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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF DABIGATRAN ETEXILATE IN PURE AND DOSAGE FORMS BY USING RP-HPLC METHOD

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ABSTRACT

A simple, precise, analytical method was developed for Dabigatran Etexilate in pure and dosage forms by using RP-HPLC method analysis initial chromatographic conditions Mobile phase Buffer: ACN: Water in gradient method, Zodiac, C18, 100×4.6mm, 3.5µ column, Flow rate 1.0 ml/min and temperature was ambient, eluent was scanned with UV- VIS detector in system and it showed maximum absorbance at 310 nm. As the mobile phase content is allowed in gradient performance Dabigatran Etexilate got eluted with good peak symmetric properties. The retention time for Dabigatran Etexilate was found to be 4.767min respectively. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 50%, 100% and 150 % levels, R2 value was found to be as 0.999. By using above method assay of marketed formulation was carried out, 99.92% for Dabigatran Etexilate was present. Full length method was not performed; if it is done this method can be used for routine analysis of Dabigatran Etexilate.

KEYWORDS: Dabigatran Etexilate, gradient method, acetonitrile.

INTRODUCTION

A variety of methods are available for analyzing pharmaceutical compounds. High Performance/Pressure Liquid Chromatography (HPLC) is one of the best methods of choice for analyzing a variety of natural and synthetic compounds. It is because it offers high performance over ambient pressure.[3] The phenomenal growth in chromatography is largely due to the introduction of the technique called high-pressure liquid chromatography, which is frequently called high-performance liquid chromatography (both are abbreviated as HPLC). It allows separations of a large variety of compounds by offering some major improvements over the classical column chromatography, TLC, GC; and it presents some significant advantages over more recent techniques such as supercritical fluid chromatography (SFC), capillary electrophoresis (CE), and electro kinetic chromatography.[4]

Effective and fast method development is of paramount importance throughout this drug development life cycle. This requires a thorough understanding of HPLC principles and theory which lay a solid foundation for appreciating the many variables that are optimized during fast and effective HPLC method development and optimization. Chromatographic separations are based on a forced transport of the liquid (mobile phase) carrying the analyte mixture through the porous media and the differences in the interactions at analytes with the surface of this porous media resulting in different migration times for a mixture components.

High surface area of the interface between mobile and stationary phases is essential for space discrimination of different components in the mixture. Analyte molecules undergo multiple phase transitions between mobile phase and adsorbent surface.

Average residence time of the molecule on the stationary phase surface is dependent on the interaction energy. For different molecules with very small interaction energy difference the presence of significant surface is critical since the higher the number of phase transitions that analyte molecules undergo while moving through the chromatographic column, the higher the difference in their retention.

Dabigatranetexilate is an inactive prodrug that is converted to dabigatran, the active form, by esterase-catalyzed hydrolysis in the plasma and liver. Dabigatran, the active principle in plasma, is a rapid -acting competitive and reversible direct inhibitor of thrombin. Thrombin, a serine protease, is responsible for the conversion of fibrinogen to fibrin in the coagulation cascade. Inhibition of thrombin consequently prevents thrombus development. Dabigatran inhibits free

thrombin, fibrin-bound thrombin and thrombin-induced platelet aggregation.

Chemical structure

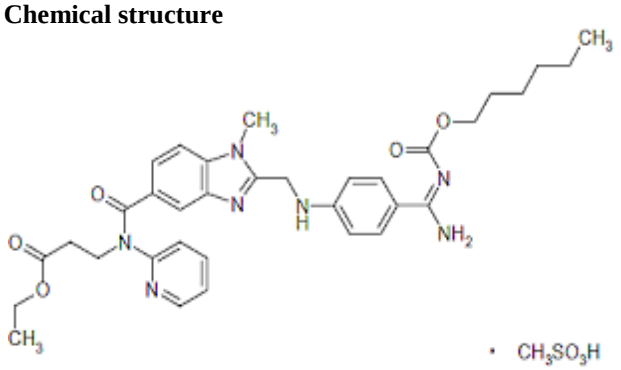


Fig. 1: Chemical structure.

