Available Online at

http://www.ijcpa.in

October-December 2017

International Journal of CHEMICAL AND PHARMACEUTICAL ANALYSIS

eISSN: 2348-0726 ; pISSN : 2395-2466

Research Article | Volume-5 | Issue-1 | Article ID: 1377

METHOD DEVELOPMENT AND VALIDATION OF APIXABAN USING RP-HPLC METHOD AND ITS STRESS STABILITY STUDIES

Akiful Haque*, Rayanni Soundharya, Julakanti Venu, Malipeddi Lakshmi Monika, Vasudha Bakshi

Department of Pharmacy, Anurag Group of Institutions (Formerly Lalitha College of Pharmacy), Venkatapur, Ghatkesar, Medchal, Telangana-500088, India

*Corresponding Author: Email: akif963@gmail.com

Received: 9 September 2017 / Revised: 26 September 2017 / Accepted: 25 November 2017 / Available online 31 December 2017

ABSTRACT

A simple, fast and precise reverse phase high performance liquid chromatographic method (RP-HPLC) good sensitivity was determined for the development and validation of Apixaban. In Water Apixaban λ_{max} was fixed as 259nm using a Shimadzu UV-Visible spectrophotometer. The chromatographic separation was achieved on a Waters C18 column, 15µm, 4.6 x 250 mm as stationary phase with mobile phase comprising of water: Acetonitrile as a mixture in the ratio of 50:50 in an isocratic elution mode at 1.0mL/min flow rate and maintaining column temperature at ambient. The detection was monitored at 259nm for Apixaban. The retention time of Apixaban were found to be 3.4mins respectively. The linearity was found to be in the range of 5-2 µg/ml. The calibration factor is 0.999. Apixaban was subjected to photolytic, acidic, basic, oxidative, and thermal degradation. The drug was found to be stable in all the conditions. The developed method was simple, accurate, precise, specific, sensitive and reproducible. Stress testing should be given importance for quantification of degraded products of drugs helps us to maintain the quality, safety and efficacy of drugs in formulations.

Keywords *– Apixaban, Spectrophotometric method, Method development, Validation.*

1. INTRODUCTION

Apixaban is one of new generation oral anticoagulant drug and is a direct highly selective reversible factor Xa inhibitor. It is indicated in the prevention of venous thromboembolism (VTE) after major orthopaedic surgery, treatment of acute VTE, prevention of stroke or systemic embolism in patients with atrial fibrillation. Apixaban has about 50% bioavailability, and is excreted by multiple elimination pathways, including renal excretion (257%) and hepatic metabolism (75%). It is also a substrate of P-glycoprotein and may be subject to drug–drug interactions. The absence of a requirement for routine anticoagulation monitoring is expected to provide a therapeutic advantage. However, in some emergency situations, a specific method of quantification is useful: before surgery or invasive procedure, when a patient is bleeding, when a patient has taken an overdose, when a patient has developed renal failure or to assess whether there is failure of therapy or lack of adherence.

Literature survey [3,4,5,6] revealed that there were methods reported for estimation of Apixaban in combined dosage forms and for individual drugs using UV, HPLC and LCMS. Here is an attempt made to develop a spectrophotometric method for determination of apixaban using water as a solvent which is very economic.

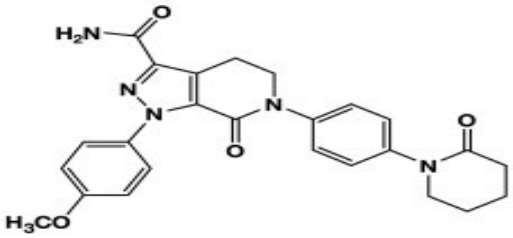

Fig. 1: Structure of Apixaban

2. MATERIALS AND METHODS

2.1 Instruments used

Analysis was carried out by Shimadzu LC-20AD HPLC with UV detector, column used was Waters X-bridge C18, 5µm (4.6 x 250mm), Shimadzu double beam UV/Visible spectrophotometer model UV1800s. Shimadzu Electronic balance model AX 200 and Ultra Sonicator (Fast clean) model 2k811056 and vacuum filter pump were also used during the analysis.

2.2 Chemicals and Reagents

Pure drug Apixaban was obtained from Hetero labs (Hyderabad, India). Eliquis tablets manufactured by Bristol Mayer's Squibb S.R.L contrda Fontana del ceraso 03012 anagni, Frosinone, Italy was purchased from local pharmacy and used for analysis. The label claim states that this formulation contains 2.5mg of Apixaban.

