AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Dissolution Method Development and Validation of Dabigatran Etexilate Mesylate Capsules by RP-HPLC

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ABSTRACT

The article aims at developing simple, fast and effective dissolution method for Dabigatran etexilate mesylate capsules by RP-HPLC and validate as per ICH guidelines. The optimized RP-HPLC method for dissolution studies uses a reverse phase column, Phenomenex Luna C18 (250 X 4.6 mm;5µ), a mobile phase of triethylammonium phosphate buffer (pH 3.0):acetonitrile in the proportion of 40:60 v/v, diluent as 0.01N HCl, flow rate of 1.0ml/min, injection volume as 20µl. and a detection wavelength of 341nm using a UV detector. The optimized dissolution conditions include, 0.01N HCl as dissolution media, apparatus as USP Type 1 Basket, rpm as 100, dissolution media temperature as 37±0.5ºC, dissolution volume as 500ml, dissolution time point as 30mts, working concentration of standard and sample as 5µg/ml and a detection wavelength of 341 nm. The developed method resulted in Dabigatran etexilate exhibiting linearity in the range 1.25-10µg/ml. System precision and intra-day precision is exemplified by relative standard deviation of 1.59% and 2.21% respectively. Method was found to be rugged/inter day precise as %RSD was found to be 3.25. Percentage Mean recovery was found to be greater than 80% at all the three levels by absolute method during accuracy studies. LOD and LOQ for Dabigatran etexilate were found to be 0.05ng/ml and 5ng/ml respectively. Hence it can be concluded that effective dissolution method by RP-HPLC is developed and validated as per ICH guidelines which can be applicable in various pharmaceutical industries.

Keywords: Dabigatran etexilate, Dissolution, RP-HPLC, ICH guidelines, Validation

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Received 30 May 2016, Accepted 14 June2016

Please cite this article as: Kumar AA *et al.*, Dissolution Method Development and Validation of Dabigatran Etexilate Mesylate Capsules by RP-HPLC. American Journal of PharmTech Research 2016.

INTRODUCTION

Dabigatran etexilate (DE) is the oral prodrug of the active moiety Dabigatran. Dabigatran etexilate pro-drug was developed due to the limited oral availability of Dabigatran, and it is converted into Dabigatran (DAB) invivo via esterases enzyme. The drug substance is the mesylate salt form of the prodrug, called Dabigatran etexilate mesylate (DEM) (Figure 1). The chemical name (IUPAC) of Dabigatran etexilate mesylate is ethyl-N-{[2-({[4-((E)-amino{[(hexyloxy)carbonyl]imino}methyl)phenyl]amino}methyl)-1-methyl-1H-benz imidazol-5-yl]carbonyl}-Npyridin-2-yl-β-alaninate methanesulfonate [1] corresponding to the molecular formula $C_{35}H_{45}N_7O_8S$. Dabigatran is an oral anticoagulant drug that acts as a direct thrombin (factor IIa) inhibitor. It was developed by the pharmaceutical company Boehringer Ingelheim. It is an anticoagulant medicine used for the prevention of clots and emboli after orthopedic surgery (hip or knee replacement) and to prevent stroke and other systemic emboli in people with non-valvular atrial fibrillation (AF), a commonly occurring abnormal heart rhythm [2].

Few analytical methods are reported for the determination of Dabigatran etexilate by UV[3], LC/MS [4] and UPLC MS/MS[5] in bulk and/or plasma. While two stability indicating assay methods are cited in the literature using HPLC in bulk [1,6] and two methods in formulations [7-8]. As there are no dissolution methods by RP-HPLC reported till now in the literature, we here report a simple, cheap and a rapid dissolution method for Dabigatran etexilate mesylate capsules by RP-HPLC and validate the method as per ICH guidelines.

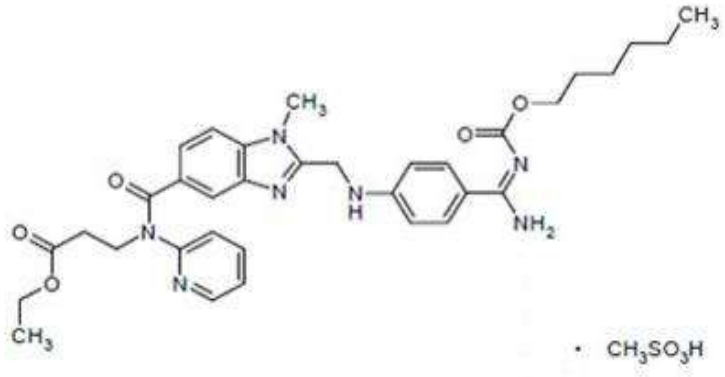

Figure 1: Structure of Dabigatran etexilate mesylate

MATERIALS AND METHOD

Materials

Chemicals and Reagents

Analytically pure drugs, Dabigatran etexilate with purity greater than 95% was obtained as gift sample from Chandra Labs, Hyderabad, India and capsule formulation [PRADAXA] was procured

from Medplus pharmacy, Hyderabad, India. Composition in one capsule include Dabigatran etexilate mesylate equivalent to 110mg of Dabigatran etexilate. Acetonitrile (HPLC grade) was obtained from Sigma aldrich (Hyderabad, India), water (HPLC grade), Triethylamine (AR grade), ortho phosphoric acid (AR Grade) were obtained from SD Fine chemicals (Hyderabad, India), 0.22 and 0.45µm Nylon membrane filters were obtained from Spincotech Private Limited, Hyderabad, India.