MATERIALS METHODS

All chemicals and reagents were purchased Dabigatran Etexilate Mesylate – NRC, Mekaguda, Hyderabad. PRADAXA Capsule (Boehringer Ingelheim Pharmaceuticals, Inc. (Germany)) containing, (From Local Pharmacyshop) Dabigatran Etexilate - 150 mg,

Methods

Diluent: Use N, N-Dimethyl formamide as diluent.

Diluted ammonia solution: Dilute 3mL of ammonia solution to 100mL with water.

Preparation of Standard stock solutions: Accurately weigh and transfer about 29mg of Dabigatran Etexilate Mesylate working standard into a 250ml volumetric flask and add 160ml of diluent and sonicatetodissolve. Cool the solution toroomtemperature and diluteto volume withdiluent.

Preparation of Sample stock solutions: Accurately weigh 20 capsules, mix the contentsand transfer the pelletsequivalentto 290mg of Dabigatran Etexilateintoa 250m Lvolumetric flask. Add 160mL of diluent and stirred on magnetic stirrer for 30minutes, removedmagnetic bar and ensure that the sample was not stick with magnetic bar. Dilute to volume with diluent. Centrifuge the solution at 3000RPM for10minutes.

Transfer 5.0mL of above solution into a 50mL volumetric flask and dilute to volume with diluent. Filter through 0.2µm Nylon filter.

Preparation of buffer: Accurately weigh and transfer about 0.63g of Ammonium formate into 1000mL Milli-Q water and dissolve. Adjust the pH of the solution to 8.5±0.05 with diluted Ammonia solution. Filter through 0.22µm PVDF membrane filter and degas.

Preparation of Mobile phase

Preparation of Mobile phase A: Buffer is used as Mobile phase A.

Preparation of Mobile phase B: Mixture of Acetonitrile and water in ratio 800:200 v/v and filter through 0.22µm PVDF membrane filter and degas.

Assay procedure: Separately inject equal volumes of 5□L of the blank, standard and a sample solution of DabigatranEtexilate was injected into the chromatograph and the chromatograms were recorded. Measure the peak area responses for the analyte peaks and calculate the amount of drug present in the capsules by the formula.

Validation

System suitability parameters: A Standard solution of DabigatranEtexilate working standard was prepared as per procedure and was injected six times into the HPLC system. The system suitability parameters were evaluated from standard Chromatograms obtained by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from six replicate injections.

Specificity: Checking of the interference in the optimized method. We should not find inter fering peaksin blank and place boat retention times of the sedrugs in this method. Sothis method was said to bespecific.

Precision

Preparation of Standard working solutions (100%): Accurately weigh and transfer about 29mg of Dabigatran EtexilateMesylate working standard into a 250ml volumetric flask and add 160ml of diluent and sonicate to dissolve. Cool the solution to room temperature and dilute to volume with diluent (116µg/mL).

Preparation of Sample working solutions (100%): Accurately weigh 20capsules, mix the contents and transfer the pellets equivalent to 290mg of Dabigatran Etexilate into a 250mL volumetric flask. Add 160mL of diluent and stirred on magnetic stirrer for 30minutes, removed magnetic bar and ensure that the sample was not stick with magnetic bar. Dilute to volume with diluent. Centrifuge the solution at 3000RPM for 10minutes.Transfer 5.0mL of above solution into a 50mL volumetric flask and dilute to volume with diluent. Filter through 0.2µm Nylon filter (116µg/mL).

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

A study was conducted to determine the effect of variation in flow rate. Standard and Test solutions of 100% concentration was prepared & injected into the HPLC system by keeping flow rates 0.8 ml/min and 1.2 ml/min. The effect of variation of flow rate was evaluated.