2.3 Method Development

2.3.1 Selection of solvent and wavelength

The UV spectra of Apixaban differ in different solvents like ethanol, methanol, 0.1N HCl, 0.1N NaOH and distilled water were recorded. The drug showed good absorbance when dissolved in distilled water. Hence, distilled water was selected as the solvent for the method because, it shows good absorbance at wavelength of 259nm, hence selected as λ_{max} of Apixaban respectively.

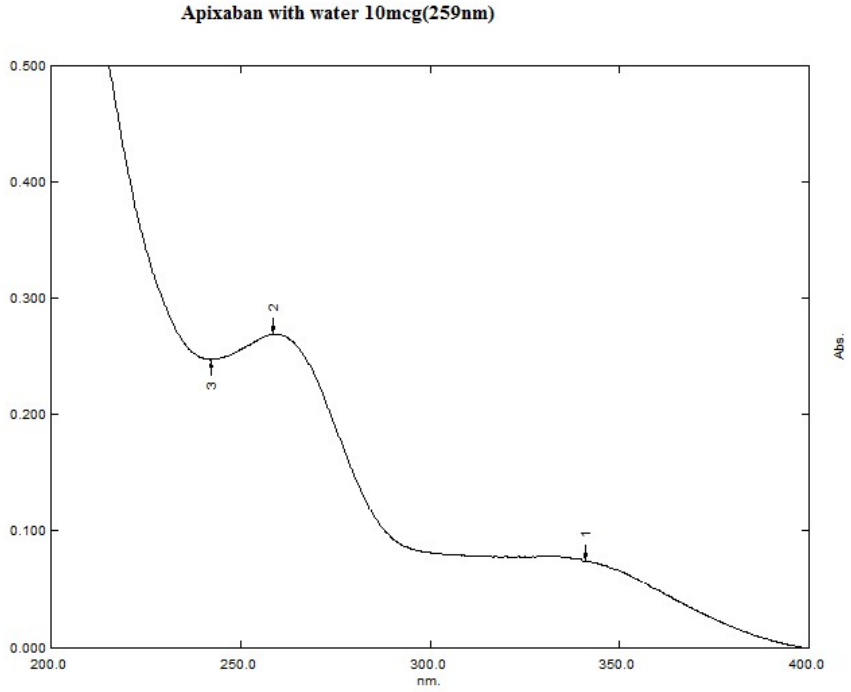

Fig.2: UV spectra of Apixaban standard in distilled water

2.3.2 Preparation of standard stock solutions

Standard stock solution of Apixaban was prepared by dissolving 10 mg of drug in a 10 ml volumetric flask and dissolved in distilled water to get a concentration of 1000µg/ml.

2.3.3 Preparation of working standard solutions

The working standard solution of Apixaban was prepared by diluting1 ml of the standard stock solution to 10 ml with distilled water in a 10 ml volumetric flask to get the concentration of 100µg/ml.

2.3.4 Preparation of sample solutions

10 tablets were weighed to get the average weight of each tablet. Tablets were placed in the mortar and finely powdered. The tablet powder equivalent to 10mg Apixaban was transferred into 10ml volumetric flask. About 5ml of mobile phase was added to the flask and sonicated for about 5mins and made up to the volume with mobile phase.

The contents were filtered through Whatmann filter paper. From this sample stock solution, 1ml was transferred into a 10ml volumetric flask. The volume was made up to the mark with mobile phase. The solution prepared was injected into the HPLC to obtain the % content of Apixaban in the tablets.