Instrument

HPLC analysis was performed on Shimadzu LC-20AD Prominence Liquid Chromatograph comprising a LC-20AD pump, Shimadzu SPD-20A Prominence UV-VISIBLE detector and a reverse phase C18 column, Phenomenex Luna (250 X 4.6 mm; 5µ). A manually operating Rheodyne injector with 20 µL sample loop was equipped with the HPLC system. The HPLC system was controlled with "Lab solutions lite" software. Dissolution studies were performed on USP Dissolution apparatus (Electrolab, Model: TDT-08L). A double beam UV-visible spectrophotometer (Shimadzu, model UV-1800) having two matched quartz cells with 1 cm light path and loaded with UV probe software (version 2.41) was used for recording of spectra and measuring absorbance. An electronic analytical weighing balance (0.1mg sensitivity, Shimadzu AY 220), digital pH meter (DELUX model 101), a sonicator (sonica, model 2200 MH).

Optimized RP-HPLC method

Selection of Wavelength

Suitable wavelength for the HPLC analysis was determined by recording UV spectrum in the range of 200-400 nm for standard solution of Dabigatran etexilate. Suitable wavelength selected is 341nm (Figure 2).

Chromatographic conditions

The developed method uses a reverse phase C18 column, Phenomenex Luna C18 (250 X 4.6 mm; 5µ), mobile phase of triethylammonium phosphate buffer (pH 3.0):acetonitrile in the proportion of 40:60 v/v. The mobile phase was set at a flow rate of 1ml/min and the volume injected was 20µl for every injection. The detection wavelength was set at 341nm.

Buffer Preparation

The buffer solution was prepared by adding 5 ml of triethylamine to 1000 ml of HPLC grade water and later pH was adjusted to 3.0 using 30% v/v of ortho phosphoric acid in water. The buffer was filtered through 0.45µ filter to remove all fine particles.

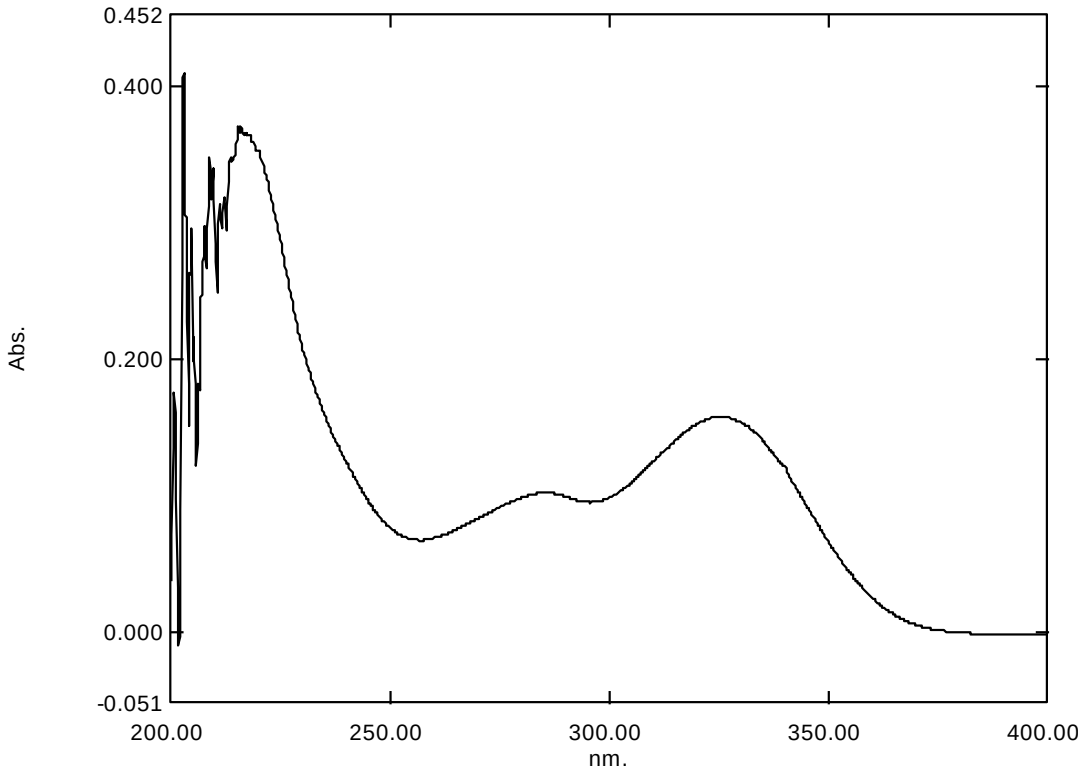

Figure 2: UV spectrum of Dabigatran etexilate standard

Mobile phase Preparation

The mobile phase was prepared by mixing buffer and acetonitrile in the ratio of 40:60 v/v and later it was sonicated for 10 minutes for the removal of air bubbles.

