Development & Validation of RP-HPLC Method for Estimation of Dabigatran Etexilate Mesylate from Capsule Dosage Form

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ABSTRACT:

An accurate, sensitive and rapid RP-HPLC method has been developed and validated for the estimation of Dabigatran etexilate mesylate from capsule Dosage form. The separation was achieved by a Zorbax C18 column (100mm X 4.6mm, 3.5µm) in isocratic mode, with mobile phase comprises of Acetonitrile : Water in proportion of 70:30 v/v. The flow rate of mobile phase was 1.0ml/min and detection was done at 225nm wavelength. The retention time of Dabigatran etexilate mesylate was 3.0 min. The calibration curve was found to be linear within the concentration range of 20µg/ml to 100µg /ml. The regression data for calibration curve shows good linear relationship with r^2 = 0.998. The method was validated as per the ICH guidelines. Moreover, the proposed analytical method can be used for routine analysis of Dabigatran etexilate mesylate in quality control labs.

INTRODUCTION:

Dabigatran etexilate mesylate (DAB) is chemically β - Alanine,N[[2[[[4[[[(hexyloxy)carbonyl]amino]iminomethyl] phenyl]amino]methyl]-1-methyl-1H-benzimidazol-5-yl]carbonyl]-N-2-pyridinyl-, ethyl ester methane sulfonate. The empirical formula is $C_{34}H_{41}N_7O_5 \cdot CH_4O_3S$ is an orally available prodrug of dabigatran, a synthetic novel, reversible ,low molecular weight direct thrombin inhibitor (DTI) that binds to thrombin with high affinity. It is a competitive DTI with 6-7% oral bioavailability and is not metabolized by cytochrome P_{450} system. It has a rapid onset of action, predictable pharmacodynamic effects and pharmacokinetics characteristics that allow once daily dosing[1-5]. Unlike heparins, It do not require antithrombin as a cofactor and do not bind to plasma proteins ,therefore they produce more predictable anticoagulant effect and variability of patient response is low relative to other drug classes[6-7]. It is indicated in patients with artrial fibrillation and prevention of venous thromboembolism in patients who have undergone total hip or knee replacement surgery. The marketed formulation is available in indian market in different strength 75 mg,110 mg,150 mg manufactured by boehringer ingelhieim.

Literature survey reveals that there are few analytical methods reported in the literature which includes UPLC MS/MS in human plasma, LC/MS based metabolite identification and semi quantitative estimation approach in the investigation of in vitro DAB[8-9] but there are No UV Spectrophotometric method available for estimation of Dabigatran etixilate mesylate. Thus the aim of this present work was to develop and validate a simple, reproducible & cost effective method to estimate Dabigatran etixilate mesylate in routine analysis.

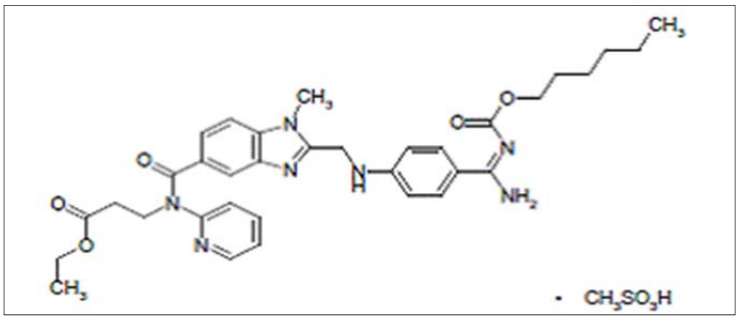

Fig 1: structure of DAB

EXPERIMENTAL:

Chemical and Reagent

Drug: DAB was kindly obtained. All other solvents were of hplc grade and were purchased from merck specialities pvt.ltd, mumbai. Hplc grade water was purchased from sisco research lab,mumbai.

Instrumentation and chromatographic conditions:

An AGILENT-1260 series (INFINITY ,agilent technologies) consisting of a degasser,zorbax eclipse plus C18 column, a manual injector rheodyne valve 20µl fixed loop and PDA detector was used for seperation and detection of analytes.Data was collected using EZ CHROME ELITE software. Hplc analysis was performed on zorbax eclipse plus C18(100 x4.6 mm,3.5 µm) column maintained at ambient conditions.chromatographic seperation was achieved with mobile phase ratio of 70:30 (v/v) mixture of acetonitrile :water at flow rate of 1ml/min.the injection volume was 20µl.The uv detection wavelength was 225nm.