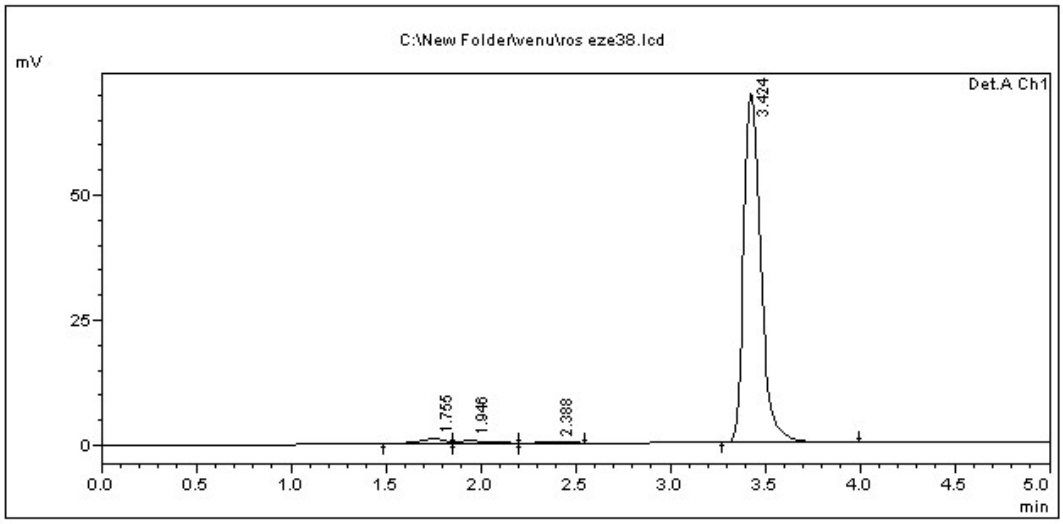

Fig.3: Chromatogram of Sample (Formulation)

2.4 Validation of the Method

The analytical method was validated with respect to parameters such as linearity, range, precision, accuracy, selectivity and robustness.

2.4.1 Linearity

The linearity of the method was determined in the concentration range of 0-25 µg/ml for Apixaban. Each solution was injected and calibration curve was plotted using peak area versus concentration, data of Apixaban was shown in fig. 4 and table 1. The correlation coefficient of drug was found to be 0.999 and the regression equation was y = 36261x + 7078 for Apixaban respectively.

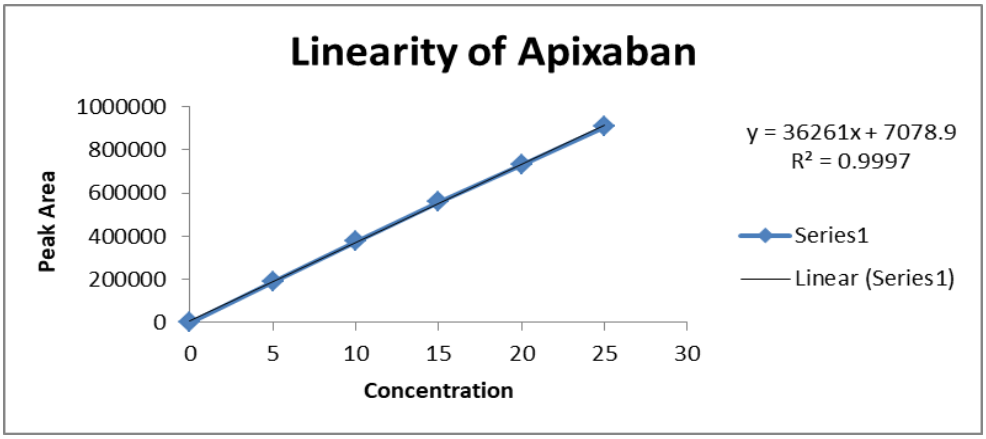

Fig.4: Linearity of Apixaban

Table 1: Linearity of Apixaban

Concentration(µg/ml)	Peak Area	Statistical Analysis	
0	0	Slope	35836
5	188707	Y-Intercept	14866
10	376895	Correlation Coefficient	0.999
15	558297		
20	730164		
25	907976		

2.4.2 Precision

Precision studies were carried out by injecting six replicate injections of the standard drug mixture on one day. This process is called intraday precision. The results were calculated in terms of %RSD. The results are shown in Table 2.

Precision studies were also carried out by injecting six replicate injections of the standard drug mixture on six different days. This process is called interday precision. The results were calculated in terms of %RSD. The results are shown in Table 3.

Table 2: Intraday Precision

S. No	Peak area
1	782217
2	789519
3	784474
4	775236
5	789542
6	776224
Mean	782868.7
S.D	6231.714
%RSD	0.79601

S. D. = Standard deviation;

% RSD =Percentage of Relative Standard Deviation

Table 3: Interday precision

S. No	Peak area
1	782217
2	783672
3	779673
4	765432
5	773621
6	764532
Mean	774857.8
S. D.	8390.848
%RSD	1.082889

S.D. = Standard deviation;

% RSD =Percentage of Relative Standard Deviation

2.4.3 Accuracy

Accuracy (% Recovery) was evaluated at three different concentrations equivalent to 50, 100 and 150% of the target concentration of active ingredients, by adding a known amount of standard and sample solutions in a same volumetric flask and calculates the % recovery for each concentration. The results are shown in Table 4.

