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Development and validation of stability indicating HPLC method for estimation of Dabigatran Etexilate Mesylate in drug substance

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Abstract: A novel high speed, high resolution Reverse phase-HPLC method was developed for estimation of assay in Dabigatran etexilate mesylate drug substance. The separation of drug from the possible impurities was achieved on an Inertsil C8 column. Ammonium formate buffer at pH 5.5 and acetonitrile mixture was selected as a mobile phase. Flow rate and detection were kept at 1.0 mL/min and 255 nm respectively. Column and sample compartment temperatures were maintained at ambient. Sample diluent was a mixture of buffer with acetonitrile. The developed HPLC method was subjected to validation parameters; Precision, Specificity, Linearity, Robustness, Ruggedness to comply with guidelines specified by ICH. Stability indicating nature of the method was also studied by exposing the sample under various conditions like acid, base, peroxide and photo stability conditions. Using the method one can carry out quantitative estimation of assay in Dabigatran etexilate mesylate drug substance, further the same method can be adopted for determination of related substances also.

Key words: Dabigatran Etexilate Mesylate, Anticoagulant, High performance liquid chromatography, Validation, Forced degradation.

Introduction

Dabigatran etexilate mesylate is chemically *Ethyl-3-[[2-[[4-[(Z)-N'-hexoxy carbonyl carbamimidoyl] anilino] methyl]-1-methyl benzimidazole-5-carbonyl]-pyridin-2-ylamino] propanoate*; methane sulfonicacid. The empirical formula is $C_{34}H_{41}N_7O_5 \bullet CH_4O_3S$ and the molecular weight is 723.86 g/mol. Figure-1 indicated chemical structure of the moulecule. Dabigatran etexilate mesylate is a yellow-white to yellow powder. A saturated solution in pure water has a solubility of 1.8 mg/mL. It is freely soluble in methanol, slightly soluble in ethanol, and sparingly soluble in isopropanol. It is sold under the brand name of Pradaxa contains 75mg, 110mg and 150mg of Dabigatran drug substance. Dabigatran is a recently developed anti thrombin anticoagulant which is used for prevention of stroke and venous embolism in patients with chronic atrial fibrillation [US FDA]. As per the literature, determination of dabigatran etexilate mesyalte in pharmaceutical dosage forms may be performed by TLC [Pintu.B], spectro photometric method [Hussain Syed shahed, Harini.U and Kumar Raja Jayavarapu] and LC-MS/MS [Nouman EG] methods. LC [Nagadeep.J, Rajesh nawale and Dare.M] related substances method was proposed for bioequivalence and pharmacokinetics evaluation.

Since this drug is being marketed in domestic an international market the present investigation by the author was to develop a rapid, accurate and precise RP-HPLC method for the determination of assay. The developed method was subjected to validation parameters [Snyder L.R and USFDA] such as precision, linearity, accuracy, robustness and ruggedness to prove accurate, precise and rugged method. The method was validated according to ICH requirements [ICH Q2, ICH Q1B and ICH Q3A (R2)]. Stability indicating nature of the method was demonstrated by exposing the drug to various stress conditions like acid hydrolysis, Base hydrolysis, heat and photo stability studies.

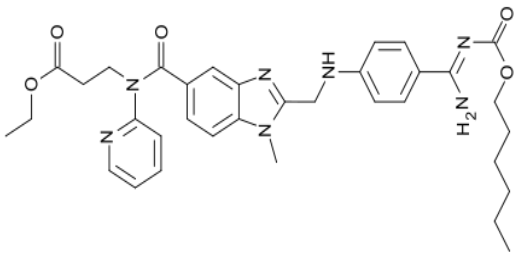

Figure 1. Chemical structure of dabigatran etexilate mesylate

Materials and Methods

Instrumentation and Reagents

Agilent Make High performance liquid chromatography with photo diode array detector was adopted for the present study. Merck AR Grade Ammonium formate and Gradient grade acetonitrile reagents were used to prepare mobile phase. Inertsil C8, 250 x 4.6 mm 5µm column purchased from advanced materials technology.

Chromatographic Conditions

Chromatographic separation was achieved on a Inertsil C8, 250 x 4.6 mm 5µm Column. Mobile phase consists of 10 mM Ammonium formate buffer and HPLC grade acetonitrile. Mobile phase flow rate was kept at 1.0mL/min with a simple gradient. Gradient program was set as Time/ % of solution B: 0/50, 15/70, 15.5/50, 20/50. Column temperature was maintained at ambient and detection was carried at 255nm. Sample compartment was maintained at ambient with an

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injection volume of 10µL. Buffer with acetonitrile in the ratio of 1:1 was used for preparation of standard and test solutions.

