IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
August 2020 Vol.:19, Issue:1

To Develop and Validate RP-HPLC Method for Dabigatran Etexilate Mesylate in Bulk and Tablet Form

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Pranali P. Kalyankar*, Charushila J. Bhangale, Kiran B. Dhamak, Mayur T. Gaikar

Pravara Rural Education Society, Pravaranagar College of Pharmacy (For Womens), Chincholi, Sinnar, Nashik. 422102. (MS), India.

Submission: 24 July 2020
Accepted: 30 July 2020
Published: 30 August 2020

Keywords: HPLC, Dabigatran Etexilate Mesylate, validation, stability studies

ABSTRACT

A simple, precise, accurate, economical and reproducible RP-HPLC method for estimation of Dabigatran Etexilate Mesylate in capsule dosage form has been developed. Quantitative HPLC was performed with HPLC Binary Gradient System using software HPLC workstation, model no. HPLC 3000 series, and company Analytical Technologies Ltd. with UV-Visible - 3000m detector using column Grace C_{18} ×4.6ID, particle size: 5 micron. The mobile phase used in study was methanol: water (90:10). The condition optimized were flow rate of 0.8ml/min, column temperature was maintained at room temperature. Retention time was found to be 4.8 min. The linearity was found to be in the concentration range of 10-50 µg/ml. The developed method was evaluated in the assay of commercially available capsules Paradaxa containing Dabigatran Etexilate Mesylate. The amount of drug in capsule was found to be 75mg. Results of analysis were validated statistically and by recovery studies. The recovery studies 96.67 % was indicative of the accuracy of proposed method. The precision studies were carried out the Interday precision and intraday precision was found to be 0.08% and 0.07%. The Limit of detection and limit of quantitation was found to be 0.51µg/ml and 1.54µg/ml. The robustness of the method was studied. The stability studies of drug was also been carried out.

INTRODUCTION

Dabigatran, sold under the brand name Pradaxa, FDA approved on October 19, 2010, is an anticoagulant medication which can be taken by mouth. It is used as an alternative to warfarin, since it does not have to be monitored by blood tests, but offers similar results in terms of efficacy. It is a direct thrombin inhibitor, and functions by directly inhibiting both free and fibrin-bound thrombin. Dabigatran is considered a "reversible" anticoagulant medication. There is a specific antidote, idarucizumab, which reverses the effect of dabigatran. Dabigatran etexilate is an oral prodrug that is metabolized by a serum esterase to dabigatran. It is a synthetic, competitive and reversible direct thrombin inhibitor. Inhibition of thrombin disrupts the coagulation cascade and inhibits the formation of clots. Dabigatran etexilate may be used to decrease the risk of venous thromboembolic events in patients who have undergone total hip or knee replacement surgery or to prevent stroke and systemic embolism in patients with atrial fibrillation, in whom anticoagulation therapy is indicated. In contrast to warfarin, because its anticoagulant effects are predictable, lab monitoring is not necessary. [12-18]

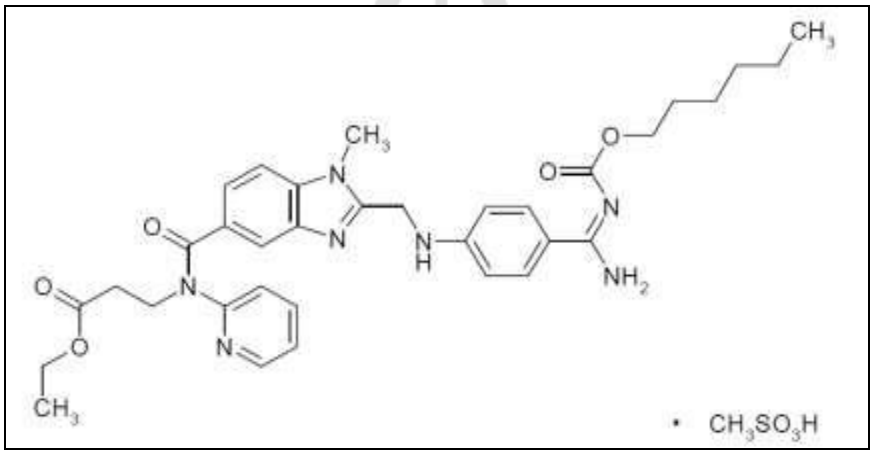

Figure No. 1: Molecular structure of Dabigatran etexilate mesylate

MATERIALS AND METHODS

Instruments Used:

HPLC system consists of Analytical Technologies Ltd. having model HPLC 3000 Series and other instruments used such as Electronic weighing balance, UV–Visible Spectrophotometer, Digital pH meter, vacuum pump and ultra Sonicator were used.

Chemicals and Reagent:

Dabigatran etixilate mesylate was purchased from vidisha analytical Pvt Ltd Nashik, HPLC grade methanol, water and all other chemicals which are analytical grade such as ortho-phosphoric acid, was used for analysis.

Preliminary analysis of drug: [19-29]

Dabigatran etixilate mesylate are official in USP. Hence, Preliminary analysis of Dabigatran were performed according to USP.

Description:

The sample of Dabigatran etixilate was observed for its colour and texture.

Solubility:

The solubility was studied in water, methanol, ethanol, and other solvents.

Identification Test:

Determine by infrared absorption Spectrophotometry. Compare the spectrum with that obtained with Dabigatran etixilate mesylate (given in USP 2002).

Assay:

10 mg of Dabigatran etixilate mesylate was weighed dissolved in 10ml of solvent (methanol: water) that give 1000ppm stock solution of given Drug. Assay was performed.

Selection of HPLC Parameter

Selection of Mobile Phase and its Strength:

Solution of Dabigatran etixilate mesylate was prepared and injected into the HPLC system. The solution was analyzed using different proportion of Methanol: HPLC Grade water such as 80:20, 90:10% v/v.

Selection of Mobile Phase pH:

pH of HPLC grade water was adjusted to 3 using 1% orthophosphoric acid. The solution of Dabigatran etixilate mesylate (10µg/ml) was prepared and injected into the HPLC system.

Selection of Flow Rate:

Chromatogram of solution of Dabigatran etixilate mesylate (10µg/ml) 1.2, 1, 0.8ml/min.

Selection of Analytical Wavelength:

The standard solution of Dabigatran etixilate mesylate (10µg/ml) in mobile phase were scanned separately in the UV region of 190 to 400 nm and the overlain spectra were recorded.

Instrumentation and Finalized Chromatographic Conditions:[3-11]

HPLC was used for analytical validation of Dabigatran etixilate mesylate having chromatographic conditions such as:

- ✓ **Analytical Column** Grace c18(250mm × 4.6ID, Particle size; 5 micron)
- ✓ **Mobile Phase:** Methanol: HPLC Grade Water (80:20 % v/v)
- ✓ **pH of the HPLC Grade Water:** Adjusted to 3.0 using 1% orthophosphoric acid
- ✓ **Injection volume:** 20µl
- ✓ **Flow Rate:** 0.8ml/min
- ✓ **Detection Wavelength:** 245nm
- ✓ **AUFS:** 0.1000
- ✓ **Pressure Adjusted:** Minimum Pressure: 2MPa

Maximum Pressure: 15MPa

Preparation of Mobile Phase:

Mobile phase was prepared by mixing 900 ml of methanol and 100 ml of HPLC grade water whose pH was adjusted to 3 using 1% orthophosphoric acid. The mobile phase was filtered through 0.2µm Supor 200 membrane filter using Vacuum Pump and ultrasonicated for 10 min.