Table 4: Recovery studies

S. No	Amount of marketed formulation added (µg/ml)	Amount of API mixture added (µg/ml)	Total amount of both the drugs (µg/ml)	Peak area at 259nm	Total amount of drug found (µg/ml)	% Recovery
1	10	5	15	561841	15.2	101.3
2	10	10	20	726362	19.8	99.25
3	10	15	25	914053	25.0	100

nm = Nano meter; µg = Micro grams; ml = Milli Litre

2.4.4 Specificity

The specificity studies were done and retention time for both sample and standard found to be same.

Table 5: Specificity studies

Name	Retention Time (mins)
Standard	3.48
Sample	3.42

min = Minutes

2.4.5 Robustness

To evaluate the robustness of the method the chromatographic conditions were deliberately varied and degree of reproducibility was evaluated. Robustness was carried out on standard drug solution and formulation. Robustness of the proposed method was assessed with respect to change in flow rate (1ml/min ± 0.2ml/min). The results are shown in Table 6.

Table 6: Robustness of Apixaban

S. No	Flow Rate	Std. Areas	Tailing Factor
1	0.8 ml/min	532541	1.3
2	1.2 ml/min	255917	1.4

ml = Milli Litre; min = Minutes

2.4.6 Forced Degradation Studies

Forced degradation studies were performed on Apixaban. The drug was subjected to stress conditions which include acid hydrolysis (0.1N HCl), base hydrolysis (0.1N NaOH), thermal (50ºC), oxidation (3% H_2O_2) and photolytic (exposure to light). The monitoring period for acid, base and oxidation was 24hrs and for thermal (50ºC in hot air oven) and photolytic (longer wavelength in UV cabinet) it was 5-6hrs. After completion of degradation processes, the solutions were neutralized and diluted with mobile phase.

2.4.7 Acid Hydrolysis

Accurately weigh 10mg of Apixaban and transferred into 10ml of volumetric flask to which 0.1N HCl was added. Shaken for some time and then the volume was made up to the mark with mobile phase and kept aside for 24hrs. Then, after completion of 24hrs, about 0.1ml of the above solution was transferred into 10ml of volumetric flask and diluted to 10ml using mobile phase (10µg/ml). Then this solution was injected into HPLC and chromatogram was recorded (Fig.5).

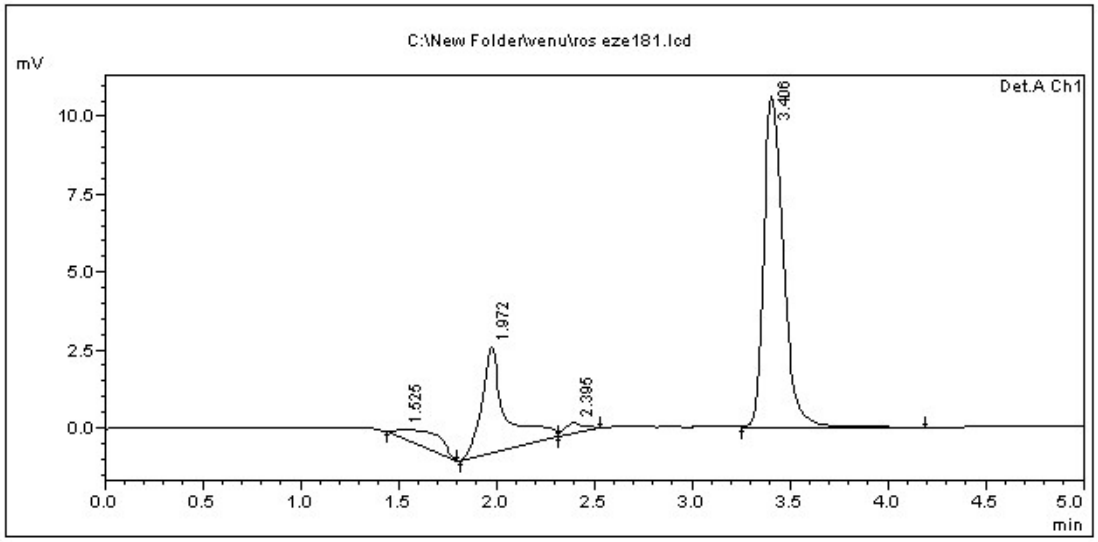

Fig.5: Chromatogram of Apixaban in acid degradation (0.1N HCl)

2.4.8 Base Hydrolysis

Accurately weighed 10mg of Apixaban and transferred into 10ml of volumetric flask to which 0.1N NaOH was added. Shaken for some time and then the volume was made up to the mark with mobile phase and kept aside for 24hrs. Then, after completion of 24hrs, about 0.1ml of the above solution was transferred into 10ml of volumetric flask and diluted to 10ml using mobile phase (10µg/ml). Then the solution was injected into HPLC and chromatogram was recorded (Fig.6).