Diluent

Diluent used is 0.01NHCl for the preparation of standard and dissolution sample.

Preparation of stock and working standard solution

10mg of Dabigatran etexilate was accurately weighed and taken in 100ml clean and dry volumetric flask containing 80ml of diluent and then the solution was made up to the mark using diluent. This is considered as standard stock solution A(100µg/ml). 1ml of the stock solution was pipette out and made up to 10 ml to get a concentration 10µg/ml, treated as stock solution B. From this stock B solution, 5ml was pipetted out and made up to 10ml to get a concentration of 5µg/ml, treated as working standard, 100% target concentration for which RP-HPLC chromatogram was recorded (Figure 3).

DISSOLUTION METHOD

Preliminary solubility studies for dissolution:

Solubility studies were explored for Dabigatran etexilate in various solvents ranging pH of 1 to 7.5.

Distilled water:

1mg of drug was added to 10ml of distilled water and found to be practically insoluble. Similar solubility procedure was followed using other solvents.

Preparation of pH 6.8 buffer as per USP:

To 50ml of mono basic potassium phosphate solution (0.2M, 22.7g/L) in a 200ml volumetric flask, was added 22.4ml of 0.2M NaOH solution and later made up to the volume with distilled water, whose pH was checked. If desired pH was not achieved, solution was adjusted to desired pH using dilute phosphoric acid and sodium hydroxide solutions.

Preparation of pH 4.5 buffer as per USP:

2.99gm of sodium acetate in 1000ml volumetric flask was taken and then was added 14 ml 2N acetic acid solution which was finally made to the volume using water, whose pH was checked. If desired pH was not achieved, solution was adjusted to desired pH using dilute acetic acid and sodium hydroxide solutions.

Preparation of pH 7.5 buffer as per USP:

To 50ml of mono basic potassium phosphate solution (0.2M, 22.7g/L) in a 200ml volumetric flask, was added 37ml of 0.2M NaOH solution and made up the volume using distilled water, whose pH was checked. If desired pH was not achieved, solution was adjusted to desired pH using dilute phosphoric acid and sodium hydroxide solutions.

0.01N HCl:

0.835 ml of concentrated HCl was made up to 1000ml using distilled water. It was concluded from the preliminary solubility studies that Dabigatran etexilate was found to be freely soluble only in 0.01NHCl and hence was taken forward for performing dissolution studies.

Optimized Dissolution method conditions:

The optimized dissolution method includes the following keeping the acceptance criteria for % drug release (Q value) as greater than 75% at dissolution sampling point (Q point), 30min. Dissolution media volume was considered based on sink conditions where in dissolution media volume should be at least 3 times of saturation volume of the dose in the formulation.

Rpm : 100

Dissolution medium: 0.01N HCl

Dissolution media volume: 500mL

Apparatus: USP Type 1 (Basket)

Sampling time point (Q point): 30 min

Sampling volume: 10ml

Temperature: 37±0.5ºC

Working concentration of standard: 5µg/ml

Working concentration of sample: 5µg/ml

Detection wavelength: 341nm

Preparation of stock and working sample solution

One capsule (dose:110mg) was studied under above dissolution conditions for 30 minutes and dissolution sample volume of 10ml was sampled out and later filtered through 0.22µm nylon filter. First few ml of the filtrate was discarded and then from the filtrate (stock solution of sample A), 1 ml was pipetted out and made up to 10ml using 0.01NHCl, to get stock solution B. 2.27ml was pipetted out from stock B and later made up to 10ml using 0.01NHCl, to get working sample solution concentration equivalent to 5µg/ml, 100% target concentration as that of standard. RP-HPLC chromatogram of this sample solution was recorded as shown in Figure 3.

% Drug release (Q value) was calculated using the formula as below:

$$\frac{\text{Peak area of sample x Concentration of standard X Molecular weight of DE X 100}}{\text{Average peak area of standard x Concentration of sample X Molecular weight of DEM}}$$

RESULTS AND DISCUSSION

Dissolution Method Development by RP-HPLC

System suitability tests are an integral part of RP-HPLC method development and are used to ensure adequate performance of the chromatographic system. A Reverse phase HPLC method was developed keeping in mind the system suitability parameters i.e. Peak Tailing factor (T), number of theoretical plates (N), runtime and the cost effectiveness. Various mobile phases at pH's of 5, 6, 7 were explored to get good peak shape for Dabigatran etexilate. Peak shapes were very poor (broad and tailing) in the above pH's. Peak shape was found to meet acceptance criteria with respect to system suitability parameters, when pH was explored at 3.0. Accordingly, the optimized method developed resulted in the elution of Dabigatran etexilate at 3.57min. Figure 3, 4 and 5 represent chromatograms of blank, standard solution and sample solution respectively. The total run time is 5 minutes. System suitability parameters for standard and sample solution are given in Tables 1 and 2.

