WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 8, Issue 1, 1249-1259 Research Article ISSN 2278 – 4357

METHOD DEVELOPMENT AND VALIDATION FOR ASSAY OF DABIGATRAN ETEXILATE MESYLATE IN PHARMACEUTICAL DOSAGE FORM BY HPLC

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Article Received on
14 November 2018,
Revised on 04 Dec. 2018,
Accepted on 25 Dec. 2018,
DOI: 10.20959/wjpps20191-13009

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ABSTRACT

A new HPLC method was developed and validated for the determination of Dabigatran Etexilate Mesylate (DEM), in tablet dosage form. The chromatographic quantification and qualification was achieved on a XTerra C18 (4.6 x 150mm, 5 µm) or equivalent with a mobile phase combination of Ph4.5 0.1%TFA buffer and Acetonitrile in isocratic mode in 45:55 ratio, employing flow rate of 1.0 ml/min, and the detection was carried out by using UV detector at 334 nm with total run time was 6 minutes. The retention time of DEM was found to be 2.163min. The performance of the method was validated according to the present ICH guidelines.

KEYWORDS: RP-HPLC, Dabigatran Etexilate Mesylate (DEM).

INTRODUCTION

DEM is an oral prodrug that is metabolized by a serum esterase to dabigatran. It is a synthetic, competitive and reversible direct thrombin inhibitor. Inhibition of thrombin disrupts the coagulation cascade and inhibits the formation of clots. DEM may be used to decrease the risk of venous thromboembolic events in patients who have undergone total hip or knee replacement surgery, or to prevent stroke and systemic embolism in patients with atrial fibrillation, in whom anticoagulation therapy is indicated. In contrast to warfarin, because its anticoagulant effects are predictable, lab monitoring is not necessary. The chemical name of DEM is ethyl 3-(1-{2-[({4-[amino ({[(hexyloxy) carbonyl] imino}) methyl] phenyl} amino)methyl]-1-methyl-1H-1,3-benzodiazol-5-yl}-N-(pyridin-2-yl)

formamido) propanoate; methanesulfonic acid. It has a molecular formula of $C_{35}H_{45}N_7O_8S$ and a molecular weight of 723.846 g/mol. It has the structural formula (Figure 1). DEM is a white to off-white crystalline solid with a solubility of approximately 1.8 mg/ml in water.[1,2,3]

Literature survey reveals that various HPLC methods.[4,5,6,7,8,9] However all the available HPLC methods for the determination of DEM in formulations were time consuming for elution and lack of reproducibility. Hence we felt to develop a stability indicating method with short run time. The method is simple, precise and accurate with short run time. The stability indicating developed method was validated as per ICH Guidelines.

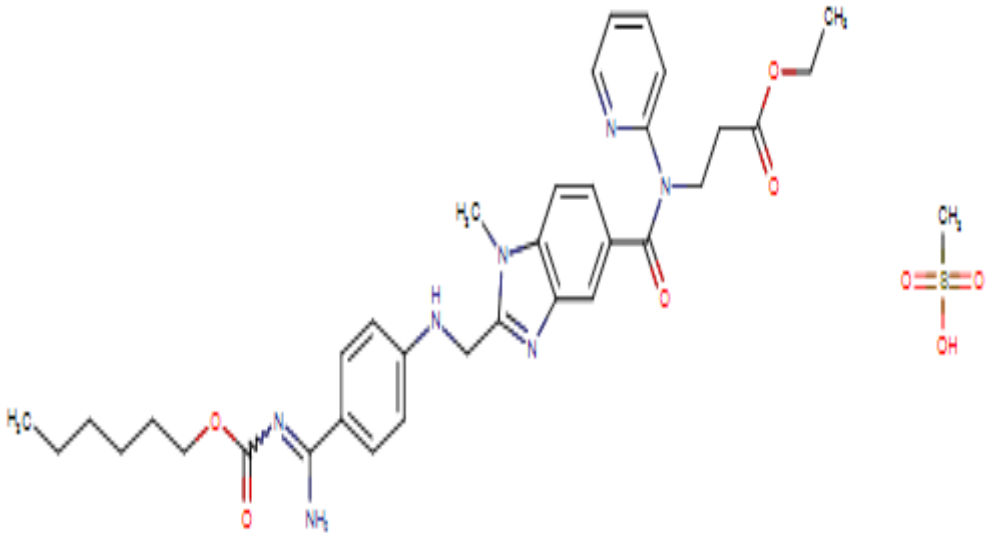

Figure 1: Dabigatran Etexilate Mesylate (DEM) Structure.