Linearity

Preparation of Standard stock solutions: Accurately weigh and transfer about 29mg of DabigatranEtexilateMesylate working standard into a 250ml volumetric flask and add 160ml of diluent and sonicate to dissolve. Cool the solution to room temperatureanddiluteto volume withdiluent.

Linearitystocksolution:

Accuratelyweighedandtransferred116mg of Dabigatran Etexilate Mesylate working standard into 10mL volumetric flask. Added 60mL of diluent to it and sonicatedto dissolve and diluted to the volume with diluent (1160µg/mL).

Linearity solution preparations

25% Standard solution: 0.25ml from standard stock solution was pipetted out and made up to 10ml (29µg/mL).

50% Standard solution: 0.50ml from standard stock solution was pipetted out and made up to 10ml (58µg/mL).

80% Standard solution: 0.80ml from standard stock solution was pipetted out and made up to 10ml (92.8µg/mL).

90% Standard solution: 0.90ml from standard stock solution was pipetted out and made up to 10ml (104.4µg/mL).

100% Standard solution: 1ml from standard stock solution was pipetted out and made up to 10ml (116µg/mL).

110% Standard solution: 1.1ml from standard stock solution was pipetted out and made up to 10ml (127.6µg/mL).

120% Standard solution: 1.2ml from standard stock solution was pipetted out and made up to 10ml (139.2µg/mL).

Accuracy

Preparation of Standard stock solutions: Accurately weighed and transferred 116mg of Dabigatran Etexilate Mesylate working standard into 10mL volumetric flask. Added 60mL of diluent to it and sonicated to dissolve and diluted to the volume with diluent and labeled as Standard stock solution 1and 2 (1160µg/mL).

Preparation of 50% Spiked Solution: 0.5ml of sample stock solutionwastakenintoa10ml volumetric flask, to that 1.0ml from standard stock solution was pipetted out, and made up to the mark withdiluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from standard stock solution was pipetted out, and made up to the mark withdiluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from standard stock solution was pipetted out, and made up to the mark withdiluent.

Acceptance Criteria: The % Recovery for each level should be in between 98 to 102.

LOD AND LOQ

LOD sample Preparation: 0.25ml of standard stock solution was pipetted out and transferred to 10ml volumetric flasks and made up with diluent. From the above solution 0.1ml of Dabigatran Etexilate Mesy latesolutionwastransferredto 10mlvolumetricflaskand made up with the samediluent.

LOQ sample Preparation: 0.25ml of standard stock solution was pipetted out and transferred to 10ml volumetric flasks and made up with diluent. From the above solution 0.3ml of Dabigatran Etexilate Mesy latesolutionwastransferredto 10mlvolumetricflaskand made up with the same diluent.

RESULTS AND DISCUSSION

Determination of Optimized Wavelength

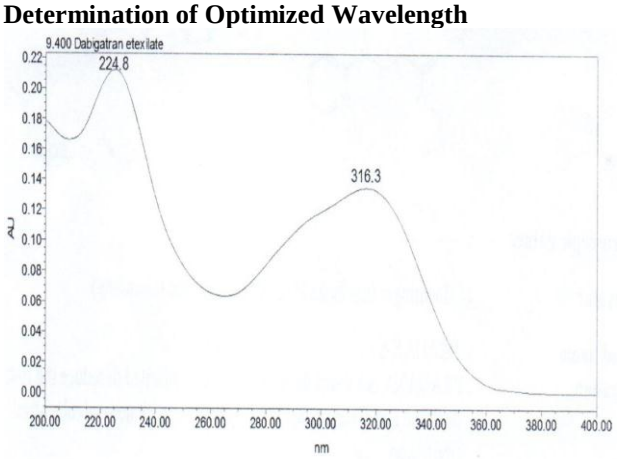


Fig. 1: UV Spectrum for.

Method Development Trials: Method development was done by changing various mobile phase ratios, buffers etc.