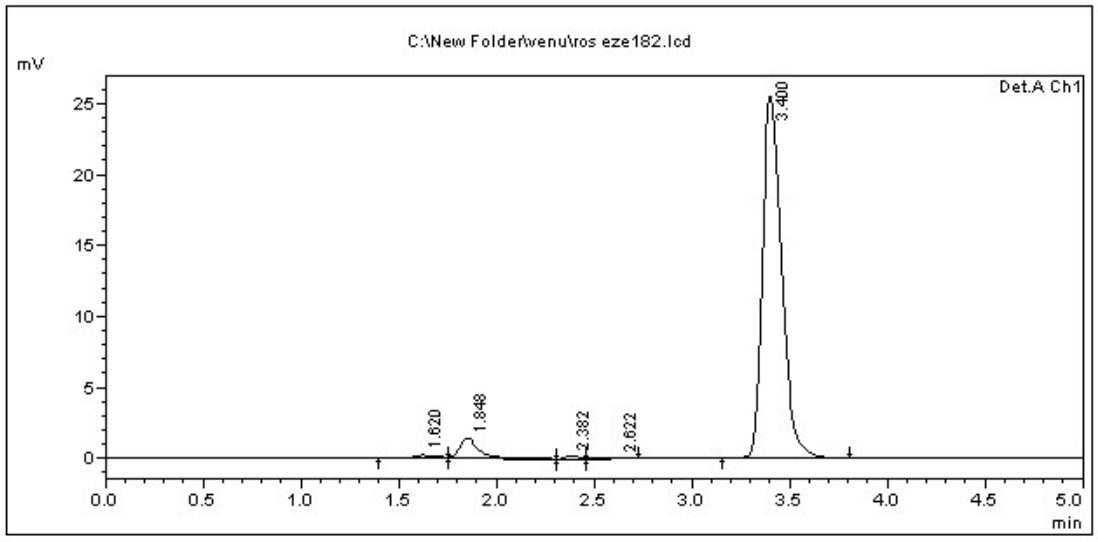

Fig.6: Chromatogram of Apixaban in base degradation (0.1N NaOH)

2.4.9 Oxidative hydrolysis

Accurately weighed 100mg of Apixaban and transferred into 100ml volumetric flask to which 30ml of 3% Hydrogen Peroxide was added. The solution was kept aside for 24hrs. at room temperature. After completion of 24hrs the volumetric flask was filled up to the mark with mobile phase. Then about 1ml of the above solution was transferred into 10ml of volumetric flask and diluted to 10ml using mobile phase (10µg/ml). Then the solution was injected into HPLC and chromatogram was recorded (Fig.7).

