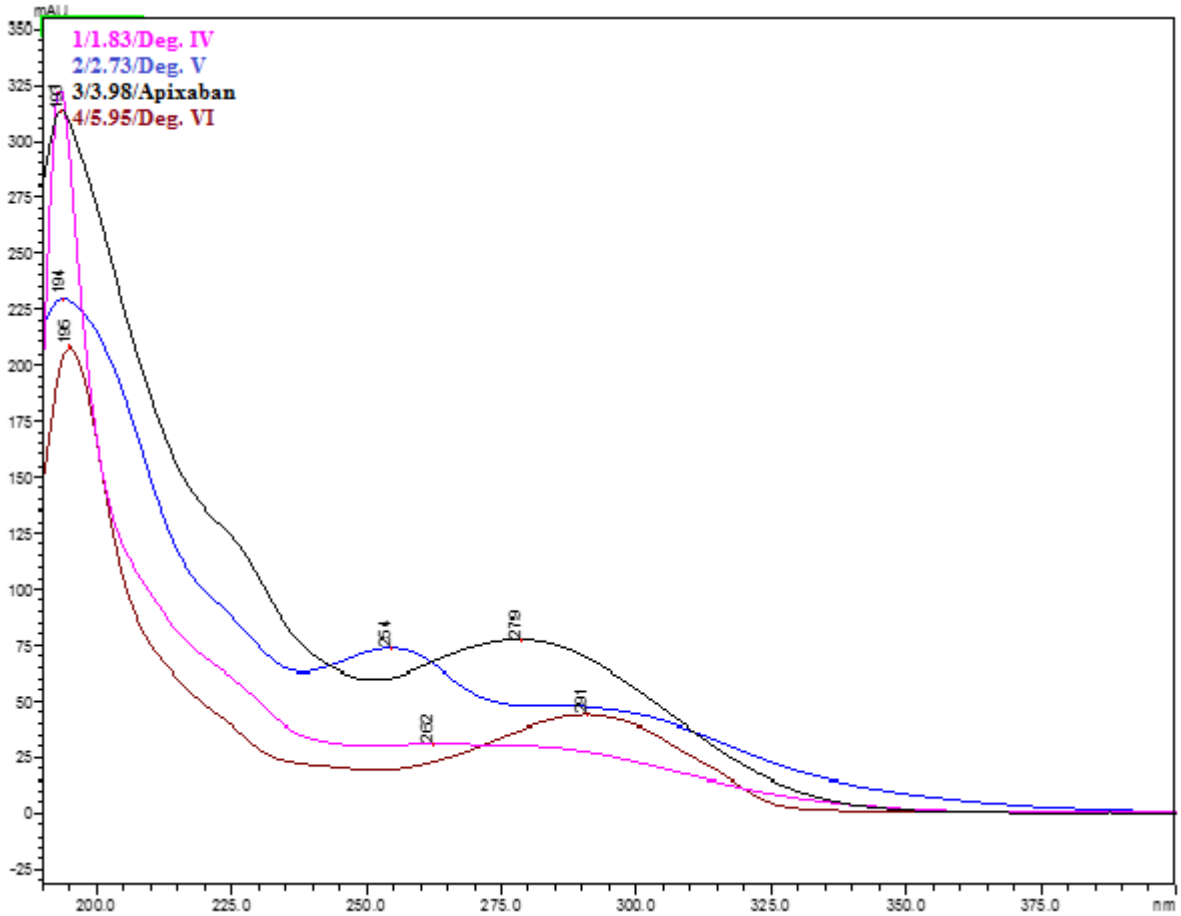

Fig. PDA Overlay spectra of Apixaban acid degradation products

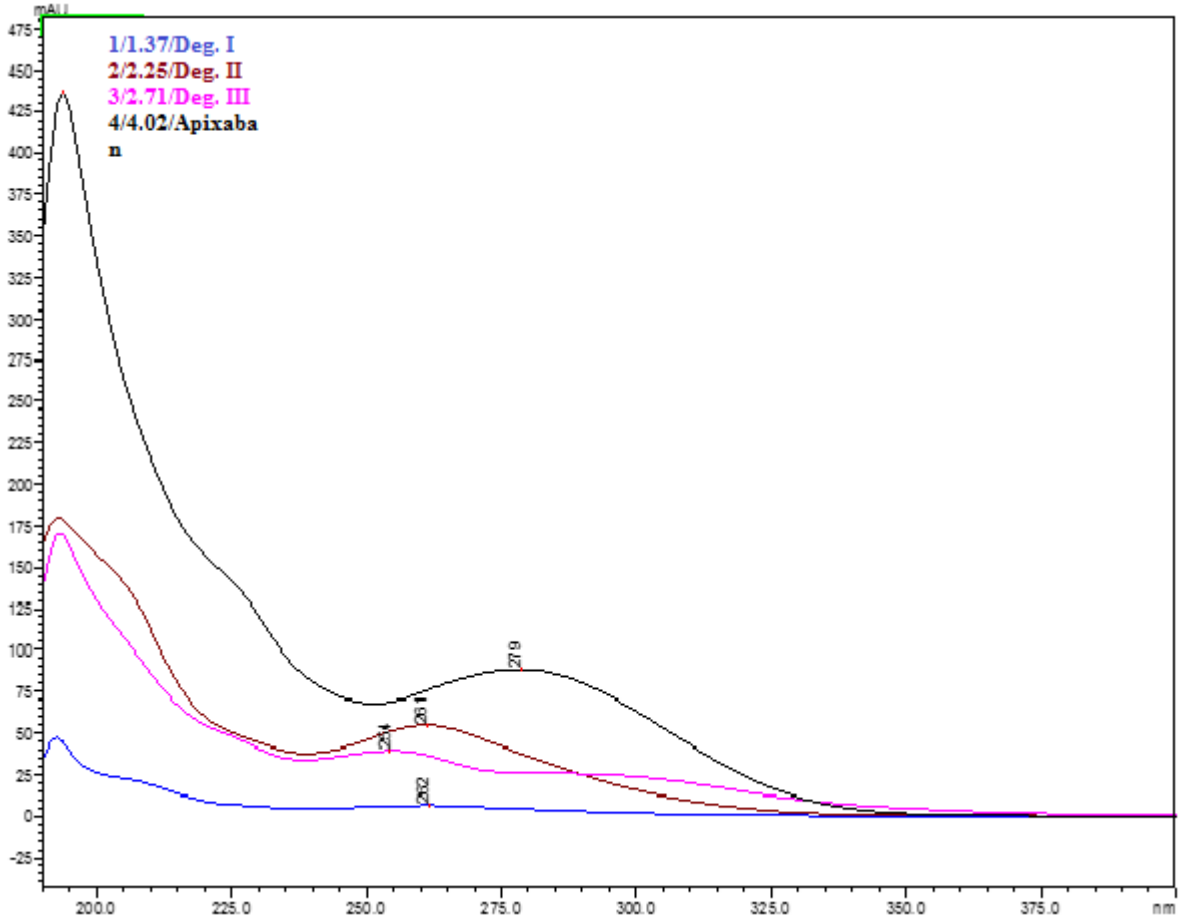

Fig. PDA Overlay spectra of Apixaban alkali degradation products