Optimized chromatographic conditions

Mobile Phase: Buffer: ACN: Water

Buffer: Ammonium formate in Milli-Q water adjust the pH to 8.2 with dilute Ammonia solution

Column: Zodiac, C18, 100x4.6mm, 3.5µ

Detector: UV-VIS

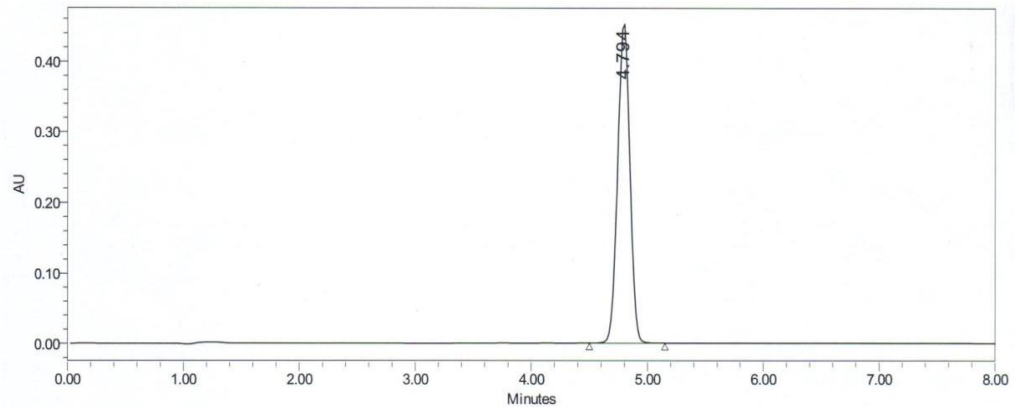
Flow rate: 1ml min^{-1} **Column temperature:** 40°C **Wavelength:** 310 nm

Type of elution: Gradient **Injection volume:** 5µl **Run time:** 8min

Needle wash: Extended.

Table Gradient table for Optimized 8min method.

Time (min)	Flow(mL/min)	Mobile Phase –A (%)	Mobile Phase –B (%)
0.0	**1.0**	**35.0**	**65.0**
1.0	1.0	35.0	65.0
3.0	1.0	25.0	75.0
5.0	1.0	20.0	80.0
6.0	1.0	20.0	80.0
6.1	1.0	35.0	65.0
8.0	1.0	35.0	65.0



	Peak Name	Vial	RT	Area	% Area	USP Tailing	USP Plate Count	USP Resolution
1	Dabigatran etexilate	3	4.794	3354449	100.00	1.0	9441	

Fig. Chromatogram of standard.

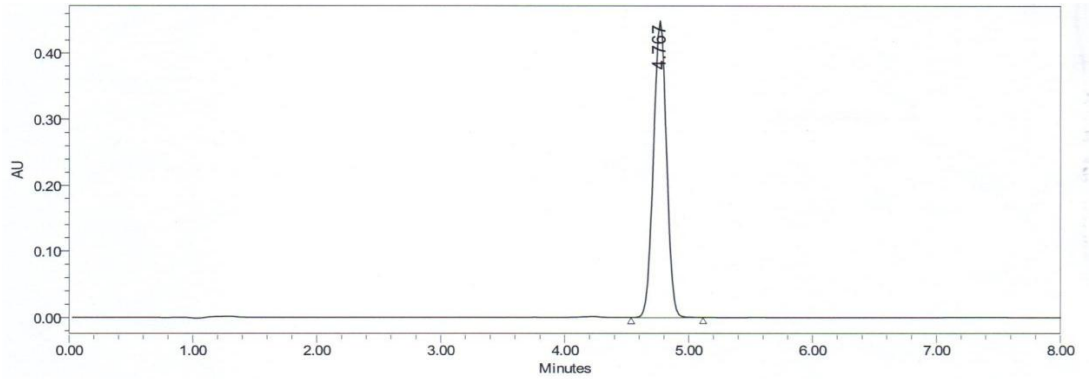


Fig. Chromatogram of Test.