Preparation of Solutions

Preparation of DAB standard stock solution (100µg/ml)

Accurately weighed 10 mg of DAB was transferred into 100 ml volumetric flask and dissolved in acetonitrile. Sonicate for 5 min and diluted up to the mark with HPLC grade acetonitrile to get a stock solution containing 0.1 mg/ml of DAB (100 µg/ml DAB).

Preparation of Calibration curve(working standard) for DAB

Transfer 2, 4, 5, 6, 8, 10 ml from standard stock solution of DAB (100µg/ml) into a 10 ml volumetric flask and diluted up to the mark with HPLC grade Acetonitrile to get the concentration range 20-100µg/ml for DAB respectively.

Preparation of sample solution of marketed formulation

Granules of 10 capsules were crushed to make fine powder. It was weighed and mixed. The net content of capsule was found. Then accurately weighed capsule content equivalent to 10 mg of DAB was transferred into 100 ml of volumetric flask containing 5 ml of Acetonitrile ,it was shaken throughly and diluted up to mark with HPLC grade acetonitrile and sonicated for 10 minutes. The resulting solution was filtered with whatmann filter paper and then 4 ml of aliquote was diluted to 10 ml with acetonitrile to get a solution containing 40 µg/ml of DAB.

Analysis of Market formulation:

Granules of 10 capsules were crushed to make fine powder. It was weighed and mixed. The net content of capsule was found. Capsule content powder quantity equivalent to 10 mg DAB was accurately weighed and transferred to volumetric flask of 100 ml containing 40 ml of acetonitrile. It was then sonicated for 10 min. The flask was shaken and volume was made up to the mark with HPLC grade acetonitrile. The above solution was filtered through whatman filter paper. From this solution, 4 ml was transferred to volumetric flask of 10 ml capacity. Volume was made up to the mark with acetonitrile to give a solution of 40µg/ml DAB. The resulting solution was analyzed by proposed method. Chromatogram was recorded at the 225 nm and the amount of the drug was estimated.

Method validation

The proposed method was subjected to validation for various parameters like linearity and range, precision, accuracy, robustness in accordance with international conference on harmonization guidelines**.**

Linearity

The linearity of analytical method is its ability to elicit test results that are directly proportional to concentration of analyte in sample within given range. The linearity is expressed in terms of correlation co-efficient of linear regression analysis. Transfer 2, 4, 5, 6 ,8, 10 ml from standard stock solution of DAB into a 10 ml volumetric flask and diluted up to the mark with HPLC grade acetonitrile to get the concentration range 20-100 µg/ml for DAB. The standard solution was run for 5 minutes using mobile phase at a flow rate of 1.0 ml/min. The graph was plotted for peak area vs. concentration for the drug.

Precision - the Repeatability of sample application was assessed by Transferring 4 ml from standard stock solutions of DAB into a 10 ml volumetric flask and diluted up to the mark with HPLC grade Acetonitrile to get

the concentration of 40µg/ml of DAB respectively. This standard solution was chromatographed for 5 minutes using mobile phase at a flow rate of 1.0 ml/min for six times. The graphs were plotted for peak area vs. concentration for the drug. The inter-day precisions of the proposed method was determined by analyzing corresponding responses in triplicate on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of DAB (20,50,100 µg/ml). All the Results were reported in terms of % RSD.

Sensitivity

The sensitivity of measurement of DAB by the use of proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ were calculated by equation. Based on the standard deviation of the response and the slope, LOD and LOQ were estimated using the formulae:

$LOD = 3.3\ \sigma/S$, $LOQ = 10\ \sigma/S$

LOD and LOQ were determined from the standard deviations of the responses for six replicate determinations.

System suitability

System suitability test was an integral part of the method development to verify that the system is adequate for analysis of DAB to be performed .Five replicate injections of standard preparation were injected and resolution, asymmetry, number of theoretical plates and relative standard deviation of peak area were determined.