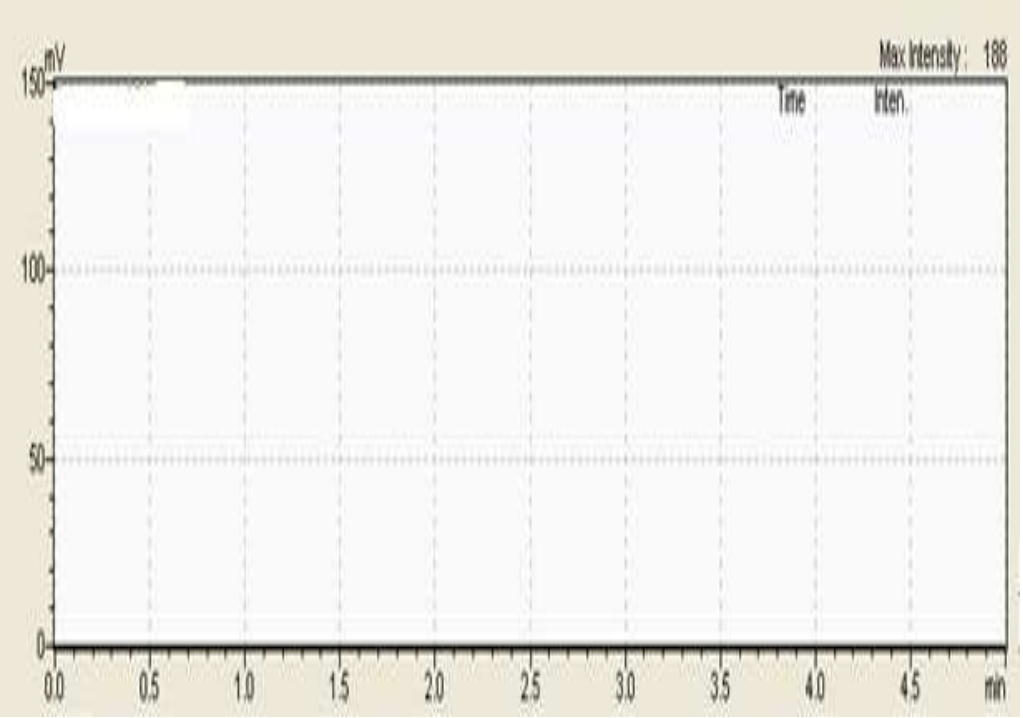

Figure 3: Typical chromatogram of blank solution

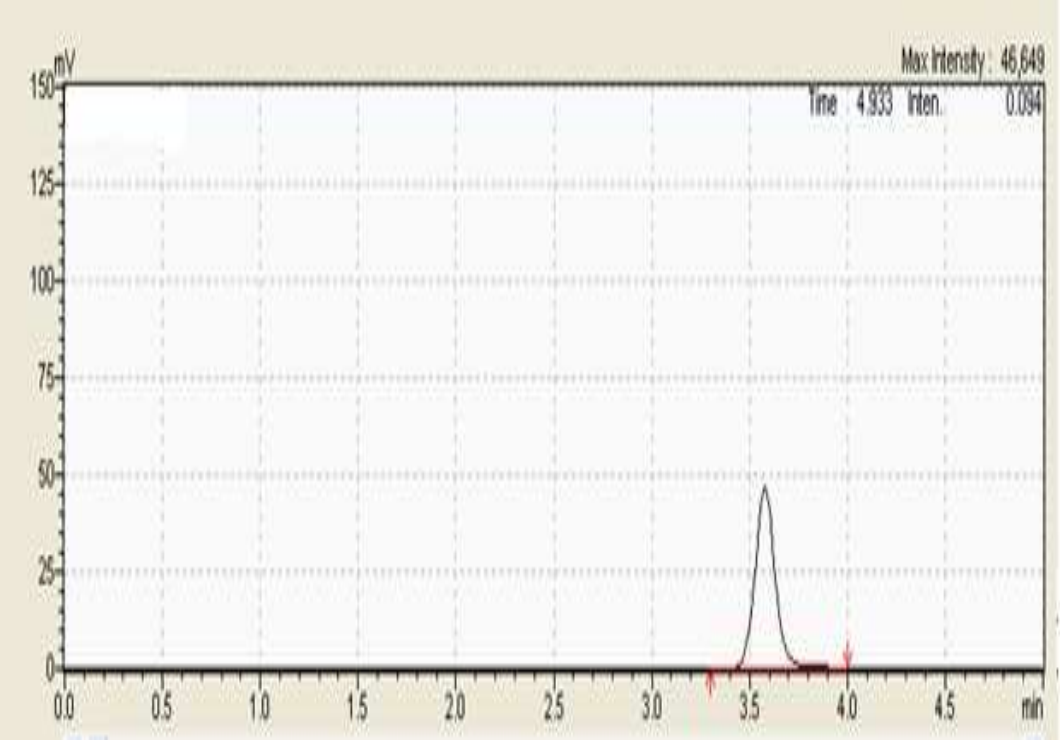

Figure 4: Typical chromatogram of standard solution

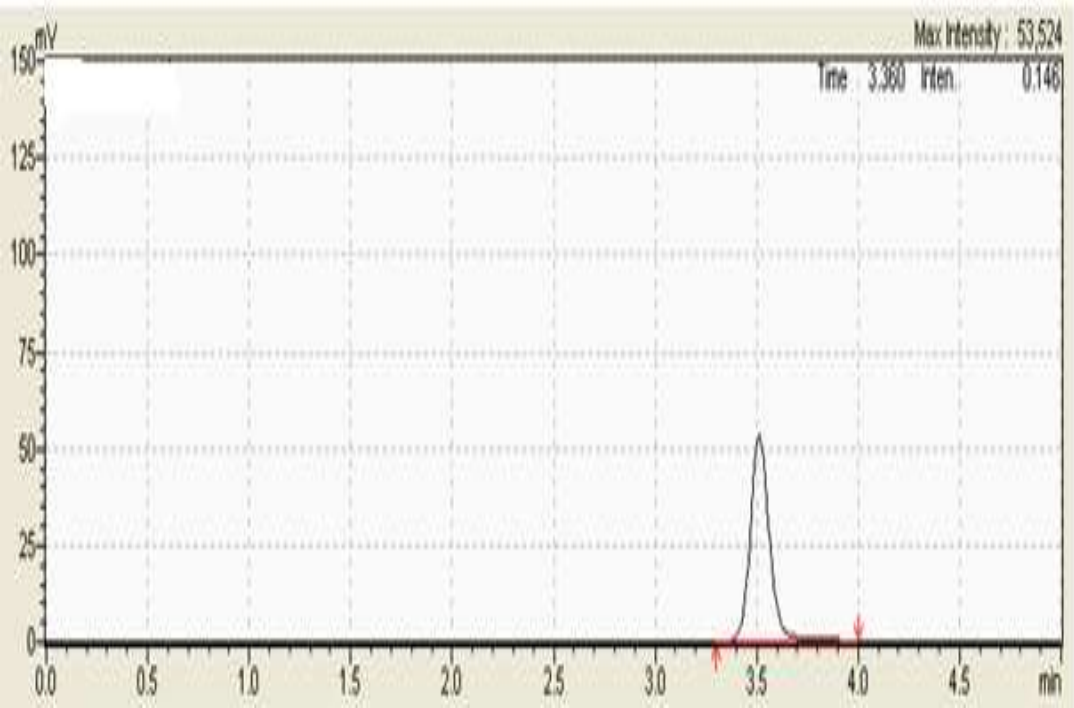

Figure 5: Typical chromatogram of sample solution

Table 1: System suitability studies results for standard.

Parameters	Acceptance Limits	Dabigatran etexilate
Retention time (min)	-	3.57
Number of theoretical plates (N)	Not less Than 2000	5600
Tailing factor (T)	Not More Than 2	1.08

Table 2: System suitability studies results for sample.

Parameters	Acceptance Limits	Dabigatran etexilate mesylate
Retention time (min)	-	3.55
Number Of Theoretical plates (N)	Not less Than 2000	6065
Tailing factor (T)	Not More Than 2	1.1

Once the peak shape of Dabigatran etexilate standard was found to be good, we have next explored for optimization of dissolution conditions. To get the optimized dissolution method, the following parameters were kept in mind.

I. Selection of dissolution medium
II. Selection of dissolution volume as per sink conditions
III. Selection of apparatus
IV. Selection of RPM (Speed)
V. Selection of dissolution time interval (single and multiple point)
VI. Selection of other parameters like sampling volume, temperature, etc.