Table: Peak results of Standard & Test Chromatograms for Assay.

	Peak Name	Vial	RT	Area	% Area	USP Tailing	USP Plate Count	USP Resolution
1	Dabigatran etexilate	12	4.767	3341753	100.00	1.0	8926	

Parameter	DabigatranEtexilate	
	Standard	**Test**
Retention time	4.794	4.767
Peak Area	3354449	3341753
USP Plate Count	9441	8926
Tailing Factor	1.0	1.0
USP Resolution	-	-

Parameters results for assay of Dabigatran Etexilate
Test peak area (mean): 341753
Average Weight: 694.2mg
Label claim: 150mg
% Purity of Standard: 99.50
% Assay: 99.93%
Amount of drug in tablet was calculated using following formula

Where,

ASP	=	Area for sample solution.
AST	=	Area for standard solution.
DST	=	Dilution factor for standard.
DSP	=	Dilution factor for sample.
LC	=	Label claim.
A	=	Average weight,
P	=	Potency

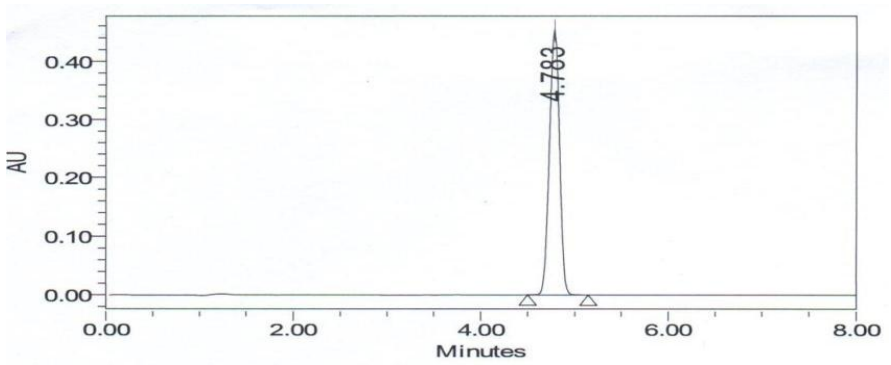


Fig. Chromatogram of system suitability.

Table: Results of System suitability Test for DabigatranEtexilate.

Injection	Retention Time(min)	Peak Area	USP Tailing	USP Plate count
1	4.783	3351679	1.01	9103
2	4.787	3354104	1.01	9356
3	4.784	3357596	1.01	9219
4	4.793	3366115	1.00	9437
5	4.782	3362016	1.00	9182
6	4.774	3347586	1.01	9399
Mean	4.784	3356516	1.01	9283
SD	0.0063	6819.4156		
% RSD	0.13	0.20		

Validation
Specificity

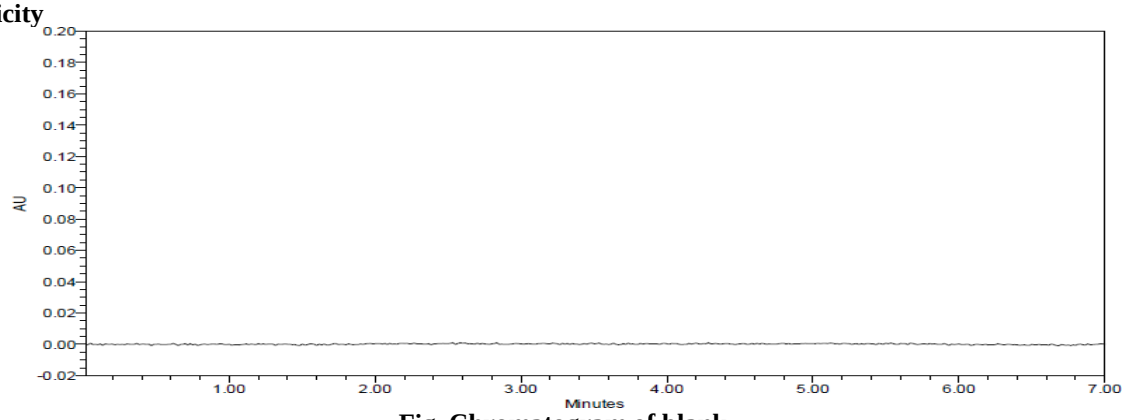


Fig. Chromatogram of blank.