Preparation of Standard Stock Solution of dabigatran elexilate mesylate:

For 1000ppm solution of drug: 0.01gm of pure drug dissolve in 10ml to get 1000ppm.

Table No. 1: Dilutions of Standard Stock Solution

Sr. No.	Concentration (ppm)	Volume of Stock sol. (ml)	Volume made up to (ml)
1	10	0.1	10ml
2	20	0.2	10 ml
3	30	0.3	10 ml
4	40	0.4	10 ml
5	50	0.5	10 ml

Analysis of capsule Formulation:

Ten capsules of Dabigatran etixilate mesylate (Paradaxa 75mg Mfg. by: Boehringer Ingelheim Pvt. Ltd.) were open and removed for its powder content. An accurately weighed powder equivalent to about 10mg of Dabigatran etexilate mesylate was transfer into 100ml volumetric flask and sonicated for the 10min 25ml of HPLC grade methanol and made up the volume with the same solvent of 100µg/ml of Dabigatran etexilate mesylate. The resulting solution filtered through Whatman filter paper no 41 and this solution was used as Stock solution means to prepare 1000ppm of stock solution 28.65mg of capsule contents were weighed and dissolved in 10ml to get 1000ppm.

Table No. 2: Calculation of average weight of capsules

Sr. No.	Weight of individual capsules content (mg)	Average
1	214	214.9mg
2	213	
3	217	
4	214	
5	216	
6	211	
7	217	
8	218	
9	214	
10	215	

Validation of Reverse phase high performance liquid chromatography method: [1,2]

Accuracy:

To study the accuracy, 10 capsules content was removed analysis of the same was carried out. Recovery studied were carried out by standard additional method by adding the known amount of Dabigatran etexilate mesylate to the pre-analyzed sample at three different concentration level i.e. 50%, 100%, 150% of assay concentration of % recoveries were calculated.

Precision:

The precision of an analytical method was studied by performing intraday and inter-day precision.

Intra-day Precision:

Intra-day precision was determined by analyzing the standard solution of Dabigatran etexilate mesylate (30 µg/ml) at three different time intervals on same day (Evening).

Inter-day Precision: Inter- day precision was determined by analyzing the standard solution of Dabigatran etexilate mesylate (30 µg/ml) on three consecutive days (Afternoon).

Linearity:

The concentration of ranges 5- 50µg/ml were prepared and analyzed. From the data linearity was determined.

Limit of Detection and Limit of Quantitation:

Detection limit and quantitation limit were determined based on the standard deviation of y-intercepts of five Calibration curve and average slope of six calibration curves as mentioned.

Robustness:

Standard solution of Dabigatran etixilate mesylate were prepared and analyzed i.e. (10 µg/ml) at different Wavelength.

RESULTS AND DISCUSSION

Preliminary analysis:

Description:

The sample of Dabigatran etixilate was observed for its colour and texture. It is yellow white to yellow powder.

Solubility:

The solubility in water is 1.8mg/ml; it is freely soluble in methanol, soluble in ethanol, sparingly soluble in isopropanol, very slightly soluble in acetone and practically insoluble in ethyl acetate.

Identification Test:

Determine by infrared absorption Spectrophotometry. Compare the spectrum with that obtained with Dabigatran etixilate mesylate (given in USP 2002).

UV- Visible Spectrometric Method:

Selection of Analytical Wavelength:

The sensitivity of HPLC method depends upon proper selection of detection wavelength. An ideal wavelength is one that gives good response for the drug that is to be detected. Appropriate dilution of stock solution with mobile phase, various concentrations of Rosuvastatin were prepared. The solution was scanned in the spectrum mode between the range 200 to 400 nm were overlaid. The wavelength selected for the analysis was 245 nm at

which drugs showed significant absorbance. The overlain UV spectrum of Dabigatran in the mobile phase is as shown in Fig. No. 2. [21]

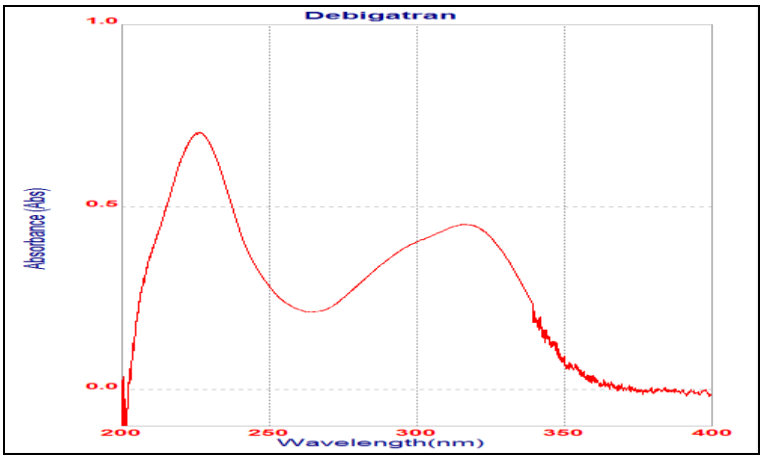

Figure No. 2: Spectra of Dabigatran etexilate mesylate

Reverse phase High performance liquid chromatography:

Results of Optimization of Chromatographic Conditions:

The optimization of HPLC method were done for the selection of proper mobile phase for method development Pure drug products were injected and run in different solvent systems. In this, different trials are taken with different ratio of mobile phase. For trials methanol and water at different flow rate and pH were used. Different combinations of mobile phases were tried for selections of proper mobile phase are given below table no. 3. [19]

Table No. 3: Optimization of Chromatographic Conditions

Parameter	**Observation**	
Mobile Phase Composition	80:20	90:10
pH	3	3
Flow Rate (ml/min)	0.8	0.8
Drug	Dabigatran	Dabigatran
Retention time (min)	11.260	6.198
Area (mV /s)	1822999	2413255
Run time	15.11	9.38
Theoretical Plates	2188	4127
Resolution	0.00	0.00
Asymmetry	1.36	1.38

Trail 1:

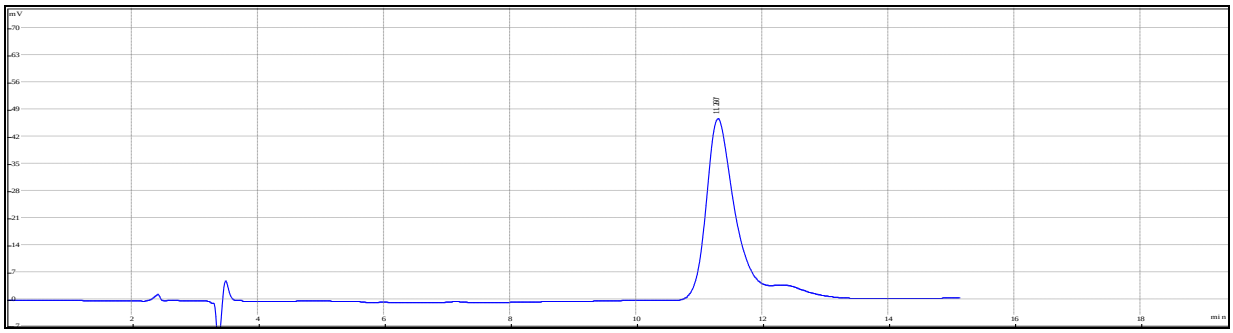

Figure No. 3: Chromatogram of Dabigatran in Methanol: HPLC Water (80:20 % v/v, pH 3) at flow rate of 0.8ml/min at 227nm.

