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Original Article

# 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***

**Department of Pharmaceutical Analysis and Quality Assurance, Vijaya College of Pharmacy, Munaganur (village), Hayathnagar (Mandal), Hyderabad 501511, India**
**Email: ashok576@gmail.com**

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**ABSTRACT**

**Objective:** To develop a rapid, sensitive, accurate, precise and linear Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method and validate as per ICH guidelines for the quantitative estimation of Dabigatran etexilate mesylate in capsules.

**Methods:** The optimized method uses a reverse phase column, Waters Symmetry C18 (250 X 4.6 mm; 5µ), a mobile phase of tri ethylammonium phosphate buffer (pH 2.5):acetonitrile in the proportion of 40:60 v/v, flow rate of 1.0 ml/min and a detection wavelength of 313 nm using a UV detector.

**Results:** The developed method resulted in Dabigatran etexilate mesylate eluting at 2.44 min. It exhibited linearity in the range 15-45µg/ml. The precision is exemplified by relative standard deviation of 0.05%. Percentage mean recovery was found to be in the range of 98-102, during accuracy studies. The limit of detection (LOD) and limit of quantitation (LOQ) was found to be 39.19µg/ml and 118.88µg/ml respectively.

**Conclusion:** A sensitive, rapid, accurate, precise and linear RP-HPLC method was developed and validated for the quantitative estimation of Dabigatran etexilate mesylate in capsules as per ICH guidelines and hence it can be used for the routine analysis in various pharmaceutical industries.

**Keywords:** RP-HPLC, Dabigatran etexilate mesylate, Method development, Validation.

## INTRODUCTION

Dabigatran etexilate (DE) is the oral prodrug of the active moiety dabigatran. The dabigatran etexilate pro-drug was developed due to the limited oral availability of dabigatran, and it is converted into dabigatran (DAB) *in vivo* via esterases enzyme. The drug substance is the mesylate salt form of the prodrug, called dabigatran etexilate mesylate (DEM) (fig. 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 methane-sulfonate [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 only two stability indicating assay methods are cited in the literature using HPLC in bulk [1,6] and only two methods in formulations [7, 8]. Literature reveals use of potassium dihydrogen orthophosphate as buffer (pH 4.5and pH 7.0) and triethyl ammonium phosphate buffer (pH 6.0) along with organic modifier as mobile phase for assay methods in formulations using RP-HPLC. As there is no literature reported on working at acidic pH using triethyl ammonium phosphate buffer (pH 2.5), we here report a new and a rapid RP-HPLC validated method for the quantitative estimation of Dabigatran etexialte in capsules using triethyl ammonium phosphate buffer (pH 2.5) along with acetonitrile as mobile phase.

## MATERIALS AND METHODS

### Chemicals and reagents

Analytically pure sample of Dabigatran etexilate mesylate with purity 95% was obtained as gift sample from Chandra labs, Hyderabad, India and tablet formulation [PRADAXA] capsules was procured from Apollo Pharmacy, Hyderabad, India with labelled amount of 110 mg of Dabigatran etexilate mesylate. Acetonitrile (HPLC grade), water (HPLC grade), Triethylamine (AR Grade) and ortho phosphoric acid (AR Grade) were obtained from SD Fine chemicals (Hyderabad, India), 0.45 and 0.22µm Nylon membrane filters were obtained from Spincotech Private Limited, Hyderabad, India.

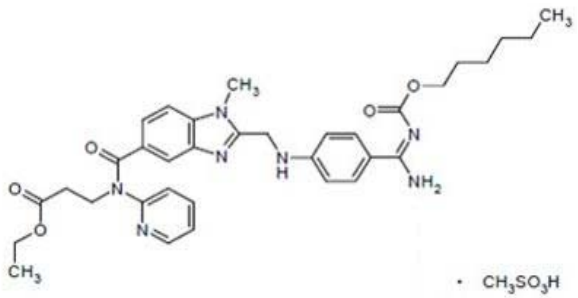


**Fig. 1: Structure of dabigatran etexilate mesylate**

### Instrument

HPLC analysis was performed on Shimadzu LC-20AD Prominence Liquid Chromatography comprising a LC-20AD pump, Shimadzu SPD-20A Prominence UV-VISIBLE detector and a reverse phase C18 column, Waters Symmetry (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. 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 (0.1 mg sensitivity, Shimadzu AY 220), digital pH meter (DELUX model 101), a sonicator (sonica, model 2200 MH).

## Methods

### Selection of wavelength

Suitable wavelength for the HPLC analysis was determined by recording UV spectrum in the range of 200-400 nm for Dabigatran. Suitable wavelength selected was 313 nm (fig. 2).