Experimental

Chemicals and reagents

DEM bulk drug was made available from Pharmatrain, Kukatpally, and Hyderabad. Trifluoroacetic Acid, Orthophosphoric acid and Acetonitrile were obtained from Fisher Scientific. All chemicals and reagent used were of HPLC grade, Milli-Q-water was used throughout the experiment.

Equipments

The Waters HPLC system with a UV or photo diode array detector was used for method development and validation. The output signal was monitored and processed by using Empower software.

Chromatographic condition

Instrument used	:	Waters HPLC with auto sampler and PAD or detector.
Temperature	:	Ambient
Column	:	XTerra C18 (150 x 4.6mm, 5µm)
Buffer	:	0.1% Octa sulphonic acid
pH	:	4.5
Mobile phase	:	45% buffer 55% Acetonitrile
Flow rate	:	1 ml per min
Wavelength	:	334 nm
Injection volume	:	20 µl
Run time	:	8 min.

Preparation of standard solution

Accurately weigh and transfer 25mg of Dabigatran EM working standard into a 25ml clean dry volumetric flask add about 10ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent (30ppm of Dabigatran EM).

Assay of Pharmaceutical Dosage form: (Sample Preparation)

Accurately weigh 5 tablets crush in mortor and pestle and transfer equivalent to 25mg Dabigatran EM (marketed formulation=42.5mg of tablet Powder) sample into a 25ml clean dry volumetric flask add about 10 ml of Diluent and sonicate it up to 30 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.44 micron Injection filter (Stock solution).

Further pipette 0.3 ml of Dabigatran EM from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent (30ppm of Dabigatran EM).

Procedure: 20µl of the standard and sample solutions were injected into the chromatographic system and the area of peak for DEM was measured and the % assay was calculated by using the formula.

Method Development

Chromatographic parameters were preliminary optimized to develop HPLC method for simultaneous estimation of DEM with short analyses time (8min). The wavelength of the DEM selected was 334 nm. In order to identify a suitable organic modifier, various compositions of acetonitrile and methanol were tested along with different buffers. Different columns like XTerra, Inertsil, Inspire columns were tried.

Finally separation of DEM was carried out by gradient elution with a flow rate of 1.0 mL/min XTerra (4.6 x 150mm, 5µm,). The standard chromatogram was shown in Fig-2. The system suitability parameters were shown in Table-1.

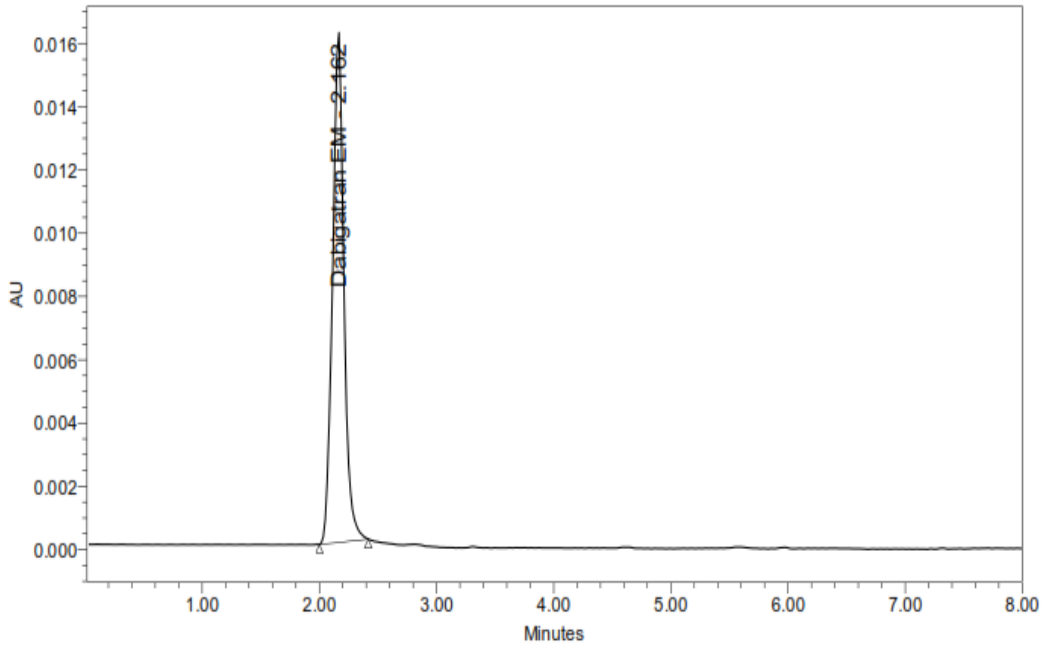

Figure 2: Standard chromatogram

Table 1: System suitability parameters.