Table No. 4: Optimization of Chromatographic Conditions: Trail 1

Time	Area	Theoretical Plate Number	Asymmetry
11.26	1822999	2188	1.36

For the RP-HPLC method development of Dabigatran etixilate mesylate two trials were conducted. In the Trial 1: the composition of mobile phase was taken as Methanol: HPLC grade water 80: 20% v/v pH:3 with the flow rate of 0.8ml/ min at 227 nm where the retention time of dabigatran etexilate mesylate found to be 11.26 min which was found to be greater. The chromatogram of Trial: 1 is shown in figure: 3 with its retention time. Hence it was decided to change the composition of the mobile phase and to carry out the Trial: 2. In the Trial: 2 the composition of mobile phase was taken as Methanol: HPLC grade water 90:10% v/v pH: 3 with the flow rate of 0.8ml/min at 227nm where resolution of peak, number of theoretical plate, asymmetry and the retention time found to be 6.19 min which was found satisfactory. The chromatogram for Trial: 2 is shown in figure no. 4.

Trail 2:

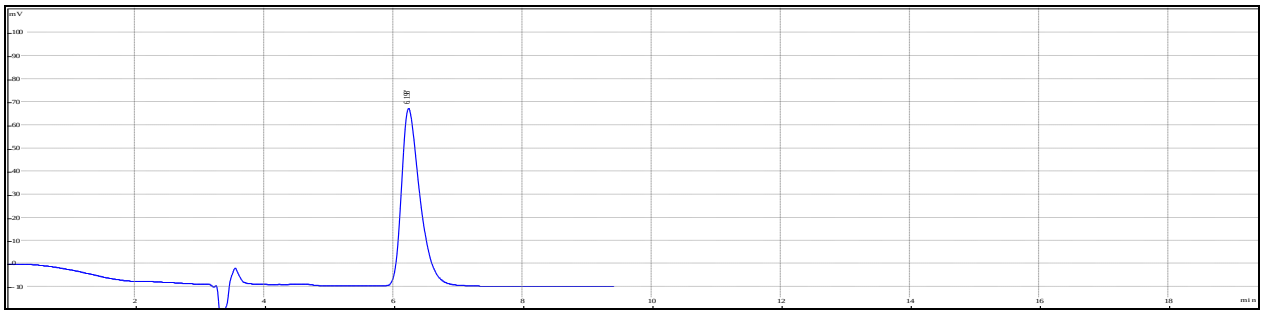

Figure No. 4: Chromatogram of Dabigatran in Methanol: HPLC Water (90:10 % v/v, pH 3) at flow rate of 0.8ml/min at 227nm.

Table No. 5: Optimization of Chromatographic Conditions: Trail 2

Time	Area	Theoretical Plate Number	Asymmetry
6.19	2413255	4127	1.38

Optimized parameters for HPLC method:

Table No. 6: Optimized parameters for HPLC method

Sr. No.	Parameter	Description
1	Stationary Phase	Grace c18 (250mm × 4.6ID, Particle size, 5 micron)
2	Mobile Phase	HPLC Grade Water (90:10 % v/v)+ 1% orthophosphoric acid
3	Flow Rate	0.8 ml/min
4	Detection wavelength	227 nm
5	Detector	UV detector
6	Injector	Rheodyne Injection
7	Injection volume	20µl
8	Column Temperature	Room Temperature
9	Run Time	10 min

Preparation of standard stock solutions:

28.65 mg of dabigatran was weighed dissolved in 10ml of solvent (methanol: water 90:10) that give 1000ppm stock solution of given Drug. From the above prepared stock solution, 30 ppm solution was prepared and used as standard stock solution.

Analysis of capsules formulation:-[15]

DEBIGATRAN ETXILATE MESYLATE CAPSULES	
PARADAXA 75mg	10 Capsules
Dabigatran Etexilate Mesylate…….75mg	Batch No: 606505 Mfg Date: 08/2016 Exp Date:07/2019 MRP: 718.00 Rs.
Colour: Yellow-white to yellow.	
Storage: Store at a temperature not exceeding 30°c. Protect from moisture.	
Dosage: As directed by Physician	
Mfg By: Boehringer Ingelheim Pvt. Ltd.	

Procedure for capsule sample preparation

214.9 mg contains 75 mg of drug, wt. of contents to take equivalent of 10 mg of drug = 28.65 mg

28.65 mg of capsule contents were weighed and dissolved in 10 ml to get 1000 ppm. Further dilutions were prepared as given.

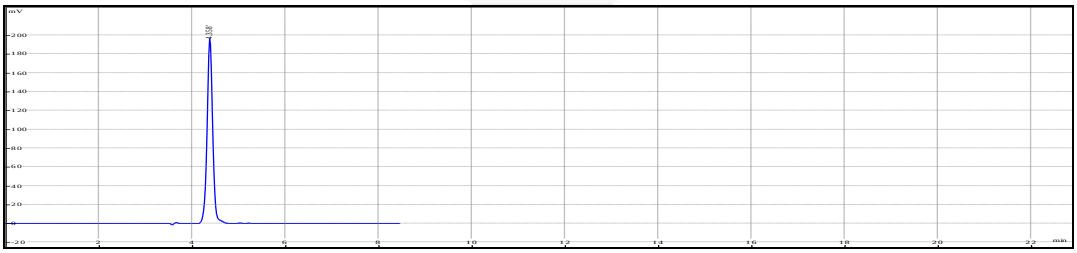

Figure No. 5: Chromatogram of standard Dabigatran

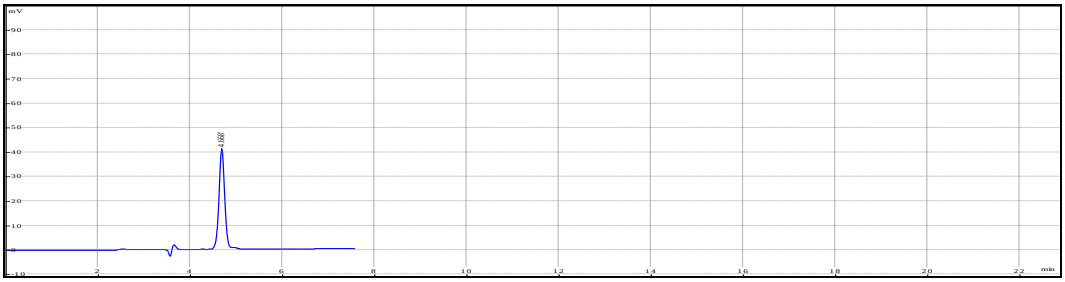

Figure No. 6: Chromatogram of sample Dabigatran

Chromatogram of Dabigetran in Methanol: HPLC Water (90:10 % v/v, pH 3) at flow rate of 1 ml/min at 227nm.

Table No. 7: Observation table of Chromatogram of standard and sample of Dabigatran

	Time	Area	Theoretical Plate Number	Asymmetry
Standard	4.78	3525522	7339	1.32
Sample	4.86	3320314	7641	1.24

Table No. 8: Result of percent assay of Dabigatran

Sr. No.	Percent Composition	Area of Standard	Area of Sample	% Assay
1	Dabigatran	3525522	3320314	94.1793584

The assay of Dabigatran was carried out for the capsule formulation the retention time of the sample Dabigatran found to be 4.86 with theoretical plate 7641 and asymmetry 1.24.