Specificity

Blank and sample preparation as per test procedure was injected and the interference of blank peaks with the analyte peak was checked. The purity for the main peak in sample preparation and standard preparation was also checked.

Robustness

Robustness was studied by changing following parameters, one by one and their effect was observed on system suitability test and assay.

1. Change flow rate by ± 2.5%.
2. Change the minor components in the mobile phase by ± 2% .

Analysis of Market formulation

Granules of 10 capsules were crushed to make fine powder. It was weighed and mixed. The net content of capsule was found. Capsule content powder quantity equivalent to 10 mg DAB was accurately weighed and transferred to volumetric flask of 100 ml containing 40 ml of acetonitrile. It was then sonicated for 10 min. The flask was shaken and volume was made up to the mark with HPLC grade acetonitrile. The above solution was filtered through whatman filter paper. From this solution, 4 ml was transferred to volumetric flask of 10 ml capacity. Volume was made up to the mark with acetonitrile to give a solution of 40µg/ml DAB. The resulting solution was analyzed by proposed method. Chromatogram was recorded at the 225 nm and the amount of the drug was estimated.

RESULT AND DISCUSSION:

Selection of Detection Wavelength

The sensitivity of RP-HPLC method with UV detection depends upon proper selection of detection wavelength. An ideal wavelength is one that gives good response for the drugs that are to be detected.

DAB showed maximum absorption at 225nm as shown in Fig 2 and it was selected as detection wavelength for RP-HPLC analysis.

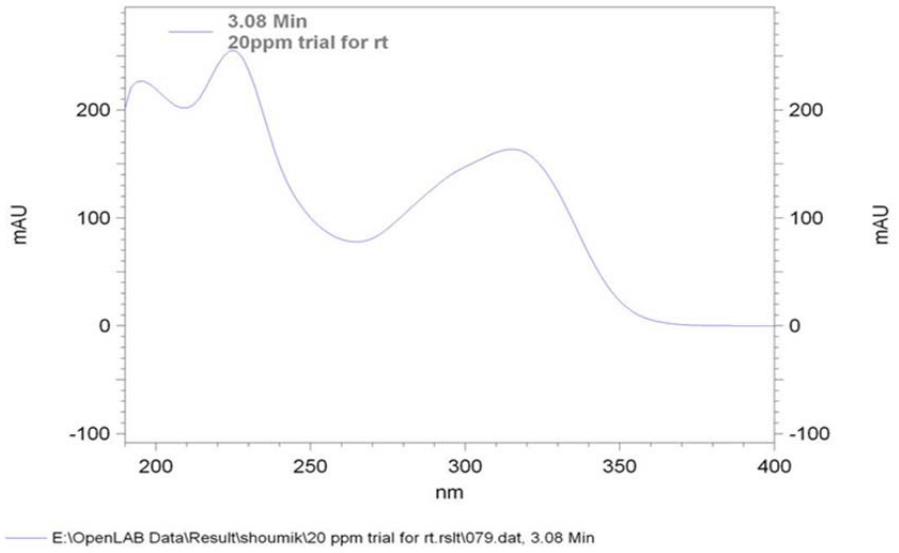

Fig 2: UV spectrum spectrum of DAB

Optimization of Mobile Phase

Proper selection of HPLC method depends upon the nature of the sample (ionic, ionizable or neutral molecule), its molecular weight and solubility. The drug selected for the present study is polar in nature and hence either reversed phase or ion pair or ion-exchange chromatography can be used. Reversed phase HPLC was selected for the initial separation because of its simplicity and suitability. The optimization of mobile phase was to resolve chromatographic peaks for active drug ingredients with less asymmetric factor. There were many mobile phases that we tried to resolve peaks ,in that first with methanol:water (50:50 v/v),methanol water (70:30) but no proper peak shape and peak shouldering was observed for DAB. To improve these ,a combination of mobile phase were tried in that Acetonitrile :methanol:water(40:10:50 v/v/v) and Acetonitrile :methanol:water(65:5:30 v/v/v) (pH 6) were tried but we found split peaks and peak tailing was observed.

Finally ,the mobile phase acetonitrile: water(70:30 v/v) was found to give a good resolution and symmetric peaks for DAB.