### Chromatographic conditions

The developed method uses a reverse phase C18 column, Waters Symmetry C18 (250 X 4.6 mm; 5µ), mobile phase of triethyl ammonium phosphate buffer (pH 2.5): acetonitrile in the proportion of 40:60 v/v. The mobile phase was set at a flow rate of 1.0 ml/min and the volume injected was 20µl for every injection. The detection wavelength was set at 313 nm.

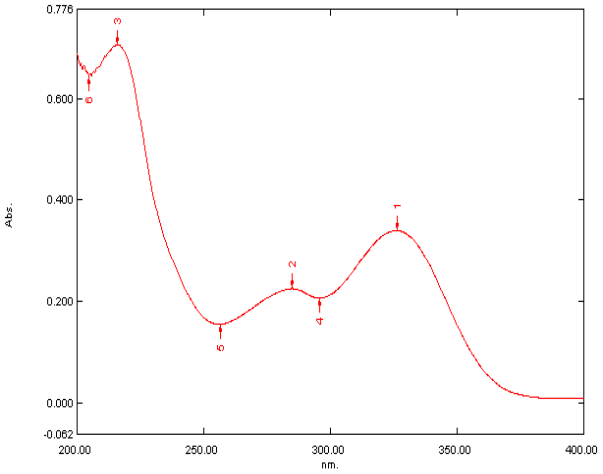


**Fig. 2: UV spectrum of dabigatran etexilate mesylate**

### 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 2.5 using 30% v/v of ortho phosphoric acid in water. The buffer was then filtered through 0.45 µm nylon membrane filter.

### 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 min for the removal of air bubbles.

### Preparation of stock and working standard solution

10 mg of Dabigatran etexilate mesylate was accurately weighed and taken in 100 ml clean and dry volumetric flask containing 50 ml of diluent (same as mobile phase) and then sonicated for 2 min to dissolve. Later the solution was made up to the mark using the mobile phase. This is considered as stock standard solution (100µg/ml). From the stock solution, 3 ml was pipetted out and to 10 ml using the mobile phase to get a concentration of 30µg/ml, treated as 100% target concentration.

### Preparation of stock and working sample solution

Not less than 20 capsules were taken, emptied and test stock solution of Dabigatran etexilate mesylate (275 µg/ml) was prepared by transferring weight equivalent to 27.5 mg of Dabigatran etexilate mesylate to 80 ml of mobile phase which is sonicated for 5 min and later made up to 100 ml with mobile phase. This solution was filtered using 0.22micron syringe filter. From the above stock solution 1.09 ml was pipetted out and made up to 10 ml to get working sample solution equivalent to a concentration of 30µg/ml for Dabigatran etexilate mesylate, concentration equal to 100% target concentration.

## RESULTS AND DISCUSSION

### Method development

A Reverse phase HPLC method was developed keeping in mind the system suitability parameters i.e. tailing factor (T), number of theoretical plates (N), runtime and the cost effectiveness. The optimized method developed resulted in the elution of Dabigatran etexilate mesylate at 2.44 min. Fig. 3 and 4 represent chromatograms of blank solution and standard solution (30µg/ml) respectively. The total run time is 5 min. System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (RT), number of theoretical plates (*N*) and peak Tailing factor (T) were evaluated for injection of the standard at working concentration. The results are given in table 1**.**

**Table 1: System suitability studies results**

| Parameters | Dabigatran etexilate mesylate |
|---|---|
| Retention time (min) | 2.44 |
| Number Of Theoretical plates (N) | 3797 |
| Tailing factor (T) | 1.739 |