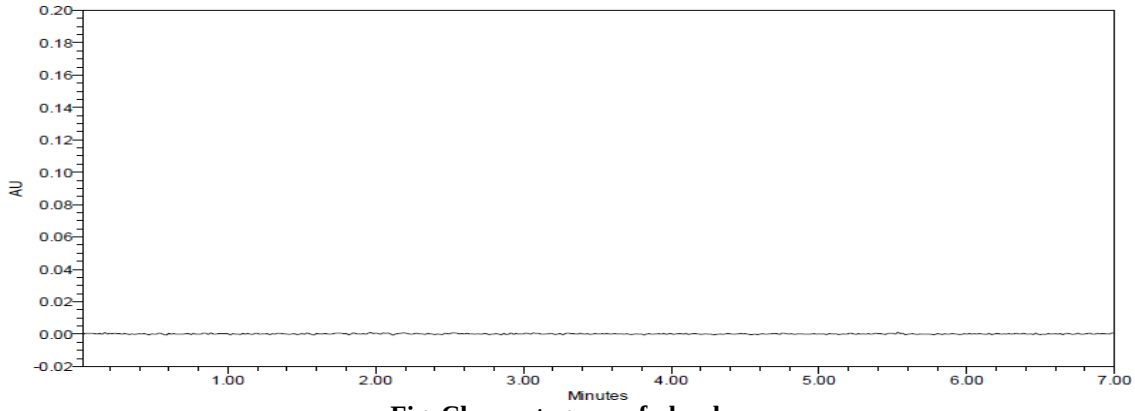
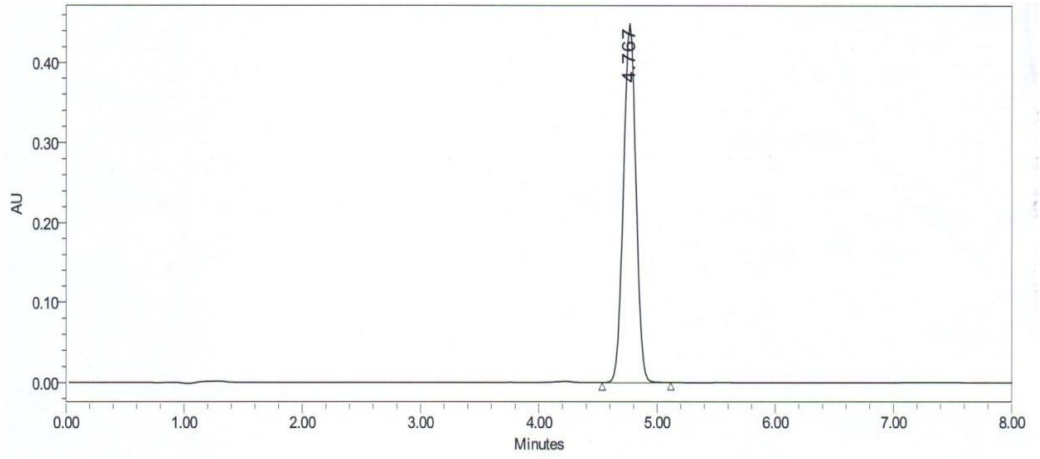


Fig. Chromatogram of placebo.