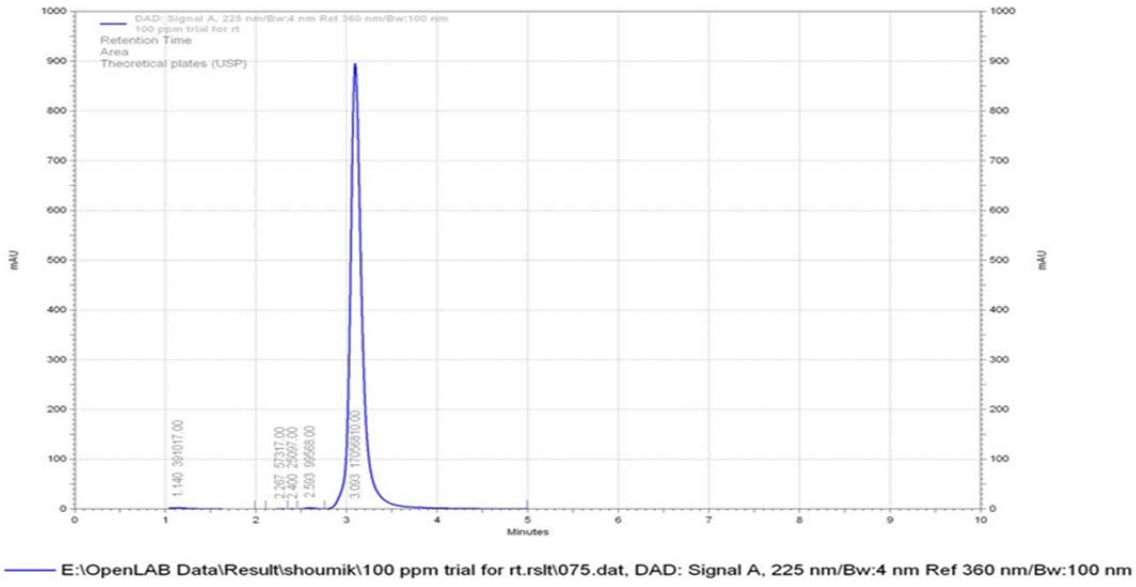

Fig3. Chromatogram of dab acetonitrile:water (70:30 v/v)

SYSTEM SUITABILITY

It was carried out using six replicates of standard mixture solution of DAB(40µg/ml). Results of System suitability study is shown in Table 1.

Table 1: System suitability

PARAMETER	DAB
RETENTION TIME	3.0 min
INJECTION REPEATABILITY(n=6)	0.16 % RSD
NO.OF THEORETICAL PLATE	6940
TAILING FACTOR	0.9

METHOD VALIDATION:

Linearity

Linear regression data for the calibration plots revealed good linear relationships between area and concentration over the ranges 20-100 µg/ml for DAB. The linear equations for the calibration plots were y = 165496x + 733282 with Regression (r^2) being 0.9981 for DAB.

Table 2: Data of linearity

Parameter for	DAB
Linearity range	20-100µg/ml
Linearity equation	y = 165496x + 733282
Correlation co-efficient	0.998
Standard deviation of slope	989.5
Standard deviation of intercept	15303.2