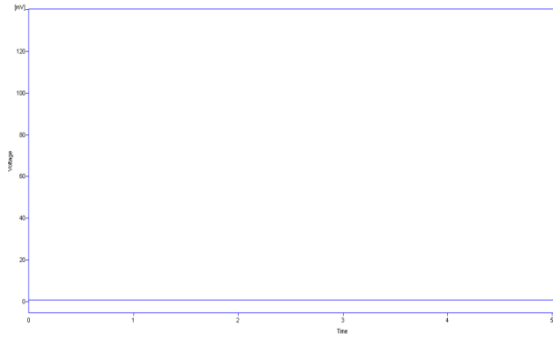

**Fig. 3: Typical chromatogram of blank solution**

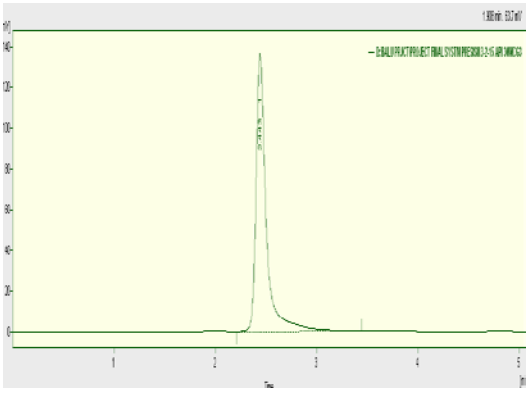

**Fig. 4: Typical chromatogram of the standard solution**

In order to test the applicability of the developed method to a commercial formulation, PRADAXA was chromato graphed at working concentration (30µg/ml) and it is shown in fig. **5.** The sample peak was identified by comparing the retention time with the standard drug fig. **4**. System suitability parameters were within the acceptance limits, ideal for the chromato graphed sample. Integration of separated peak area was done and drug concentration was determined by using the peak area concentration relationship obtained in the standardization step. The protocol affords reproducible assay of the drug in the sample ranging between 98

and 102%, which is the standard level in any pharmaceutical quality control.

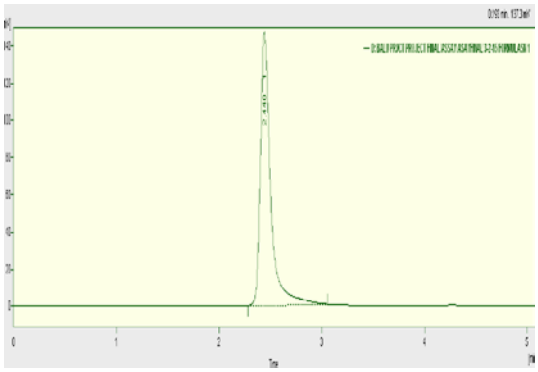

**Fig. 5: Typical chromatogram for the tablet formulation**

**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. RP-HPLC method developed was validated according to International Conference on Harmonization (ICH) guidelines [9] for validation of analytical procedures. The method was validated for the parameters like system suitability, specificity, linearity, accuracy, precision, and ruggedness, limit of detection (LOD) and limit of quantitation (LOQ).

**Specificity**

Fig. 3-5 for blank, standard drug solution and sample chromatogram reveal that the peaks obtained in the standard solution and sample solution at working concentrations are only because of the drug as blank has no peak at the retention time of Dabigatran etexilate mesylate. Accordingly it can be concluded that, the method developed is said to be specific.

**Precision**

**System precision**

Six replicate injections of the standard solution at working concentration showed % RSD (Relative Standard Deviation) less than 2 concerning peak area for the drug, which indicates the acceptable reproducibility and thereby the precision of the system. System precision results are tabulated in table 2.

**Method precision**

Method precision was determined by performing assay of sample under the tests of repeatability (Intraday precision) and intermediate precision at working concentration.

**Repeatability (Intraday precision)**

Six consecutive injections of the sample from the same homogeneous mixture at working concentration showed % RSD less than 2 concerning % assay for the drug which indicate that the method developed is method precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results (table 3).

**Table 2: System precision results of dabigatran etexilate mesylate**

| Injection no. (n) | RT | Peak area |
|---|---|---|
| 1 | 2.443 | 962.79 |
| 2 | 2.442 | 962.87 |
| 3 | 2.443 | 961.04 |
| 4 | 2.443 | 961.22 |
| 5 | 2.446 | 961.08 |
| Average | 2.443 | 961.8 |
| Standard Deviation | 0.001 | 0.943 |
| %RSD | 0.061 | 0.098 |

**Table 3: Intraday precision results of dabigatran etexilate mesylate**

| n | RT | Peak area | % Assay |
|---|---|---|---|
| 1 | 2.441 | 1018.95 | 100.64 |
| 2 | 2.441 | 1019.16 | 100.66 |
| 3 | 2.401 | 1007.82 | 99.54 |
| 4 | 2.441 | 1019.16 | 100.66 |
| 5 | 2.441 | 1018.36 | 100.58 |
| 6 | 2.46 | 1007.82 | 99.54 |
| Average | 2.437 | 1015.21 | 100.27 |
| Standard deviation | 0.019 | 5.733 | 0.566 |
| %RSD | 0.798 | 0.564 | 0.055 |

**Intermediate precision**

Intermediate precision was evaluated by performing assay of the formulations by different analyst on a different day by injecting six consecutive injections of the sample at working concentration from the same homogeneous mixture of tablets. This study showed % RSD less than 2 concerning % assay for both the drugs which indicate that the method developed is rugged and hence can be understood that the method gives reproducible results irrespective of analyst (table 4).