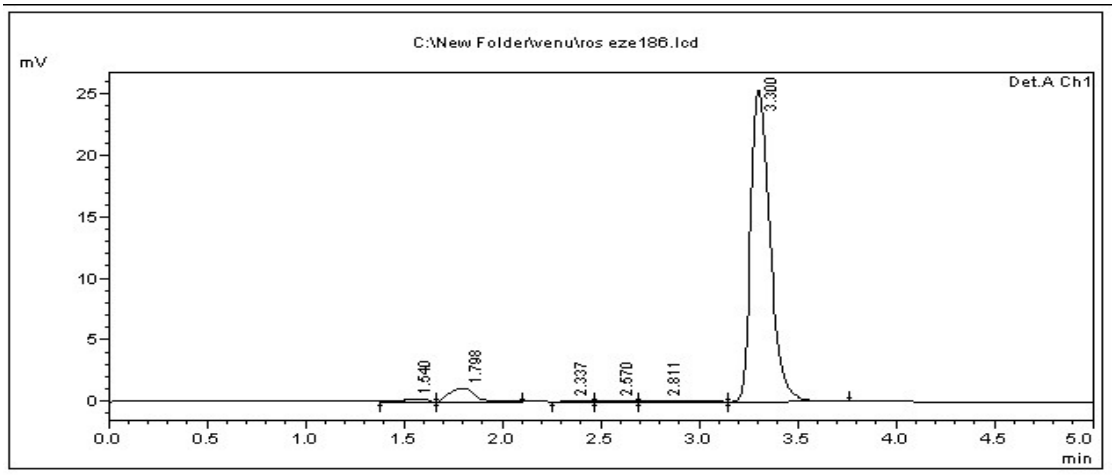

Fig.7: Chromatogram of Apixaban in oxidation condition

2.4.10 Thermal degradation

Accurately weighed 100mg of Apixaban and transferred into a clean, dry petri dish. Petridish was placed in the oven at a temperature of 50 ^{0}C for 5-6 hrs. After completion of 5-6hrs the sample was removed. About 10mg of drug was transferred into 10ml volumetric flask and make up the volume with mobile phase. Then about 0.1ml is transferred into a 10ml volumetric flask and make up to the mark with mobile phase (10µg/ml). Then the solution was injected into the HPLC system and the chromatogram was recorded (Fig.8).

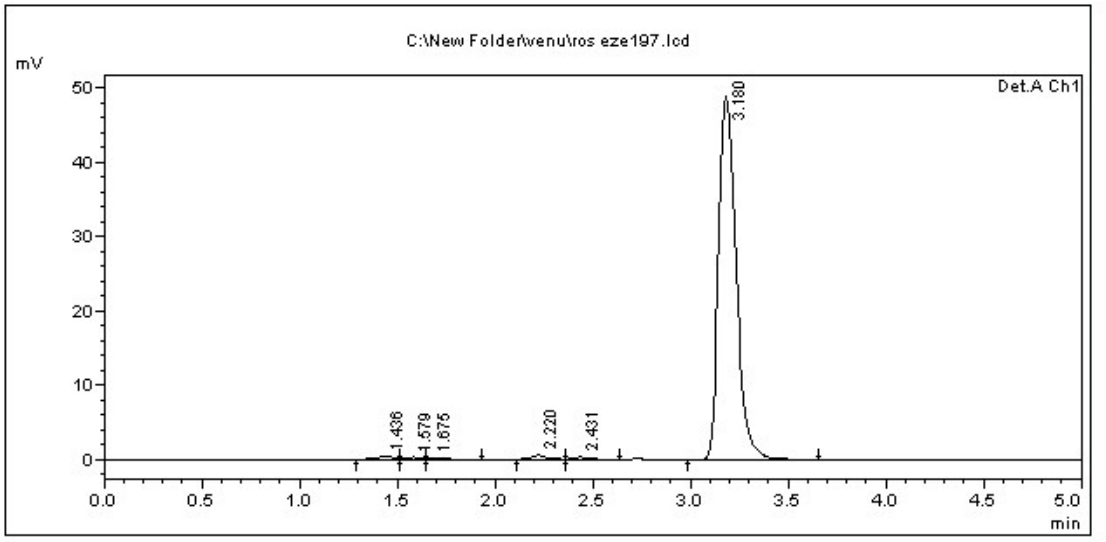

Fig.8: Chromatogram of Apixaban of Thermal degradation (50^{0}C)

2.4.11 Photolytic degradation

Accurately weighed 100mg Apixaban and transferred into a clean, dry petri dish. Petridish was placed in the UV Cabinet at long wave for about 5-6 hrs. After completion of 5-6hrs the sample was removed. About 10mg of drug was transferred into 10ml volumetric flask and make up the volume with mobile phase. Then about 0.1ml is transferred into a 10ml volumetric flask and make up to the mark with mobile phase (10µg/ml). Then the solution was injected into the HPLC system and the chromatogram was recorded (Fig.9).