Preparation of Standard and Sample

Accurately weighed and transferred 25 mg of Standard solution and test solutions in a 100 mL volumetric flask separately, dissolved and diluted to the volume with diluent. Final concentration of dabigatran in the sample and standard was 0.25 mg/mL.

Method Validation

System suitability

To ensure system suitability, a standard solution was injected on to the system and verified spectral purity of individual peaks to confirm that no co elution has been occurred. Tailing factor (T) column efficiency (N) and resolution (R) were calculated for Dabigatran.

Specificity

Specificity is the ability of method to measure the analyte response in the presence of its potential impurities. The specificity of the developed RP-HPLC method was demonstrated by forced degradation studies to prove stability indicating nature of the method.

Precision

Precision of the method was reported by injecting five replicates of standard solution consecutively under the same analytical conditions. The % RSD of individual peaks was calculated. Intermediate precision of the method also evaluated using different analyst, different day and different make of instrument in the same laboratory.

Linearity

Linearity for the related substances method was prepared by serially diluting the impurity stock solution to required concentration levels. The solutions were prepared at five different concentration levels ranging from 60% to 140% with respect to specification limits. The calibration curve was drawn by plotting the peak area versus its corresponding concentrations. Correlation coefficient of the calibration curve and slope were reported.

Accuracy

Dabigatran standard solution was prepared at three concentration levels varying from 60%, 100% and 140% of concentration. The % recovery of three levels were reported. Percentage recovery was derived based on amount of standard addition and amount of recovery in the test sample solution. % Recovery should be not less than 80% and not more than 120%.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Results and Discussion

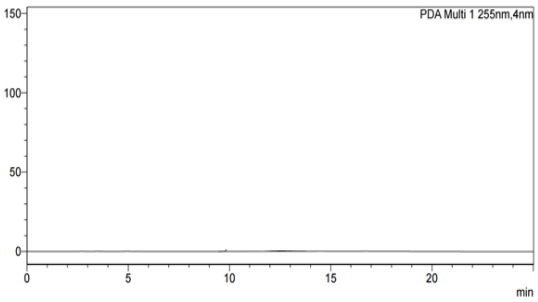
Method Development and optimization

The objective of the development is to have more specific method to achieve the separation between all impurities from the drug substance effectively and to support routine quality check at competitive time period. The wavelength of detection was finalized at 255 nm as all the degradant and dabigatran shows maximum absorbance at selected wavelength. Resolution and peak symmetry are optimal in Inertsil C8. A simple gradient was selected to resolve all the degradants and to eliminate interference with diluent and unidentified peaks from sample.

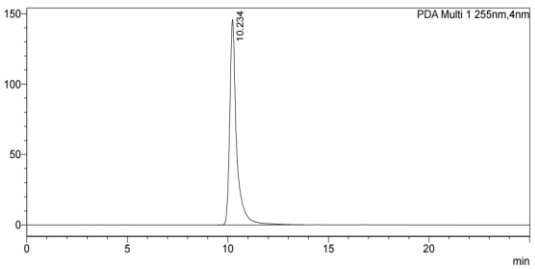
System Suitability Results

The peak shape of Dabigatran drug substance was found symmetric and well separated by its degradant impurities. A typical system suitability chromatogram of sample diluent, standard solution and test solution chromatograms are shown in Figure 2(a), 2(b) and 2(c). In the optimized conditions, Dabigatran and its related substances were well resolved with a resolution of more than 2.0. The tailing factor is in the range of 1.0 - 1.2 which indicates symmetry of peaks. Theoretical plates more than 10000 show the efficiency of the column. System suitability parameters for Dabigatran drug substance are tabulated in Table 1.

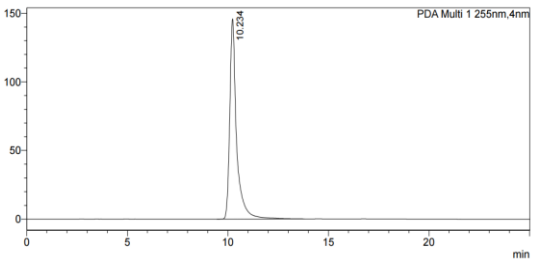
Figure 2. A typical chromatogram of Dabigatran



a) Sample diluent



b) Standard solution



c) Test solution

Table 1. System Suitability Results

Component	RT	Tailing factor	Plate count	Peak angle	Peak threshold
Dabigatran Etexilate	2.6	1.0	13293	5.523	9.151

Method Validation Results

Precision

System precision was executed by performing five replicates of the standard solution at specification level. The % relative standard deviation of 5 injections was within the acceptable limit. Which indicates the precision of the system to proceed for analysis. Results are tabulated in Table 2.