VALIDATION:-[25, 26, 27, 28, 29]

Accuracy:

Table No. 9: Results of Accuracy for RP-HPLC Method

Sr. No.	Conc.	Area	Standard Deviation		Accuracy	Precision
			Mean	SD	%SD	%RSD
	10	1169014				
1	10	1172591	1170659.333	1805.608577	0.1542386	0.154238601
	10	1170373				
	30	3525522				
2	30	3522464	3523323	1919.515824	0.05448027	0.054480268
	30	3521983				
	50	5723150				
3	50	5737425	5730603.667	7158.476817	0.12491663	0.124916627
	50	5731236				

Precision:

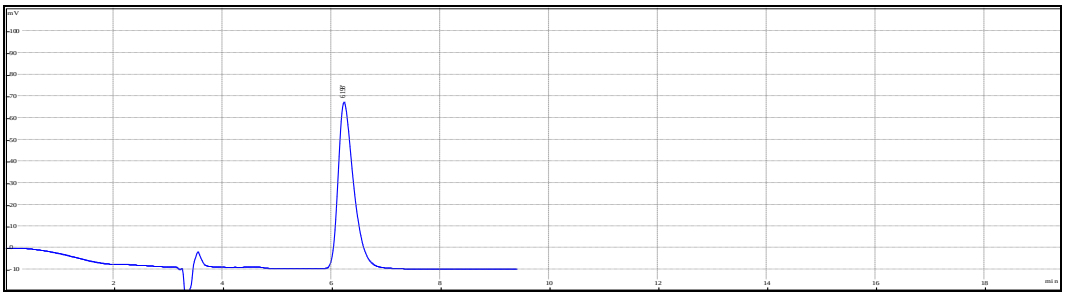

Figure No. 7: Chromatogram of Dabigatran in Methanol: HPLC Water (90:10 % v/v, pH 3) at flow rate of 0.8ml/min at 227nm.

Inter-day Precision

Table No. 10: Observations table of Chromatogram of inter-day precision of Dabigatran

	Sr. No.	Time	Area	Theoretical Plate Number	Asymmetry
Day 1	**1**	4.787	3525522	7339	1.32
	2	4.828	3522464	7190	1.31
	3	4.837	3521983	7224	1.28
Day 2	**4**	4.885	3522313	7686	1.23
	5	4.863	3529134	6598	1.23
	6	4.888	3534395	6987	1.21

Intra-day Precision:

Table No. 11: Observation table of Chromatogram of intraday precision of Dabigatran

	Sr. No.	Time	Area	Theoretical Plate Number	Asymmetry
Morning	**1**	4.787	3525522	7339	1.32
	2	4.828	3522464	7190	1.31
	3	4.837	3521983	7224	1.28
Evening	**4**	4.906	3527544	6965	1.20
	5	4.903	3524719	7995	1.19
	6	4.908	3528323	7563	1.19

Table No. 12: Results of Precision of Dabigatran

Intraday	Morning			Evening			Mean	% RSD
	3525522	3522464	3521983	3527544	3524719	3528323	3525093	0.07 %
Interday	**Day 1**			**Day 2**				
	3525522	3522464	3525522	3522464	3525522	3522464	3524395	0.08 %

Linearity:

Table No. 13: Observation table of Chromatogram of linearity of Dabigatran

Sr. No.	Conc.	Area	Time	Area	Theoretical Plate Number	Asymmetry
1	**10**	1169014	4.798	1169014	7383	1.19
2	**20**	2341385	4.778	2341385	7995	1.23
3	**30**	3525522	4.787	3525522	7339	1.32
4	**40**	4541880	4.886	4541880	7294	1.18
5	**50**	5723150	4.915	5723150	7585	1.19

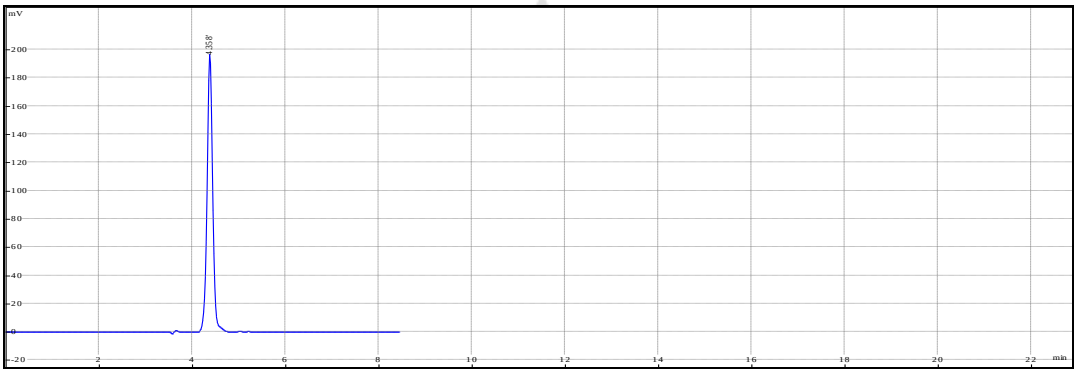

Figure No. 8: Chromatogram of Dabigatran in Methanol: HPLC Water (90:10 % v/v, pH 3) at flow rate of 0.8ml/min at 227nm

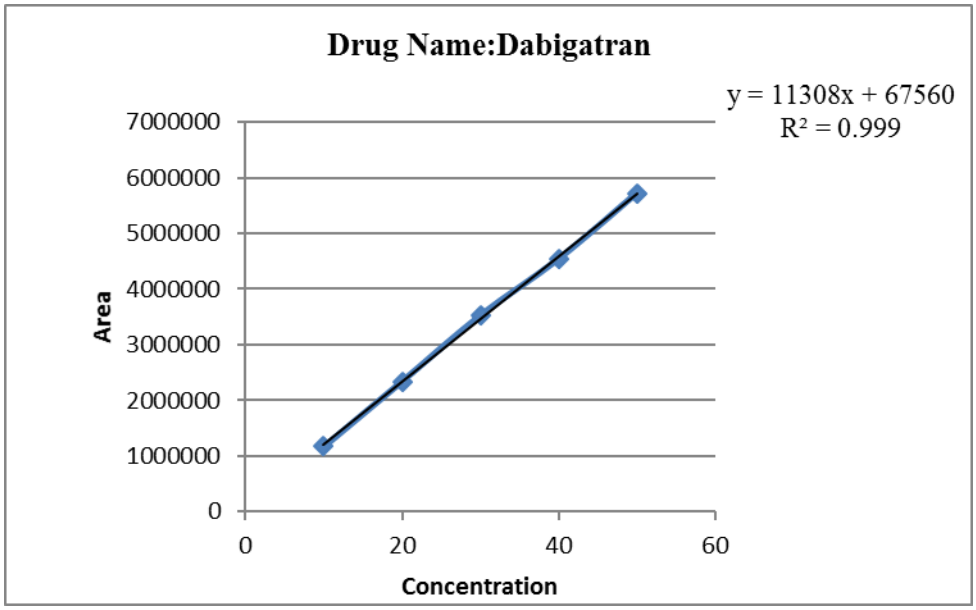

Figure No. 9: Calibration curve of Dabigatran

Limit of Detection and Limit of Quantitation:

Limit of Detection:

$$LOD = \frac{3.3 \times \%\ \text{Std.Deviation}}{\text{Slope}}$$

$$\frac{3.3 \times 0.1542}{0.999} = 0.509\mu g/ml$$

Limit of Quantitation:

$$LOQ = \frac{10 \times \%\ \text{Std.Deviation}}{\text{Slope}}$$

$$\frac{10 \times 0.1542}{0.999} = 1.5435\mu g/ml$$

Robustness:

Chromatograms for Change in flow rate:

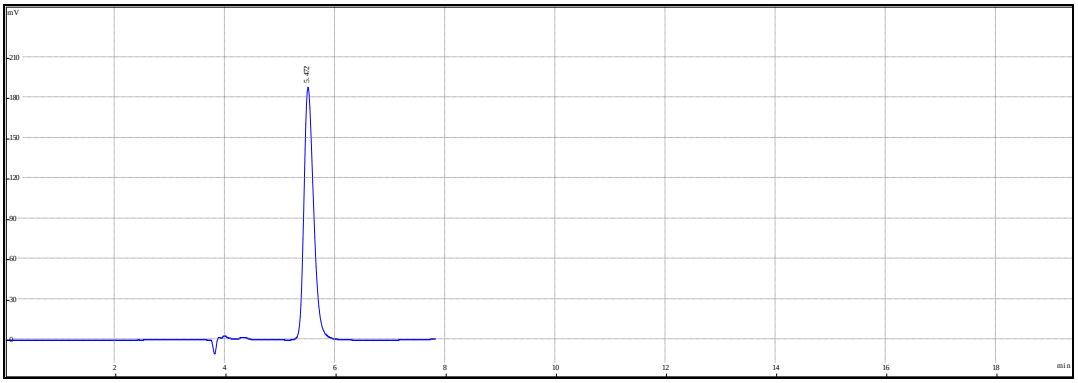

Figure No. 10: Chromatogram of Dabigatran in Methanol: HPLC Water (90:10 % v/v, pH 3) at flow rate of 0.8ml/min at 227 nm.

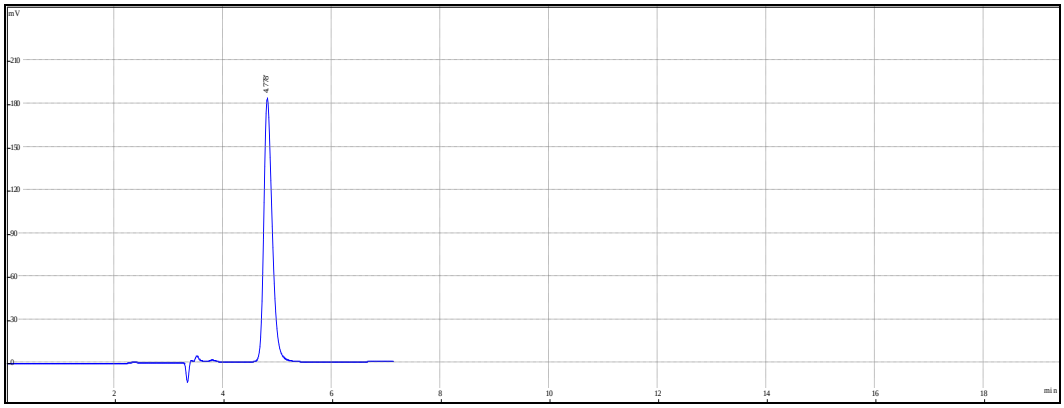

Figure No. 11: Chromatogram of Dabigatran in Methanol: HPLC Water (90:10 % v/v, pH 3) at flow rate of 1ml/min at 227nm.

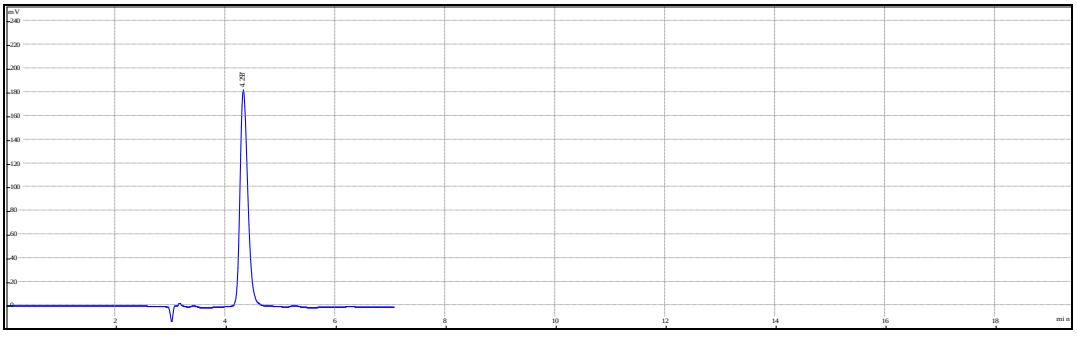

Figure No. 12: Chromatogram of Dabigatran in Methanol: HPLC Water (90:10 % v/v, pH 3) at flow rate of 1.2 ml/min at 227nm.

Table No. 14: Observation table of Chromatogram of Robustness of Dabigatran (change in flow rate)

Sr. No.	Flow rate	Retention Time	Area	Theoretical Plate Number	Asymmetry
1	0.8	5.472	2340669	6632	1.22
2	1.0	4.778	2341385	7995	1.23
3	1.2	4.293	2344535	7152	1.30

Chromatograms for Change in wavelength:

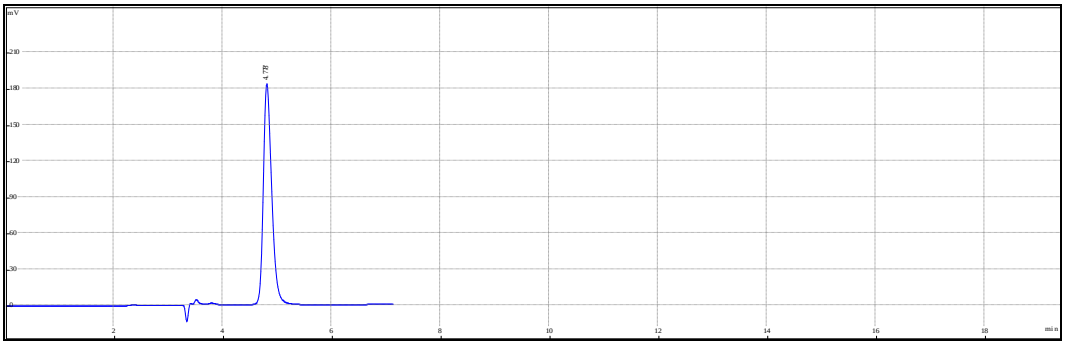

Figure No. 13: Chromatogram of Dabigatran in Methanol: HPLC Water (90:10 % v/v, pH 3) at flow rate of 0.8 ml/min at 225nm.

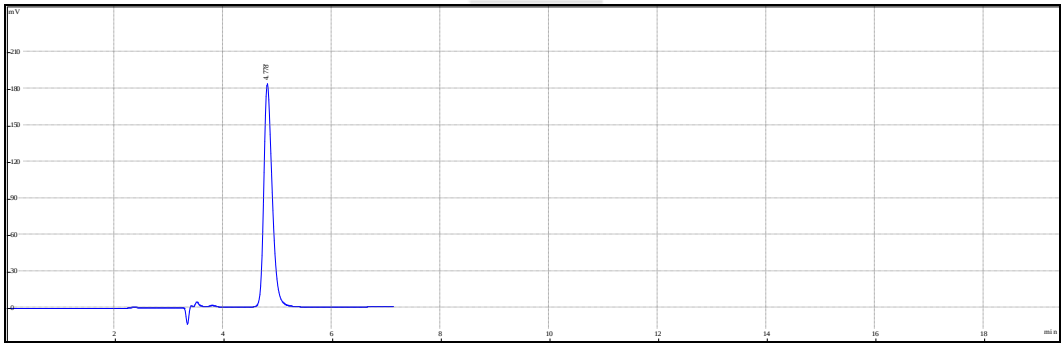

Figure No. 14: Chromatogram of Dabigatran in Methanol: HPLC Water (90:10 % v/v, pH 3) at flow rate of 0.8 ml/min at 227nm.