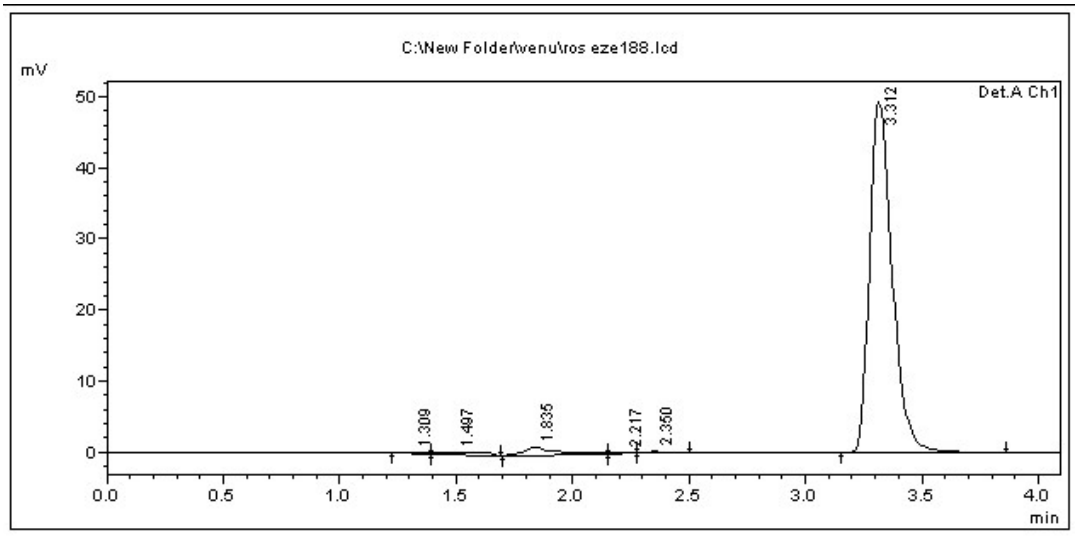

Fig.9: Chromatogram of Apixaban of Photolytic degradation

3. RESULTS AND DISCUSSION

A simple, accurate reverse phase high performance liquid chromatographic method for determination of Apixaban has been developed. In RP-HPLC various columns are available, but here Waters C_{18}, 5µm, 4.6 x 250 mm i.e. column was preferred because using this column peak shape, resolution and absorbance were good and a mobile phase comprising of water and acetonitrile as a mixture in the ratio of (50:50) in an isocratic elution mode with maintaining the column temperature at ambient were preferred (Table 7).

Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, acetonitrile, water, 0.1N NaOH, 0.1NHCl).

The drug was found to be soluble in Water. Using these solvents with appropriate composition newer methods can be developed and validated.

Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Apixaban it is evident that most of the HPLC work can be accomplished in the wavelength range of 259nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 20 µl were found to be the best analysis.

Table 7: Chromatographic Conditions

HPLC System	**Shimadzu Corporate LC-20AD**
Column	Waters X-bridge C18, 5µm (4.6 x 250mm)
Mobile Phase	Acetonitrile: water (50:50% v/v)
Flow rate	1ml/min
Injection Volume	20µl/min
Total run time	5.0 mins
Mode of separation	Isocratic
Detector	UV Detector

ml = Milli Litre; min = Minutes; µm = Micro meter; µl = Micro Litre; mm = Milli meter

The retention time of Apixaban was 3.4mins. the linearity was found to be in the range of 5-25µg/ml. the calibration factor was 0.999, the proposed method was validated according to the ICH guidelines and successfully applied for the determination of all the validation techniques such as Accuracy studies, Robustness, Ruggedness, Linearity, Precision etc., for the determination of Apixaban (Table 8 and 9).

Table 8: System Suitability Test Parameters

S. No	Parameters	Apixaban
1	Range	0-25µg/ml
2	Detection Wavelength	259nm
3	Theoretical Plates	5475.19
4	Tailing Factor	1.3
5	Retention Time	3.4

nm = Nano meter; µg = Micro grams; ml = Milli Litre

Table 9: Assay of formulation

Drug	Amount labelled (mg)	Amount taken to examine (mg)	Peak area	Amount found (µg/ml)	% Assay
Apixaban	2.5	10	377167	10.1	101

mg = Milli gram; µg = Micro grams; ml = Milli Litre

Forced Degradation Studies were carried out at different stress conditions like acidic, alkaline, thermal and photostability and oxidative condition for Apixaban and to study the percentage degradation of Apixaban.

- Apixaban was found to be in the range of 0-25µg/ml.
- The slope, intercept and correlation coefficient values were found to be 35836, 14866 & 0.999 respectively.
- The Limit of Detection was 0.49.
- The Limit of Quantification was 1.49.
- Precision of developed method was studies as:
  a. Intraday precision
  b. Interday precision

Low %RSD values indicate that the method is precise.

- The recovery studies were carried out in three levels i.e. 50%, 100% and 150% by adding known amount of standard drugs. The recovery values were found to be within the limits indicating that the method is accurate.
- In Acidic Condition: 65.9% of drug was undegraded. It was degraded more under Acidic condition.
- In Alkaline Condition: 91.5% of drug was undegraded. It was stable under Alkaline condition.
- In Thermal Studies: 96.35% of drug was undegraded. It was stable under Thermal condition.
- In Photodegradation Studies: 93.9% of drug was undegraded. It was stable under Photolytic condition.
- In Oxidation: 90.0%of drug was undegraded. It was stable under oxidation.