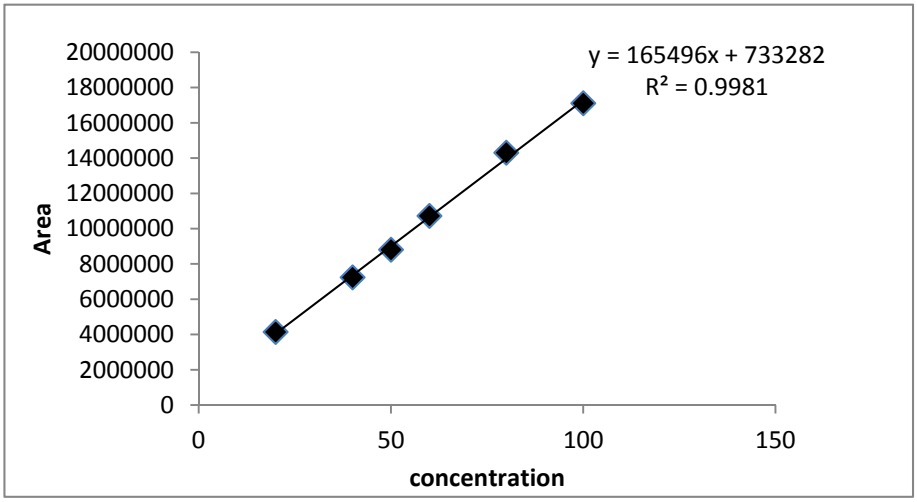

Fig .4: Calibration data of DAB

Precision

Precision was checked in terms of intra assay precision (repeatability), interday precision. Relative standard deviation was calculated and was within limit (Not more than 2).

Repeatability

The repeatability was evaluated by assaying 6 times of samples solution prepared for assay determination. % Relative standard deviation (% R.S.D) was calculated which is within limit (less than 2) as shown in Table 3.

Table.3: Repeatability Data of DAB (*n=6)

Sr no.	Concentration (µg/ml)	Mean ± S.D*	%R.S.D
1	40	7218162±12046.8	0.16

The interday precision study of DAB was carried out by estimating different concentrations of DAB (40 µg/ml) 3 times on 3 different days and the results were reported in terms of Relative standard deviation as shown in table 4 .

Table 4: Interday precision Data of DAB*(n=3)

Sr no.	Concentration (µg/ml)	Mean* ± S.D	%R.S.D
1	20	4116421±26349.8	0.64
2	50	8755783±54155.35	0.61
3	100	16720884±290963.7	1.74

Accuracy

When the method was used for accuracy and subsequent analysis of DAB, and spiked with 50, 100, and 150% of additional pure drug, the recovery was found to be 99.05- 100.41% for DAB (Table 5). The closeness of the result nearly to 100 % assured the accuracy of the developed method for the purpose.

Table.5: Determination of Accuracy for DAB *(n=3)

Concentration of Sample Taken (µg/ml)	Concentration of Pure API spiked (µg/ml)	Total Concentration (µg/ml)	Mean Total Concentration Found (n=3) (µg/ml)	%Recovery Mean (n=3)	%RSD
40	20	60	60.25	100.41	0.67
40	40	80	79.55	99.43	0.57
40	60	100	99.05	99.05	1.05

Sensitivity

The LOD and LOQ were calculated by equation. The LOD and LOQ values were 1.38 µg/ml and 4.17µg/ml for DAB.

Specificity

The peak purity index for the main peak in standard preparation and sample preparation was determined and recorded in table 6. Suggested that no interference from blank and impurity so method is specific.

