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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD TO ASSESS STABILITY OF DABIGATRAN HYDROCHLORIDE BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

The current paper describes a reversed phase high performance liquid chromatographic stability indicating assay method for the estimation of dabigatran hydrochloride in formulations. The separation was achieved on the LUNA C18 column 5µ (250 x 4.6 mm id), using phosphate buffer (pH 3.6): methanol, 70:30 as the mobile phase at 1 ml/min flow rate and 226 nm as detection wavelength. The retention time of was found to be 5.317 min. The method was validated in terms of linearity, accuracy, precision, as per ICH Guidelines. The calibration curve was linear in the concentration range from 10-50 µg/ml. The method was used for analyzing the concentration of dabigatran in capsule sample.

Keywords: Dabigatran, Estimation, HPLC, Validation, ICH

1. INTRODUCTION

Dabigatran Etexilate **(Figure 1)** is a newly approved oral direct thrombin inhibitor which is indicated for anticoagulation therapy to reduce the risk of strokes and systemic embolism in patients with non-valvular atrial fibrillation **[1]**. It is a competitive DTI with 6-7% oral bioavailability and is not metabolized by cytochrome P450 system. It has a rapid onset of action, predictable pharmacodynamic effects and pharmacokinetics characteristics that allow once daily dosing **[2]**.

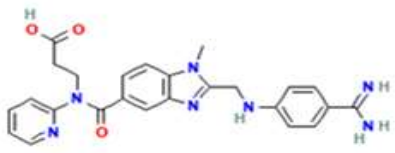

Figure 1: Chemical structure of dabigatran

Literature reveals that different assay methods such as UV and HPLC have been developed for estimation of Dabigatran from finished dosage form and bulk API **[1-9]**. These methods utilize strongly alkaline buffers as eluent which presents the need for higher precaution for the separating columns. Also, some of these methods rely on specialized detectors that makes the routine use of these methods a big problem.

The present study was aimed for the development of a new rapid, simple, sensitive and reproducible RP-HPLC method for the analysis of dabigatran that offer certain advantages in its simplicity and sensitivity and applicable in routine analysis. It also describes the development of validation work as per ICH guidelines recommended by the Food and Drug Administration (FDA) of the United States.

2. MATERIAL AND METHODS

Dabigatran etexilate pure drug was purchased from Yarrow Pharmaceuticals. The pure sample was used without further purification. Capsule formulation (Brand Name-Dabistar 150- manufactured by Lupin) was procured from local pharmacy and was used in the present study. HPLC grade water and methanol were purchased from Qualigens chemicals, potassium dihydrogen phosphate, orthophosphoric acid, sodium hydroxide, hydrochloric acid and hydrogen peroxide used in this study were of analytical grade and obtained from S.D. Fine chemicals, Mumbai. All dilutions were performed in standard class-A, volumetric glassware. All other materials used were of pharmacopoeial grade.

2.1 Preparation of pH 3.6 Phosphate buffer

2.7218g of KH_2PO_4 was weighed and transferred into a 1000ml beaker, later it was dissolved and diluted to 1000ml with HPLC water and the pH was adjusted to 3.6 with orthophosporic acid.

2.2 Preparation of mobile phase

A mixture of pH 3.6 Phosphate buffer 600 ml (60%) and 400 ml of HPLC grade methanol (40%) was taken and degassed in ultrasonic water bath for 5 min. Later it was filtered through 0.45µ filter under vacuum filtration. The mobile phase was used as diluent in the analysis.

2.3 Preparation of Standard Solution

Dabigatran etexilate (10 mg) was accurately weighed and transferred into a 10 ml clean, dry volumetric flask and about 7ml of diluent was added and sonicated to dissolve the drugs completely and the volume was made upto the mark with the same solvent. (Stock solution I). This was followed by pipetting out 5ml from the above stock solution into a 25 ml volumetric flask and the volume was made upto the mark with the diluents (stock solution II) Further 1.5 ml of solution was pipetted out from stock solution II into a10ml volumetric

flask and diluted to the mark with diluent (stock solution III).

2.4 Preparation of Sample Solution

Capsule powder equivalent to 50 mg of dabigatran was accurately weighed and transferred into a 100 ml clean dry volumetric flask and about 70 ml of diluent was added and sonicated to dissolve the drug completely and the volume was made upto the mark with the same solvent. (Stock solution I). Further 5 ml of solution was pipetted out from the above stock solution into a 25 ml volumetric flask and diluted up to the mark with diluent (Stock solution II). An accurately measured quantity of 1 ml of above solution was pipetted out from the above stock solution into a 10 ml volumetric flask and diluted up to the mark with diluents (Stock solution III).

2.5 Analysis of capsule formulation

20 µl each of the standard and sample solutions of dabigatran were injected into the chromatographic system using the optimized conditions and the area for the dabigatran peak was measured and the drug content of the tablets was calculated by comparing the areas of standard and sample solutions.

2.6 Validation of the method

The method was validated as per the ICH guidelines for validation of analytical methods for system suitability, specificity, linearity, accuracy, precision, robustness and ruggedness, limit of detection and limit of quantitation **[10-13]**.

3. RESULTS AND DISCUSSION

The wavelength for detection of dabigatran by HPLC was selected on the basis of the absorption maxima obtained from UV spectrum scan of the drug. The maximum absorption in phosphate buffer pH 3.6 was obtained at 226 nm.