Drug Name	Retention time	USP Plate count	USP Tailing
DEM	2.163	2334.25	1.07

Assay of Pharmaceutical Dosage form: The proposed validated method was successfully applied to determine DEM in their tablet dosage form. The result obtained for DEM was comparable with the corresponding labeled amounts. The sample chromatogram was shown in Fig-3. The labeled amounts and % assay were shown in Table-2.

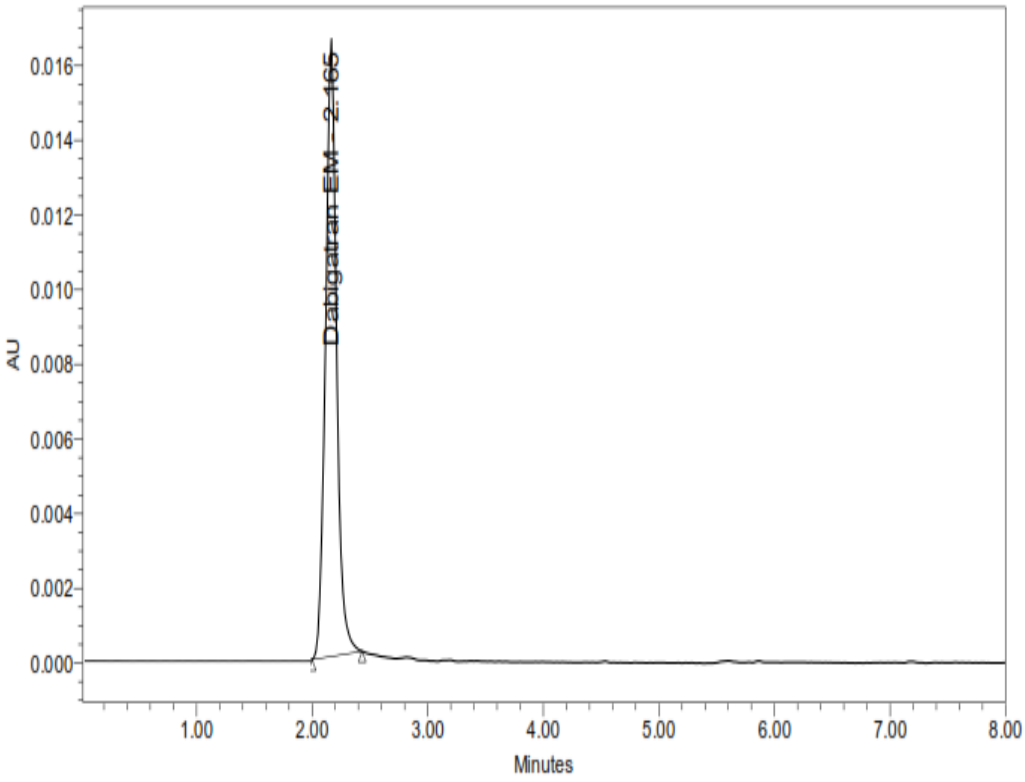

Figure 3: Sample chromatogram

Table 2: Assay Results

Drug Name	Label claim	% Assay
DEM	75 mg	100.50

RESULTS AND DISCUSSION

Method Validation

The above method was validated according to ICH guidelines to establish the performance characteristics of a method (expressed in terms of analytical parameters) to meet the requirements for the intended application of the method.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity of detector response for DEM was established by analyzing serial dilutions of a stock solution of the working standard. Five concentrations ranging from 10-50 µg/ml for were prepared and analyzed. The linearity graph was plotted using concentration Vs peak area and they were shown in Fig-4. Slope, correlation coefficient (R) and intercept were calculated and the results were shown in Table-3.

Table 3: Linearity Results for DEM.