Table 2. System Precision Results

System precision	
No. of Injections	**Peak area**
1	3431189
2	3429966
3	3392211
4	3494455
5	3388877
AVG	**3427340**
STD DEV	42542.87
% RSD	**1.24**

Table 3. Linearity Results

Linearity		
Standard weight		25.16mg
Potency of standard		0.9890
No. of Inj	**Conc (Ppm)**	**Response**
1	150.00	2058713
2	200.00	2744951
3	250.00	3431189
4	300.00	4117426
5	350.00	4803663
Slope		**13724.75**
Y-intercept		**0.90**
Correlation coeffficient		**1.0000**

Linearity

Linearity of the method is to establish a linear relationship of concentration against response. Solutions of dabigatran are prepared from 60% level to 140% of the specification limit. The obtained correlation coefficient was greater than 0.99. The regression statistics for dabigatran drug substance are tabulated in Table-3. Linearity plot is shown in Figure-3. The result shows that an excellent correlation between the peak response and concentration of the analyte.

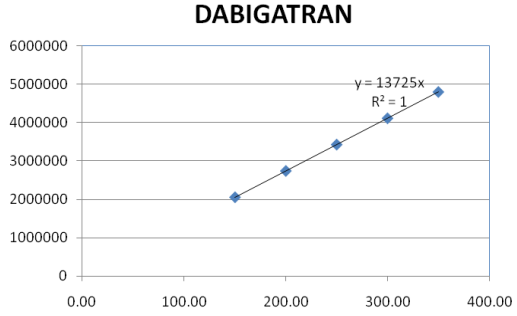


Figure 3. Linearity plot of Dabigatran

Accuracy

Accuracy of the method can be determined by spiking known concentrations of standard solution. The obtained recovery value indicates the trueness of the method to estimate drug. Dabigatran standard was prepared in a concentration range varying from 60% to 140% of their respective target analyte concentrations. The Acceptance criteria for the accuracy is 80% to 120%. The obtained percentage recovery value is in the range of 96.5 % to 99.2% which declares the method accuracy. Accuracy results are reported in Table 4.

Table 4. Accuracy Results

S.No.	Accuracy Level	Dabigatran recovery	Criteria
1	60%	96.5%	80% to 120%
2	100%	98.4%	
3	140%	99.2%	

Forced degradation study

Degradation studies were performed to demonstrate stability indicating nature of the method. Dabiagtarn test sample was exposed to various stress conditions like heat & humidity (40°C & 70% RH for 7 days), thermal (60°C for 7 days) and photolytic conditions of fluorescent light (1.2x106 LUX hours), UV light for a total exposure of 200 W·hr/m2, acid hydrolysis (0.1N HCl 80°C for 24 Hrs), base hydrolysis (0.1N NaOH, 80°C for 24 Hrs) and oxidative stress. Testing of peak purity concludes the homogeneity.

Table 5. Forced degradation studies

Stress condition	Conc. µg/mL	Purity angle	Purity Threshold	Mass balance
Non-stressed	250	0.07	5.21	100
Acid hydrolysis	254	0.01	2.44	99.2
Base hydrolysis	256	0.08	3.21	98.9
Oxidation	248	0.02	4.22	99.9
Heat and humidity	249	0.09	1.25	98.8
Photo stability	255	0.07	2.45	98.5
Dry heat	251	0.09	3.42	98.6

Peak obtained in all the stress conditions was homogenous and unaffected by the presence of its degradation impurities, confirming the stability indicating nature of the method. Mass balance also established to match up the sum of impurities with its assay value against reference unstressed sample. The results from forced degradation studies are summarized in Table 5.

Conclusion

A Reverse phase-HPLC method was developed for the estimation of assay in dabigatran drug substance. The developed method was subjected to method validation parameters as recommended by ICH. Stability indicating nature of the method is also established by applying forced degradation studies. The developed method was specific, precise, accurate and linear to estimate accurate amount of drug present in the sample. Degradation studies confirmed the homogeneity and free of interferences with the peak of interest. The method can be adopted to

determine the assay of drug substance in quality control labs.

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