Preliminary solubility studies revealed that Dabigatran etexilate was found to be freely soluble only in 0.01NHCl as detailed in the method section. Various dissolution media were explored for dissolution studies including 0.01N HCl, pH 4.5, pH 6.8, water and pH 7.5. Percentage drug release was found to be greater than 75% (Q value) between 30-60minutes when 0.01N HCl was used as dissolution media. Percentage drug release was found to be less than 80% in case of pH 4.5, pH 6.8, water and pH 7.5 as dissolution media despite extreme conditions were explored such as media volume:900ml (maximum), rpm:150 and time point as 60minutes for immediate release formulations (Table 3). Accordingly, optimized dissolution method includesoptimized dissolution method conditions include the following: Dissolution media:0.01NHCl; apparatus type:Type 2 USP Paddle; media volume:500ml; rotation speed:100rpm; time point(Qt):30min, sampling volume:10ml; media temperature as 37±0.5ºC; concentration of standard:5µg/ml and concentration of sample:5µg/ml. Dissolution profile for the optimized method is shown in **Fig 6.**

Table 3: Trials explored for optimization of dissolution conditions

Dissolution media	Media volume (ml)	RPM	Time point (min)	% Drug release
pH 4.5	900	150	60	28.22
pH 6.8	900	150	60	5.37
Water	900	150	60	57.39
pH 7.5	900	150	60	8.17
0.01N HCl	900	100	60	104.1
0.01N HCl	500	100	60	103.5
0.01N HCl	500	100	45	102.2
0.01N HCl	**500**	**100**	**30**	**96.59**
0.01N HCl	500	100	15	75
0.01N HCl	500	100	5	11
0.01N HCl	500	100	0	0.23

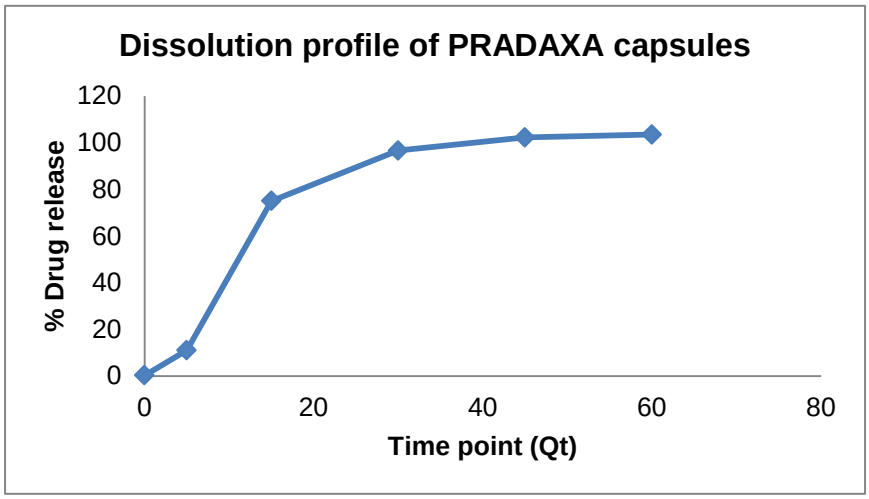

Figure 6: Dissolution profile for PRADAXA capsules employing optimized method

Method validation:

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application [9]. RP-HPLC dissolution method developed was validated according to International Conference on Harmonization (ICH) guidelines. The method was validated for the parameters like specificity, sensitivity, linearity, accuracy, system precision, intra-day precision, inter-day precision / intermediate precision/ruggedness, stability and filter validation.

Precision

System precision

Six replicate recording of peak areas at 341nm of standard solution at working concentration showed % RSD (Relative Standard Deviation) less than 2, which indicates method is system precise. System precision results are tabulated below (Table 4).

Method precision

Method precision was determined by performing dissolution studies of sample under the test of (i) Repeatability (Intraday precision) and (ii) Intermediate precision (Inter day precision or ruggedness) performed during 2 consecutive days by two different analysts at working concentration.

Repeatability (Intraday precision)

Repeatability was performed by conducting dissolution studies on six capsules on the same day and recording of peak area at 341nm of every dissolution sample at working concentration and calculating % RSD of % drug release at 30 minutes. % drug release was greater than 75 and % RSD was found to be less than 5, which indicate that the dissolution method developed is method precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results (Table 5).

Table 4: System precision results of Dabigatran etexilate

n	RT	Peak Area	Tailing factor	NTP	S/N ratio
1	3.579	332574	1.07	5356	1483.58
2	3.585	323650	1.073	5446	1271.88
3	3.579	317895	1.083	5564	1576.53
4	3.57	319753	1.086	5600	1287.74
5	3.566	321158	1.091	5637	1321.51
6	3.561	322259	1.098	5809	1317.75
Average	3.57	322881.5			
STDEV	0.009	5148.704			
%RSD	0.255	1.594			

Table 5: Intraday precision results of Dabigatran etexilate

n	RT	Peak Area	% Drug release	Tailing factor	NTP	S/N ratio
1	3.513	353330	93.94	1.137	6094	1765.01
2	3.554	344717	91.65	1.121	6067	1936.53
3	3.553	363292	96.59	1.114	6065	1398.69
4	3.551	363534	96.66	1.131	6222	1309.89
5	3.551	350166	93.10	1.136	6257	1283.7
6	3.54	361737	96.18	1.135	6210	1257.22
Average	3.54	356129.33	94.69			
STDEV	0.015	7889.458	2.09			
%RSD	0.447	2.21	2.21			

Intermediate precision (Inter day precision/Ruggedness)

Dissolution studies were performed on six capsules by different analysts on two consecutive days and % RSD of percentage drug release was calculated and was found to be less than 5, which indicate the method developed is inter day precise/rugged (**Table 6**).