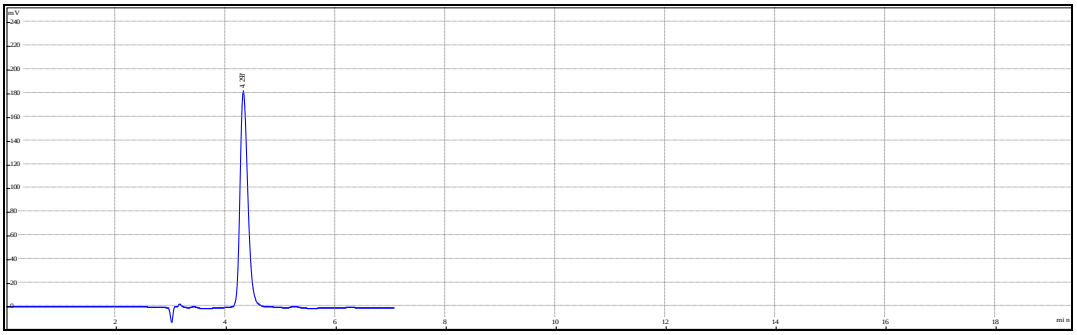

Figure No. 15: Chromatogram of Dabigatran in Methanol: HPLC Water (90:10 % v/v, pH 3) at flow rate of 0.8 ml/min at 229nm.

Table No. 15: Observation table of Chromatogram of Robustness of Dabigatran (change in wavelength)

Sr. No.	Wavelength	Time	Area	Theoretical Plate Number	Asymmetry
1	225	4.778	2341385	7995	1.23
2	227	4.751	2343064	6542	1.32
3	229	4.762	2329106	6964	1.33

Table No. 16: Result of Robustness of Dabigatran

Parameters	Conc.	Area	Mean	SD	%SD
Change in flow rate	10	2340669	2342196	2056.74	0.08781251
	10	2341385			
	10	2344535			
Change in wavelength	10	2341385	2337852	7620.35	0.32595535
	10	2343064			
	10	2329106			

Percent Recovery:

A) Sample Name: Dabigatran20+10ppm 50% Recovery

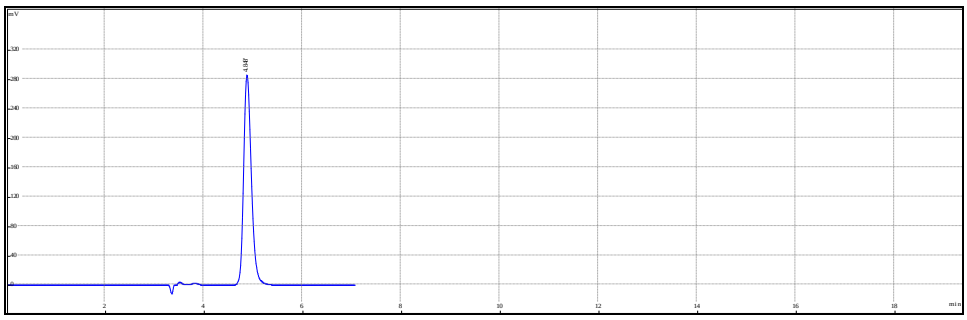

Figure No. 16: Chromatogram of 50% Recovery of Dabigatran in Methanol: HPLC Water (90:10 % v/v, pH 3) at flow rate of 1 ml/min at 227nm

B) Sample Name: Dabigatran20+20ppm 100% Recovery

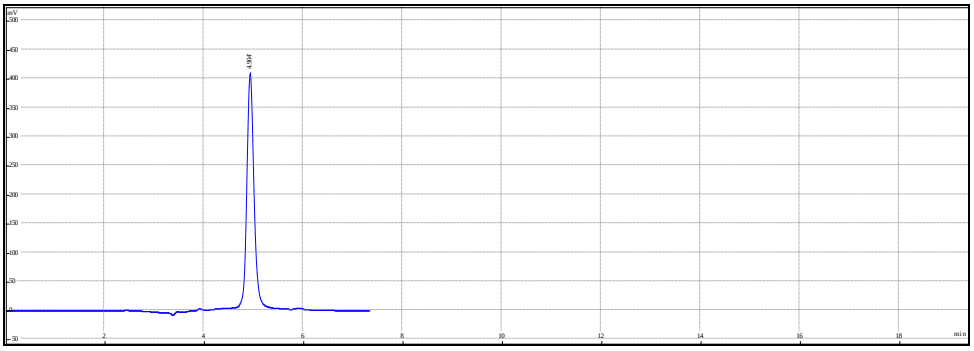

Figure No. 17: Chromatogram of 100% Recovery of Dabigatran in Methanol: HPLC Water (90:10 % v/v, pH 3) at flow rate of 1 ml/min at 227nm

C) Sample Name: Dabigatran 20+30ppm 150% Recovery

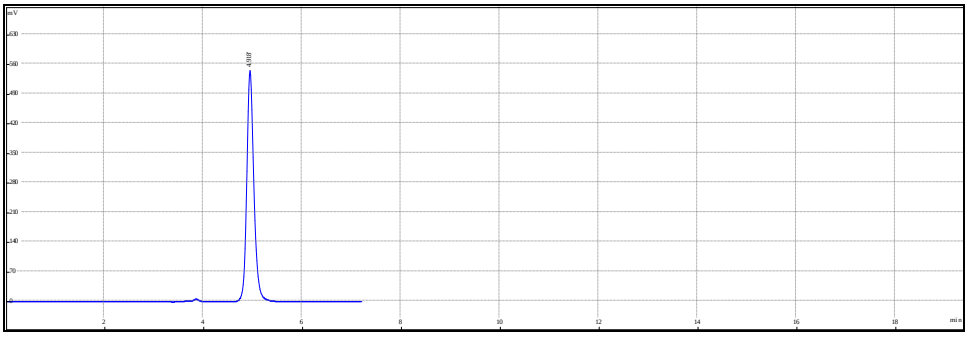

Figure No. 18: Chromatogram of 150% Recovery of Dabigatran in Methanol: HPLC Water (90:10 % v/v, pH 3) at flow rate of 1 ml/min at 227nm

Table No. 17: Observation table of chromatogram of % Recovery of Dabigatran

Sr. No.	Time	Area	Theoretical Plate Number	Asymmetry	% Recovery
1	4.848	3524691	7229	1.26	50
2	4.904	4539894	7495	1.13	100
3	4.918	5716981	7403	1.19	150

Table No. 18: Result of % Recovery of Dabigatran

Sr. No.	% Composition	Area of Standard	Area of Sample	% Recovery
1	50	3525522	3524691	99.9742902
2	100	4541880	4539894	99.95627361
3	150	5723150	5716981	99.89220971

DEGRADATION STUDIES

Acid degradation study:

Sample Name: Dabigatran 50ppm Acid Degradation

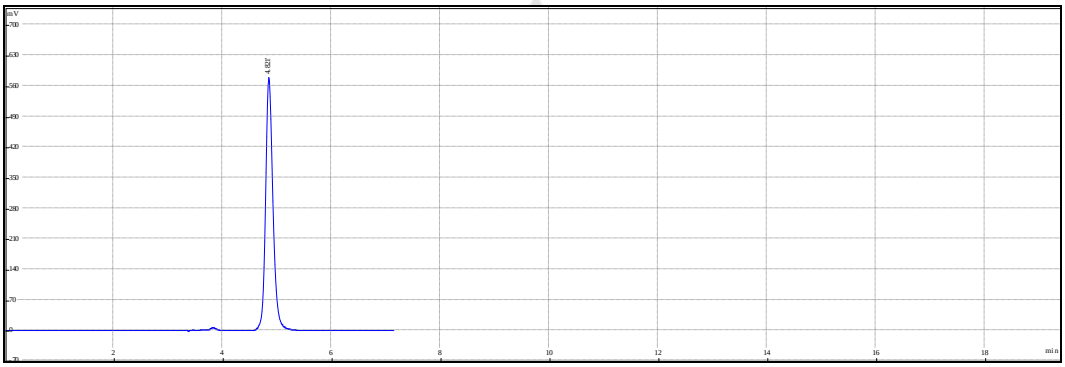

Figure No. 19: Chromatogram of Acid degradation of Dabigatran in Methanol: HPLC Water (90:10 % v/v, pH 3) at flow rate of 0.8 ml/min at 227nm

Table No. 19: Observation table of degradation studies of Dabigatran

Sr. No.	Type of degradation	Time	Area	Theoretical plate number	Asymmetry
1	Acid	4.821	5479354	6106	1.19
	Base	4.817	5504936	6099	1.20
	H_2O_2	4.783	5227490	6477	1.21
	Photolytic	4.821	5632716	7385	1.19
	Thermolytic	4.911	5579810	6439	1.18

Table No. 20: Result of Acid degradation of Dabigatran

Sr. No.	Type of Degradation	Area of Standard	Area of degraded Sample	Degraded up to %	Actual degradation %
1.	Acid	5723150	5479354	95.74017805	4.259821951
2.	Base	5723150	5504936	96.18716995	3.812830347
3.	H_2O_2	5723150	5227490	91.3398478	8.660615221
4.	Photolytic	5723150	5632716	98.4198562	1.580143802
5.	Thermolytic Degradation	5723150	5579810	97.49543521	2.504564794

Base degradation study:

Sample Name: Dabigatran50ppm Base Degradation

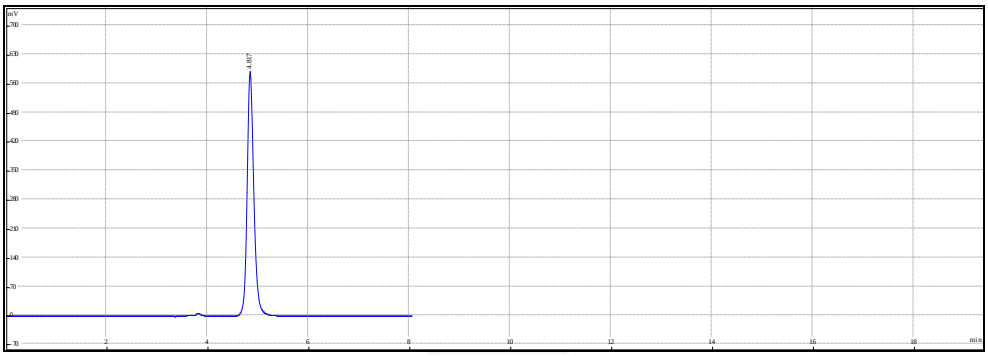

Figure No. 20: Chromatogram of Base degradation of Dabigatran in Methanol: HPLC Water (90:10 % v/v, pH 3) at flow rate of 0.8 ml/min at 227nm

H_2O_2 degradation study:

Sample Name: Dabigatran 50ppm H_2O_2 Degradation

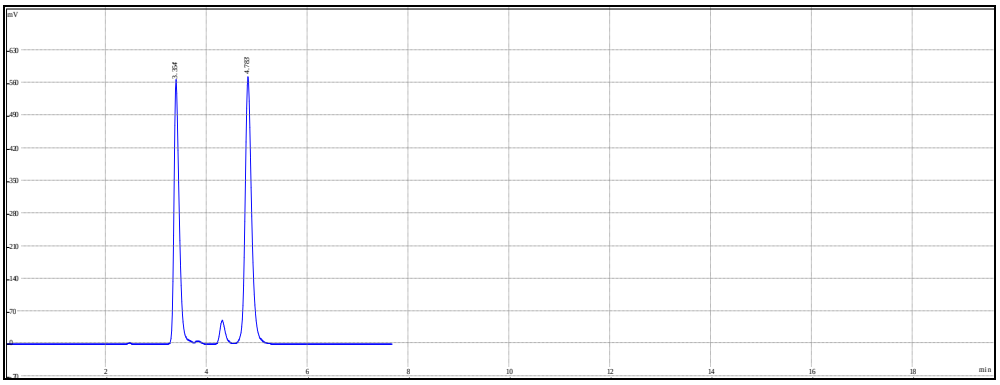

Figure No. 21: Chromatogram of H_2O_2 degradation of Dabigatran in Methanol: HPLC Water (90:10 % v/v, pH 3) at flow rate of 0.8 ml/min at 227nm

Photolytic degradation study:

D) Sample Name: Dabigatran50ppm Photolytic Degradation

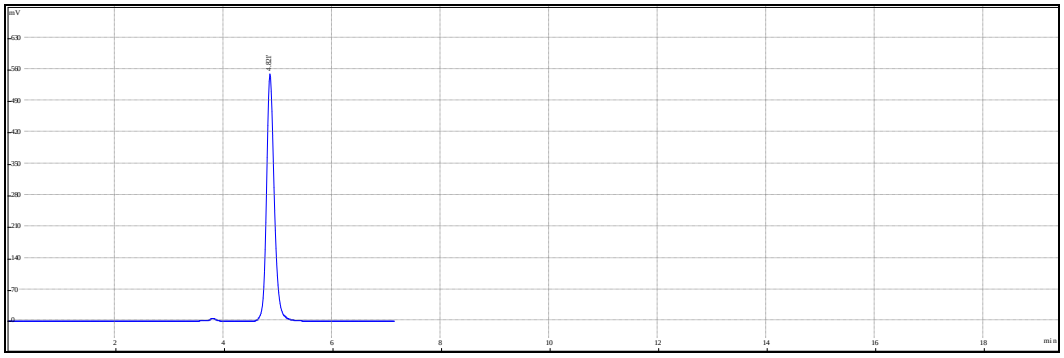

Figure No. 22: Chromatogram of Photolytic degradation of Dabigatran in Methanol: HPLC Water (90:10 % v/v, pH 3) at flow rate of 0.8 ml/min at 227nm

Thermolytic degradation study:

Sample Name: Dabigatran50ppm Thermolytic Degradation

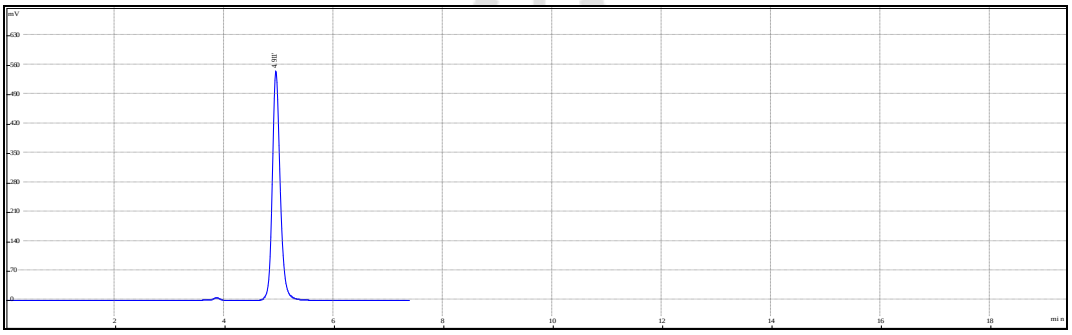

Figure No. 23: Chromatogram of Thermolytic degradation of Dabigatran in Methanol: HPLC Water (90:10 % v/v, pH 3) at flow rate of 0.8 ml/min at 227nm

CONCLUSION

The proposed method is simple sensitive and reproducible and hence can be used in routine analysis for determination of Dabigatran etixilate mesylate in bulk as well as in pharmaceutical formulations. Statistical analysis of the results has been carried out revealing high accuracy and good precision.