The chromatogram obtained using the selected chromatographic parameters (LUNA C18 column 5µ (250 x 4.6 mm id), phosphate buffer (pH 3.6):methanol, 70:30 mobile phase) is presented in Figure 2. The retention time was found to be 5.317 min.

3.1 Validation of the method

The system suitability parameters proved that the proposed method is suitable for estimation of dabigatran. Tailing factor for the peak was found to be 1.26 and the theoretical plates for separation were found to be 8884.

The method was found to be linear in the range of 10-50µg/ml **(Figure 3)**. The precision of the method was good and the recovery of drugs is well within the acceptance limits of 80-120%. The LOD and LOQ were found to be 0.024µg/ml and 0.08 µg/ml respectively.

Accuracy of the method was adjudged on the basis of recovery studies at different spiking levels **(Table 1)**.

Robustness was assessed by deliberate variation in optimized conditions of flow

rate and mobile phase ratios over small range **(Table 2 & 3)**.

3.2 Assay of marketed formulation

The developed and validated method was applied for the analysis of the two different marketed formulations of dabigatran and the results obtained are presented in **Table 4**. The percentage recovery of dabigatran 99.86 %.

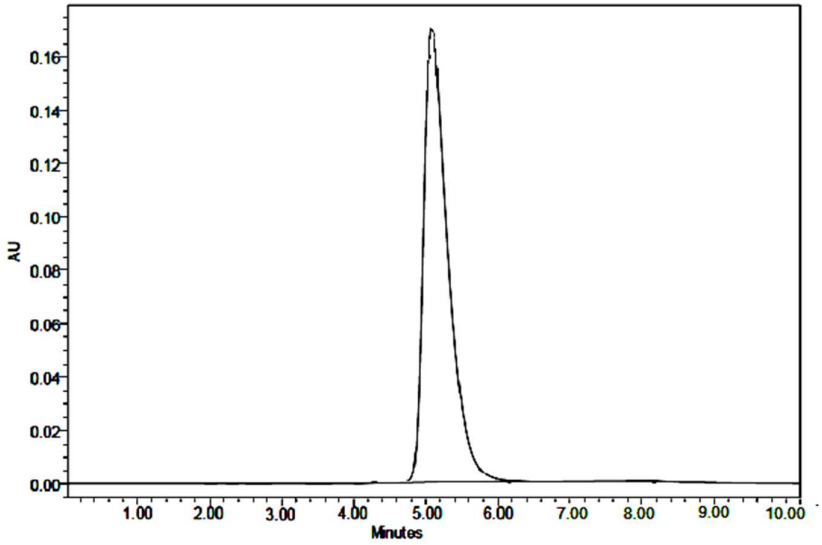

Figure 2: Chromatogram of dabigatran in optimized experimental conditions

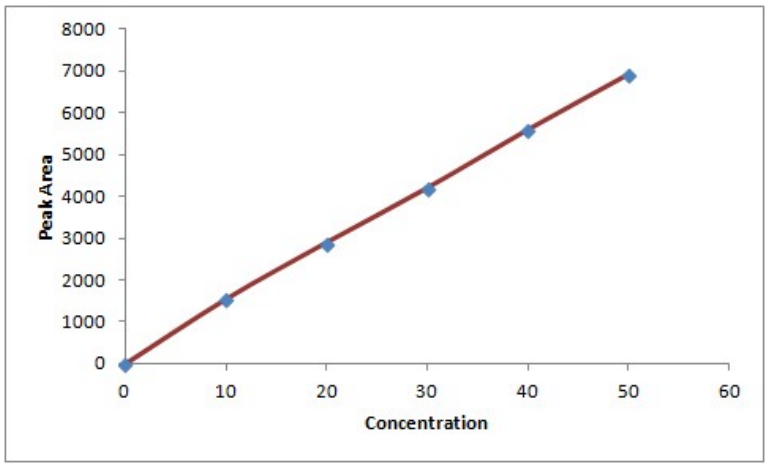

Figure 3: Linearity of the proposed method

Table 1: Recovery data obtained from the method

Conc. of drug in capsule sample µg/ml)	Conc. of drug added to final (µg/ml)	% Recovered (mean), n = 6
150	120	100.01
150	150	100.04
150	180	99.97
	Mean Recovery	100.00
	SD	0.0246
	%RSD	0.0246

Table 2: Robustness data obtained from the method by altering flow rate

Concentration (µg/ml)	Retention time at flowrate (-0.1ml/min)	% RSD	Retention Time at flowrate (+0.1ml/min)	% RSD
20	5.337	0.215	4.997	0.258
30	5.339	0.21	5.001	0.205
50	5.334	0.173	4.999	0.187

*** Average of six replicate values**

Table 3: Robustness data obtained from the method by altering mobile phase ratio

Mobile Phase ratios	Retention time (min)*	% RSD
80-20	5.319	0.857
75-25	5.317	0.598
65-35	5.319	0.251

*** Average of six replicate values**

Table 4: Results of assay of marketed formulation

Brand name & label content	Amount found (mg)*	Standard deviation	% RSD	Percentage recovery
Dabistar 150	149.79	0.397	0.795	99.86

*** Average of six replicate values**

4. CONCLUSION

The objective of the present work was to develop an accurate, precise, simple and cost effective method for routine estimation of dabigatran in capsule form. The results obtained indicate that the method is precise and accurate over a wide range of concentrations of the drug and also that the method is specifically able to estimate the drug from the capsule formulation. Thus the method can be easily used for routine estimation of dabigatran etexilate.

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