**Table 4: Inter day precision results of dabigatran etexilate mesylate**

| n | RT | Peak area | % Assay |
|---|---|---|---|
| 1 | 2.47 | 997.99 | 98.57 |
| 2 | 2.47 | 998.82 | 98.65 |
| 3 | 2.47 | 997.99 | 98.57 |
| 4 | 2.47 | 995.23 | 98.30 |
| 5 | 2.47 | 997.52 | 98.46 |
| 6 | 2.47 | 993.39 | 98.12 |
| Average | 2.47 | 995.02 | 98.28 |
| Standard deviation | 0 | 4.553 | 0.449 |
| %RSD | 0 | 0.456 | 0.045 |

**Linearity**

Standard solutions of Dabigatran etexilate mesylate at different concentrations level (50%, 75%, 100%, 125% and 150%) were prepared. Calibration curve was constructed by plotting the concentration level of drug versus corresponding peak area.

The results show an excellent correlation between peak area and concentration level of drug within the concentration range (15-45µg/ml) for the drug and the results are given in table 5 **and** fig. **6.** The correlation coefficient of Dabigatran etexilate mesylate is 0.996 and hence the method is said to be linear.

**Table 5: Calibration data of dabigatran etexilate mesylate**

| % Level | Concentration (µg/ml) | Peak area |
|---|---|---|
| 50 | 15 | 495.09 |
| 75 | 22.5 | 695.8 |
| 100 | 30 | 973.48 |
| 125 | 37.5 | 1218.32 |
| 150 | 45 | 1509.63 |
| Regression coefficient | | 0.996 |
| Regression equation | | y=34.021x-42.176 |

**Table 6: Results of accuracy studies for dabigatran etexilate mesylate**

| % Level | Peak area | %Recovery | % Mean recovery | %RSD |
|---|---|---|---|---|
| 50 | 525.81 | 100.87 | | |
| | 525.78 | 100.84 | 101.19 | 0.573 |
| | 525.96 | 101.87 | | |
| 100 | 1018.95 | 100.64 | | |
| | 1019.16 | 100.66 | 100.63 | 0.242 |
| | 1018.36 | 100.58 | | |
| 150 | 1584.63 | 101.3 | | |
| | 1585.38 | 101.2 | 100.56 | 1.173 |
| | 1509.63 | 99.2 | | |

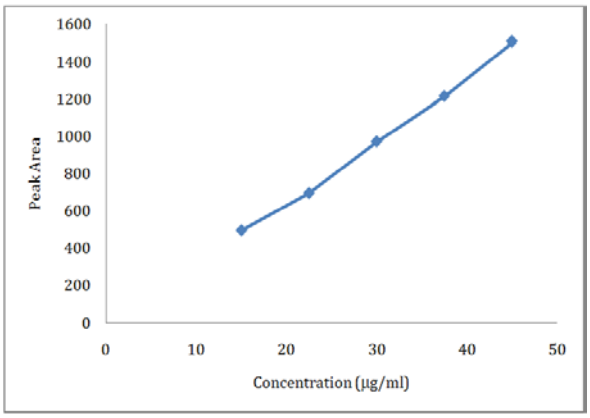


**Fig. 6: Linearity graph of dabigatran etexilate mesylate**

**Accuracy**

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at three different levels (50-150%). At each level, three determinations were performed. Percent mean recovery was calculated as shown in table 6**.** The accepted limits of recovery are 98%-102% and all observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed.

**Sensitivity**

The sensitivity of measurement of Dabigatran etexilate mesylate by use of the proposed method was estimated in terms of the limit of quantitation (LOQ) and the limit of detection (LOD). LOQ and LOD were calculated by the use of the equations LOD = 3.3σ/S and LOQ = 10σ/S where σ is the standard deviation of response of calibration plot and S is the slope of the corresponding calibration plot. The limit of detection (LOD) and limit of quantitation (LOQ) was found to be 39.19µg/ml and 118.88µg/ml respectively.

**CONCLUSION**

A reverse phase HPLC isocratic method developed has been validated as per ICH guidelines in terms of specificity, accuracy, precision, and linearity, limit of detection and limit of quantitation, for the quantitative estimation of Dabigatran etexilate mesylate in tablets. The precision is exemplified by relative standard deviation of 0.05%. A good linear relationship was observed for the drug between concentration ranges of 15 and 45µg/ml. Accuracy studies revealed that mean recoveries were between 98 and 102%, an indicative of accurate method. The limit of detection (LOD) and limit of quantitation (LOQ) was found to be 39.19(µg/ml and 118.88µg/ml respectively. Accordingly it can be concluded that the developed reverse phase isocratic HPLC method is sensitive, accurate, precise and linear and therefore the method can be used for the routine analysis of Dabigatrn etexilate mesylate in capsules.

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**CONFLICT OF INTERESTS**

Declared None

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