The validated liquid chromatographic method was applied for determination of Apixaban. Apixaban degraded more in Acidic Condition and was stable under Alkaline, Oxidation, Thermal and Photolytic conditions (Table 10 and 11).

Table 10: Forced degradation studies of Apixaban

Name	Retention Time	Peak Area	%Purity	Amount found (µg/ml)	% Not degraded
Blank	3.4	355077	96.8	9.49	94.6
Acid Degradation	3.4	75385	65.9	1.68	16.8
Base Degradation	3.4	175093	91.5	4.47	44.7
Thermal Degradation	3.1	302153	96.3	8.01	80.1
Photolytic Degradation	3.3	331360	93.9	8.83	88.3
Oxidation Degradation	3.3	171218	90.0	4.36	43.6

µg = Micro grams; ml = Milli Litre

Table 11: Study on Degradation

S. No	Stress conditions	% Degradation of Apixaban
1	Acid hydrolysis	83.2
2	Base hydrolysis	55.3
3	Oxidation	19.9
4	Thermal	11.7
5	Photolytic (UV light)	56.4

UV light = Ultra Violet light

4. CONCLUSION

A simple, accurate stability indicating RP-HPLC analytical method has been developed and validated in tablet dosage form of Apixaban. The results of degradation studies reveal that the drug has mostly degraded during acid degradation process.

5. ABBREVIATIONS

HPLC	High Performance Liquid Chromatography
UV	Ultra Violet
LC-MS	Liquid Chromatography – Mass Spectroscopy
µm	Micro Meter
mm	Milli Meter
µg	Microgram
Nm	Nanometer
Cm	Centimeter
µl	Microliters
Ml	Milliliters
Min	Minutes
%	Percentage
^{0}C	Degree Celsius/ centigrade
RSD	Relative Standard Deviation

6. ACKNOWLEDGEMENT

We are very much thankful to Anurag Group of Institutions (Formerly Lalitha College of Pharmacy), Hyderabad, for giving permission to carry out my project work.

7. REFERENCES

1. https://www.drugbank.ca/drugs/DB06605
2. http://www.rxlist.com/eliquis-drug.html
3. Prabhune SS, Jaguste RS, Kondalkar PL, Pradhan NS. Stability-indicating high-performance liquid chromatographic determination of apixaban in the presence of degradation products. Scientia pharmaceutica. 2014 May 22;82(4):777-86.
4. Delavenne X, Mismetti P, Basset T. Rapid determination of apixaban concentration in human plasma by liquid chromatography/tandem mass spectrometry: application to pharmacokinetic study. Journal of pharmaceutical and biomedical analysis. 2013 May 5;78:150-3.
5. Baldelli S, Cattaneo D, Pignatelli P, Perrone V, Pastori D, Radice S, et al. Validation of an LC–MS/MS method for the simultaneous quantification of dabigatran, rivaroxaban and apixaban in human plasma. Bioanalysis. 2016 Feb;8(4):275-83.
6. El-Bagary RI, Elkady EF, Farid NA, Youssef NF. Validated spectrofluorimetric methods for the determination of apixaban and tirofiban hydrochloride in pharmaceutical formulations. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2017 Mar 5;174:326-30.

7. Gouin-Thibault I, Flaujac C, Delavenne X, Quenet S, Horellou MH, Laporte S, et al. Assessment of apixaban plasma levels by laboratory tests: suitability of three anti-Xa assays. Thrombosis and haemostasis. 2014;111(2):240-8.
8. Gurdeep Chatwal and Sham Anand, Instrumental methods of chemical analysis, 1st Edition, Himalaya publishing House, Delhi, 1999.
9. Synder LR and Kirkland JJ, Introduction to modern liquid chromatography, Wiley interscience, New York, 1979.
10. ICH harmonized tripartite guideline, Text on validation of analytical procedures, Recommended for adoption at step 4 of the ICH process by the ICH steering committee
11. International Conference on Harmonization: Guidelines of Photostability testing of new drug substances and Products (Q1B), 1996
12. ICH Guideline, Q2 (R1), Validation of Analytical Procedures, Text and Methodology, November 2005.