Table.6: Peak purity index of DAB

Sample	Peak purity index
Standard preparation	1.000
Sample preparation	1.000

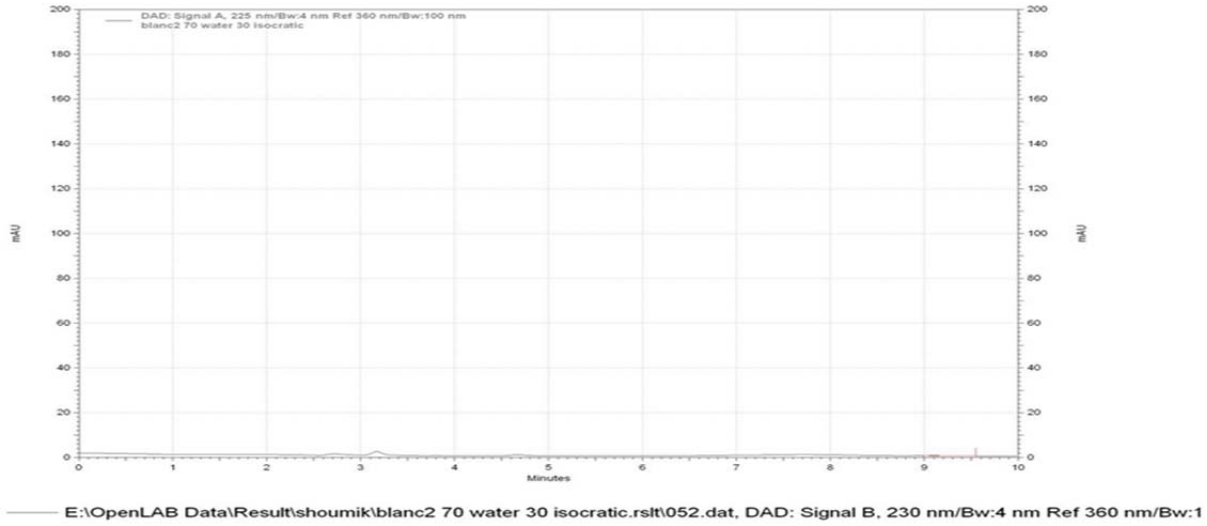

Fig.5: Chromatogram of Blank

Robustness The results of robustness study are shown in table and %RSD for each parameter is less than 2%.

Table.7 :Results of robustness study

Parameter	% RSD (*n= 5)		
	Normal Condition	Changed Condition	
Flow Rate	Normal	(-2.5%)	(+2.5%)
	0.16	0.63	0.29
Mobile phase ratio	Normal	(-2%)	(+2%)
	0.16	1.60	1.11

Analysis of Marketed Formulation

Here, 40 µg/ml solutions of DAB of marketed formulations PRADAXA were prepared three times, and absorbance measured and assay was carried out result of assay are shown in Table 8.

Table.8: Assay Result of marketed formulation

Parameters	DAB
Actual Concentration (µg/ml)	40
Concentration Obtained (µg/ml)	40.10
%Purity	100.25
%RSD	0.67

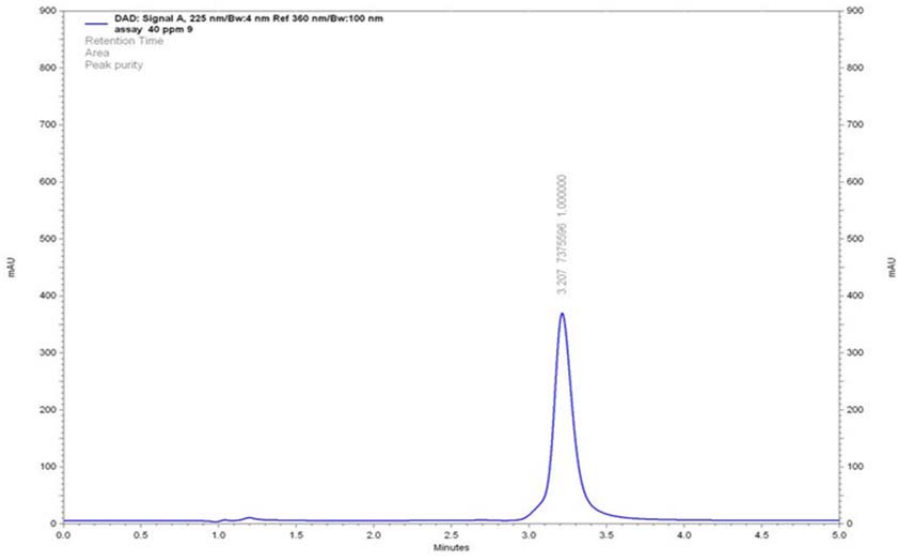

Fig.6: Chromatogram of DAB (40µg/ml) of marketed formulation

Table .9:Summary data of validation parameters

Sr. no.	Parameters	DAB
1	Linearity range	20-100 µg/ml
2	Regression equation	y=165496x+733832
3	correlation co-efficient	0.998
4	Precision	
4.1	Interday (n=3)	0.61-1.74 %RSD
4.2	Repeatability	0.16 % R.S.D
5	Accuracy or % Recovery	99.05-100.41 %
6	LOD	1.38 µg/ml
7	LOQ	4.17 µg/ml
8	Specificity and selectivity	No interference
9	Robustness	%RSD of five replicates <2%

Conclusion:

The concentration of DAB in pharmaceutical dosage form could be satisfactorily determined using isocratic RP-HPLC system with PDA detector.This study had shown that PDA detector was sensitive, accurate, simple and rapid method for the determination of the DAB from capsule dosage form. This method has been found suitable for the routine analysis of pharmaceutical dosage forms in QC and R & D Laboratories for product of similar type and composition.

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