Linearity

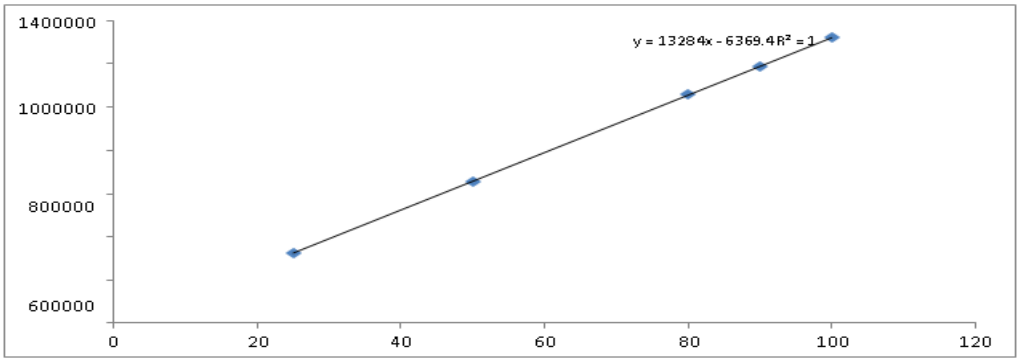


Fig. Calibration curve of Dabigatran Etexilate.

Table: Results of Linearity

Sample ID	DabigatranEtexilate	
	Concentration (ppm)	Area
25% of operating concentration	24.9984	326412
50% of operating concentration	49.9968	656047
80% of operating concentration	79.9949	1057608
90% of operating concentration	89.9942	1190503
100% of operating concentration	99.9936	1320573
110% of operating concentration	109.9929	1451616
120% of operating concentration	119.9923	1579893
Correlation Coefficient(R)=1.0000 Regression Coefficient(R^2)=1.0000 y-intercept=6369.4		

Table Accuracy Study of DabigatranEtexilate

% Concentration	Area	Amount Added (mg)	Amount Found	% Recovery	Mean Recovery (%)
50%	174472	14.5	14.11	100.38	99.92
100%	348159	29	28.09	100.15	
150%	517343	58	42.29	99.21	

Sensitivity
Limit of detection and Limit of quantification
Table: LOD and LOQ Data of Dabigatran Etexilate.

SNO	Sample	LOD	LOQ
1.	**DabigatranEtexilate**	**0.5 µg/ml**	**0.15 µg/ml**

Table: Robustness data for Dabigatran Etexilate.

S. No	Condition	%RSD
1	Flowrate (-) 0.8mL/min	0.1
2	Flow rate(+)1.2mL/min	0.2

Summary Table

Parameters		Dabigatran Etexilate	LIMIT
Linearity Range (µg/ml)		29-139.2µg/ml	R< 1
Regression coefficient		0.999	
Slope(m)		13284	
Intercept(c)		6369.4	
Regression equation (Y=mx+c)		y = 13284x + 6369.4	
Assay (% mean assay)		99.3%	90-110%
Specificity		Specific	No interference of any peak
System precision %RSD		0.17	NMT 2.0%
Method precision %RSD		0.19	NMT 2.0%
Intermediate precision %RSD		0.18	NMT 2.0%
Accuracy %recovery		99.92%	98-102%
LOD		0.5	NMT 3
LOQ		0.15	NMT 10
Robustness	**FM**	0.1	% RSD NMT 2.0
	FP	0.2	

CONCLUSION

The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for estimation of DabigatranEtexilatein capsule dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims. From literature review and solubility analysis initial chromatographic conditions Mobile phase Buffer: ACN: Water in gradient method, Zodiac, C18,100×4.6mm, 3.5µ column, Flow rate 1.0 ml/min and temperature was ambient, eluent was scanned with UV- VIS detector in system and it showed maximum absorbance at 310 nm. As the mobile phase content is allowed in gradient performance Dabigatran Etexilate got eluted with good peak symmetric properties. The retention time for Dabigatran Etexilate was found to be 4.767min respectively. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 50%, 100% and 150 % levels, R2 value was found to be as 0.999. By using above method assay of marketed formulation was carried out, 99.92% for Dabigatran Etexilate was present. Full length method was not performed; if it is done this method can be used for routine analysis of Dabigatran Etexilate.

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