REFERENCES

1. Guideline IH. Text on validation of analytical procedures. International Conference on Harmonization, Geneva 1994 Oct (pp. 1-5).
2. Code Q2B, Validation of Analytical Procedures, Methodology. ICH Harmonized Tripartite Guidelines; Geneva, Switzerland, 6th November: 1996, p. 1-8.
3. Sharma, B.K. Instrumental method of chemical Analysis. 17th edn. Krishna Prakashan Media Pvt. Ltd., Meerut, 1997, 8.
4. Snyder LR, Schunk TC. Retention mechanism and the role of the mobile phase in normal-phase separation on amino-bonded-phase columns. Analytical Chemistry. 1982 Sep 1; 54(11):1764-72.
5. Sethi PD. High Performance Liquid Chromatography: Quantitative Analysis of Pharmaceutical Formulations. New Delhi: CBS Publishers; 2001, p.13-4.
6. Gurdeep Chatwal, K. Anand, Instrumental methods of Chemical Analysis, 5th edition, Himalaya publishing house New Delhi, 2002, pp.1.1-1.8,2.566-2.570.
7. William Kemp, Organic Spectroscopy, Palgrave, New York, 2005, pp.7-10,328-330.
8. D.A. Skoog. F.J. Holler and T.A. Nieman, Principle of Instrumental Analysis, 5th edition, Saunders college publishing, 1998, pp.778-787.
9. A recent book provides a comprehensive treatment of the theory of high-performance gradient chromatography: Lloyd R. Snyder and John W. Dolan (2006). High-Performance Gradient Elution: The Practical Application of the Linear-Solvent-Strength Model. Wiley Interscience.
10. P.D. Senthi, HPLC: Quantitative Analysis Pharmaceutical Formulations, CBS Publishers distributors New Delhi (India), 2001, pp.3-137.
11. H.H. Willard, L.L. Merritt, J.A. Dean, F.A. Settel, Instrumental Method of Analysis, 7th edition, CBS Publishers distributors New Delhi(India), 1986, pp.518-521, 580-610.
12. "FDA Drug Safety Communication: Pradaxa (dabigatran etexilate mesylate) should not be used in patients with mechanical prosthetic heart valves". U.S. Food and Drug Administration (FDA), Retrieved October 29, 2014.
13. Eikelboom JW, Connolly SJ, Brueckmann M, Granger CB, Kappetein AP, Mack MJ, Blatchford J, Devenny K, Friedman J, Guiver K, Harper R. Dabigatran versus warfarin in patients with mechanical heart valves. N Engl J Med. 2013 Sep 26; 369: 1206-14.
14. Bapat P, Kedar R, Lubetsky A, Matlow JN, Aleksa K, Berger H, Koren G. Transfer of dabigatran and dabigatran etexilate mesylate across the dually perfused human placenta. Obstetrics & Gynecology. 2014 Jun 1; 123(6):1256-61.
15. Pradaxa Full Prescribing Information Archived 2015-08-10 at the Wayback Machine. Boehringer Ingelheim. October 2010.
16. Pollack, Charles V.; Reilly, Paul A.; Eikelboom, John; Glund, Stephan; Verhamme, Peter; Bernstein, Richard A.; Dubiel, Robert; Huisman, Menno V.; Hylek, Elaine M. (2015-08-06). "Idarucizumab for Dabigatran Reversal". The New England Journal of Medicine. **373** (6): 511–520.
17. Gómez-Outes, A; Terleira-Fernández, AI; Calvo-Rojas, G; Suárez-Gea, ML; Vargas-Castrillón, E (2013). "Dabigatran, Rivaroxaban, or Apixaban versus Warfarin in Patients with Nonvalvular Atrial Fibrillation: A Systematic Review and Meta-Analysis of Subgroups". Thrombosis. **2013**: 640723.
18. Kumar Raja Jayavarpu *et al*., "Validated UV-spectroscopic method for the estimation of Dabigatran Etixilate Mesylate in formulation and dosage form", Indo American Journal of Pharmaceutical Research Vol.6, 2016, pp. 4952-4956.
19. A Rapid RP-HPLC Method Development And Validation For The Quantitative Estimation of Dabigatran Etexilate Mesylate in capsules, D. Vijay Kumar, K. Balaraju, A. Ashok Kumar, *International Journal of Pharmacy and Pharmaceutical science*., Vol 7, issue:10,2015.
20. A. Srinivas, K. Sridhar Reddy, "Method Development and Validation of Dabigatran Etixilate Mesylate by RP-HPLC method and its Degradation studies," International Journal of Trends in Pharmacy and Life Sciences Vol.2, Issue:1, 2016, pp.769-777.
21. Valko K, Snyder LR, Glajch J. Retention in reversed-phase liquid chromatography as a function of mobile phase composition. J Chromatogr A 1993; 656(1-2): p.501–20.

22. A. Srinivas, K. Sridhar Reddy. "Method Development and Validation of Dabigatran Etixilate Mesylate by RP-HPLC method and its Degradation studies," International Journal of Trends in Pharmacy and Life Sciences Vol.2 Issue: 1, 2016 pp.769-777.
23. Dare *et al.*, "Method Validation for Stability Indicating Method of Related Substances in Active Pharmaceutical Ingredients Dabigatran Etixilate Mesylate by Reverse Phase Chromatography", J Chromatogr. Sep Tech 2015, p. 6.2.
24. Bhavna Patel *et al.*, "Development and validation of RP-HPLC method for estimation of Dabigatran Etixilate Mesylate from capsule dosage form," International Journal of Pharmacy And Analytical Research Vol.5 Issue:1 Jan-Mar.2016 pp.143-151.
25. Kumar Raja Jayavarpu *et al.*, "Validated UV-spectroscopic method for the estimation of Dabigatran Etixilate Mesylate in formulation and dosage form", Indo American Journal of Pharmaceutical Research Vol.6 2016 pp. 4952-4956.
26. Damle *et al.*, "Development and validation of stability indicating RP-HPLC method for estimation of Dabigatran Etixilate Mesylate," Journal of Advanced Scientific Research, 2014 5(3) pp.39-44.
27. Madhavi L.N. *et al.*, "Development and validation of RP-HPLC method for the estimation of Dabigatran Etixilate Mesylate in bulk form" International Journal Pharmaceutical and Research, Vol.5 issue 1 Jan-Mar-2016, pp.141-147.
28. Ashok Kumar *et al.*, "A rapid stability indicating RP-HPLC assay method and validation for the Quantitative estimation of Dabigatran Etixilate Mesylate in a capsules" Indo American Journal of Pharmaceutical Sciences, 2015, 2(10), pp.1382-1392.
29. A Rapid RP-HPLC Method Development And Validation For The Quantitative Estimation of Dabigatran Etexilate Mesylate in capsules, D. Vijay Kumar, K. Balaraju, A. Ashok Kumar, International Journal of Pharmacy and Pharmaceutical science., Vol 7, issue:10,2015.Fghh