Table 6: Intermediate precision / ruggedness results of Dabigatran etexilate

N	% Drug release	
	Day 1 and Analyst 1	Day 2 and Analyst 2
1	93.94	89.8
2	91.65	93.07
3	96.59	88.26
4	96.66	89.82
5	93.1	92.36
6	96.18	89.25
Average	94.686	90.426
Intermediate mean	92.556	
STDEV	3.012	
%RSD	3.254	

Linearity

Various dilutions of Dabigatran etexilate at different concentrations level (25%, 50%, 75%, 100%, 125%, 150%, 175% and 200%) were prepared from stock B of standard solution (10µg/ml). Stock A and stock B were prepared as per the procedure given in method section of standard preparation. Calibration curve (Figure 7) was constructed by plotting the concentration of drug versus peak area. Results show an excellent linear relationship between peak area and concentration of drug within the concentration range of 1.25-10µg/ml (Table 7). The correlation coefficient was found to be 0.998, which meet the method validation acceptance criteria and hence the method is said to be linear in the range of 1.25-10µg/ml. Figure 8 presents overlay of chromatograms of linearity range concentrations.

Accuracy

Accuracy was determined by means of recovery experiments by the determination of % mean recovery of dissolution sample by absolute method at three different levels 50, 100% and 150%. At each level, three determinations were performed. Individual recovery and % mean recovery was found to be greater than 75% at 30 minutes, which indicates good recovery values and hence the accuracy of the method developed (Table 8).

Table 7: Calibration data for Dabigatran etexilate

%Level	pipette out volume from stock (10µg/ml)	Actual concentration (µg/ml)	Peak area (341nm)
25	1.25ml to 10	1.25	104724
50	2.5ml to 10	2.5	184071
75	3.75ml to 10	3.75	251898
100	5ml to 10	5	304789
125	6.25ml to 10	6.25	392800
150	7.5ml to 10	7.5	452340
175	8.75ml to 10	8.75	527865
200	10ml to 10	10	602081
Regression Coefficient			0.998
Slope (m)			56093.39
Intercept (c)			37045.678
Regression Equation			y=37045.678x+56093.39

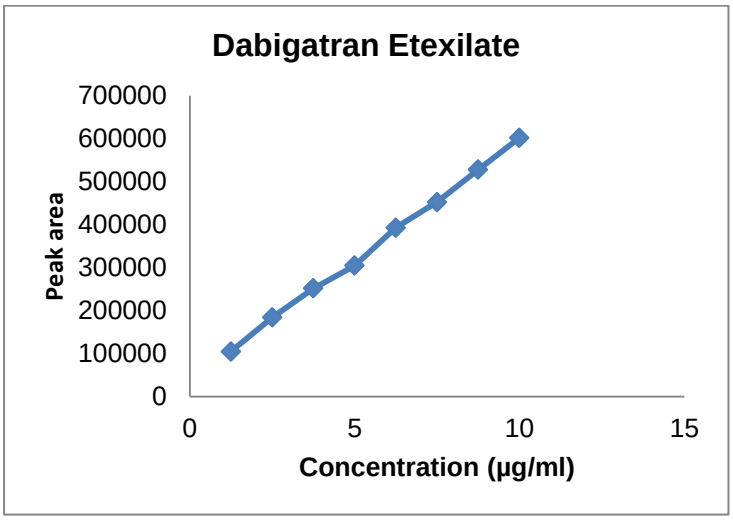

Figure 7: Calibration of Dabigatran etexilate

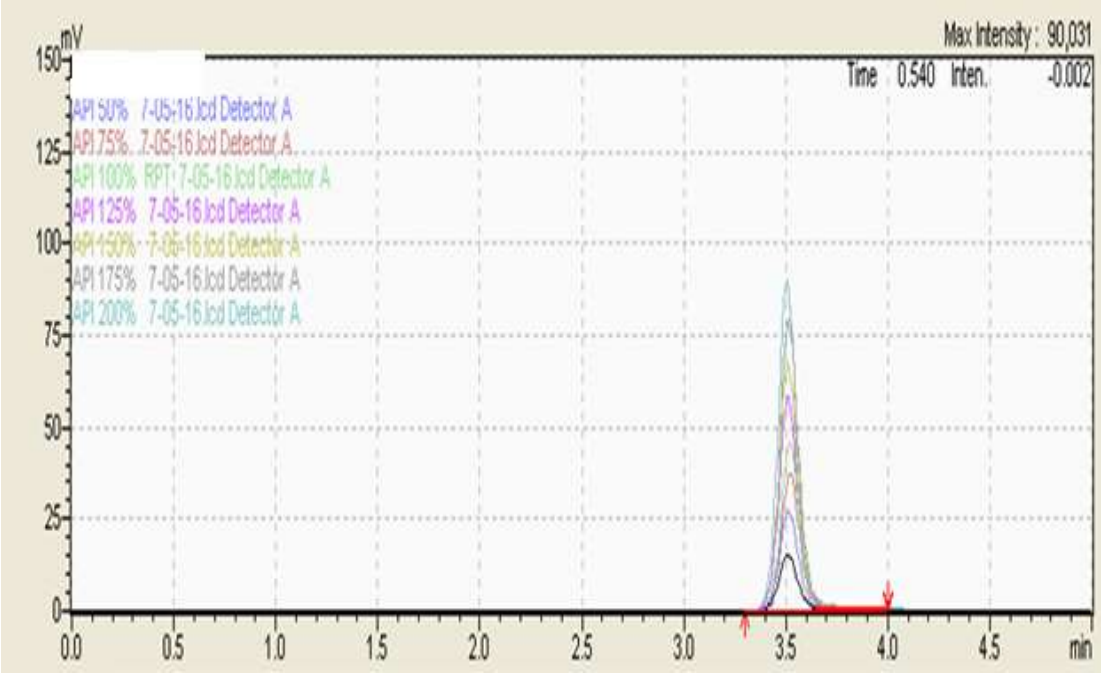

Figure 8: Overlay of linearity chromatograms

Table 8: Results of accuracy

n	Peak Area	%Recovery	% Mean Recovery	Stdev	% RSD
50-1	185291	94.1360578	98.94	4.28	4.332096
50-2	201480	102.360789			
50-3	197508	100.342836			
100-1	334278	84.9140355	85.7	0.69	0.813238
100-2	339504	86.2415556			
100-3	338340	85.9458737			
150-1	505896	84.8158204	84.7	2.59	3.062822
150-2	520397	87.2469806			
150-3	489467	82.061422			

Sensitivity

Sensitivity of the method was determined by the calculation of limit of detection) (LOD) and limit of quantitiation (LOQ). LOQ and LOD were calculated by the use of signal to noise ratio method. The detection limit (LOD) of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. A signal-to-noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit (LOD). The quantitation limit (LOQ) of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. A typical signal-to-noise ratio is 10:1 for estimating the quantitation limit (LOQ). LOD and LOQ

for Dabigatran etexilate were found to be 0.05ng/ml (S/N ratio is 3.43) and 5ng/ml (S/N ratio: 11.44) respectively.

Specificity

Blank, standard drug solution and sample solution chromatograms (Figure 3-5)reveal that the peaks obtained in the standards solution and sample solution at working concentrations are only because of the drugs as blank had no peak at the retention time of Dabigatran etexilate . Accordingly it can be concluded that, the method developed is said to be specific for the analyte of interest.

Stability studies:

Both standard and sample were studied for stability by RP-HPLC at concentration of 5µg/ml in 0.01NHCl and found to be minimum stable for 3 hours and 1 hour respectively at room temperature as percentage degradation was within 2% and accordingly concluded to use this solvent for dissolution studies (Tables 9-10). As the sample was found to be stable only up to 1 hour using 0.01NHCl, higher strengths of HCl (0.05N and 0.1N) were not explored for performing dissolution studies.

Table 9: Stability of standard at room temperature

n	Time	Peak area	Difference in area	% Drug Degradation
1	0 min	326986	0	0
2	1 hr	323254	3732	1.14
3	2 hr	322259	4727	1.44
4	3 hr	323411	3575	1.09
5	4:30 min	306564	20422	6.24

Table 10: Stability of sample at room temperature

n	Time	Peak area	Difference in area	% Drug Degradation
1	0 min	367780	0	0
2	1 hr	364241	3539	0.96
3	2 hr	344717	23063	6.27

Filter Validation

Filter validation was performed by filtration of the dissolution sample using two different filter papers of 0.45 micron filter paper, one with PVDF membrane filter and the other cellulose nitrate membrane filter. In both the cases, % drug release was found to be greater than 75% at 30 minutes and % RSD is less than 5 (Table 11). Hence concludes the filter validation parameter.

Table 11: Percentage drug release under two different membrane filters

n	% Drug release	
	PVDF	Cellulose nitrate
1	96.59	89.6
2	96.66	95.82
Average	94.66	
Std dev	3.39	
%RSD	3.59	

CONCLUSION

A reverse phase HPLC isocratic dissolution method has been developed and validated as per ICH guidelines in terms of specificity, accuracy, system precision, intra day precision, linearity, ruggedness, stability and filter validation for quantitative estimation of Dabigatran etexilate mesylatein capsules and therefore the method can be employed for the routine dissolution analysis of Dabigatran etexilate mesylate in capsules in various pharmaceutical industries.

ACKNOWLEDGEMENT

The authors thank the management of Vijaya College of pharmacy (VJYH), Hyderabad, for providing the necessary facilities to carry out of this research work. The authors are grateful to Chandra labs, Hyderabad for providing drug in form of gift sample.

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