S. No.	Linearity Level	Concentration	Area
1	I	0	0
2	II	10	34608
3	III	20	72855
4	IV	30	108936
5	V	40	147300
6	VI	50	182696
Correlation Coefficient			0.999
Intercept			908.24
Slope			3679

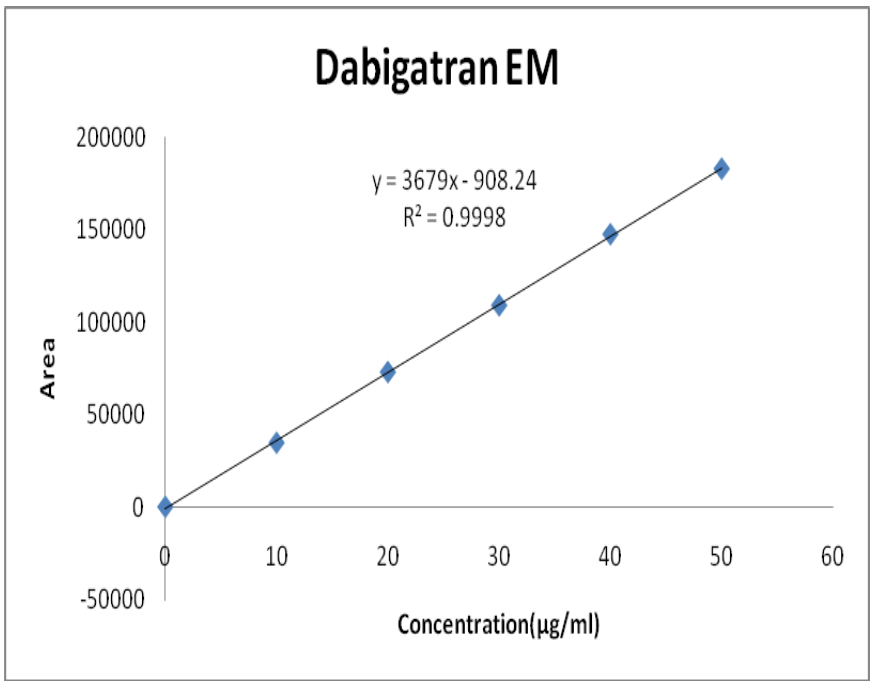

Figure 4: Linearity plot for DEM.

Precision

For the precision study, repeatability study was carried out for short time interval under the same chromatographic conditions. For the intermediate precision study, repeatability study was carried out in different day under the same chromatographic conditions and in different systems under the same chromatographic conditions. The sample was injected in six replicate for intermediate precision and six replicate for precision. The peak area for injections was recorded. The mean and % relative standard deviation (%RSD) was calculated. From the data obtained the developed RP-HPLC method was found to be precise. The results were shown in Table-4 and ID Precision in Table-5.

Table 4: Precision results for DEM.

Injection	Area
Injection-1	108897
Injection-2	108982
Injection-3	108500
Injection-4	110188
Injection-5	108944
Injection-6	108537
Average	109008.0
Standard Deviation	614.7
%RSD	0.6

Table 5: System ID Precision results for DEM.

Injection	Area
Injection-1	109237
Injection-2	108617
Injection-3	108805
Injection-4	108943
Injection-5	109173
Injection-6	108915
Average	108948.3
Standard Deviation	230.3
%RSD	0.2

Method precision

The samples are prepared as per the procedure mentioned in assay under sample solution preparation by weighing the individual tablet powder as follows

Method precision 1- 42.8mg

Method precision 2- 42.4mg

Method precision 3- 42.6mg

Method precision 4- 42.5mg

Method precision 5- 42.5mg

Method precision 6- 42.4mg

The above procedure used for 6 different preparations and injected, % relative standard deviation (%RSD) was calculated. From the data obtained the developed RP-HPLC method was found to be precise. The results were shown in Table-6.

Table 6: Method Precision results for DEM

Sample Name	Area	% Assay
Method precision-1	109098	99.34
Method precision-2	109136	100.31
Method precision-3	109158	99.86
Method precision-4	109089	100.03
Method precision-5	109072	100.01
Method precision-6	109176	100.35
Average	109121.5	99.98
Standard deviation	41.4	0.37
% RSD	0.0	0.37

Accuracy

The accuracy of the method was determined by recovery experiments. Known concentration of working standard was added to the fixed concentration of the pre-analyzed tablet sample. Percent recovery was calculated by comparing the area with pre analyed sample. The recovery studies were performed in triplicate. This standard addition method was performed at 50%, 100%, 150% level and the percentage recovery was calculated by subtracting the total area from pre analyed sample area. The results were shown in Table-7.

Table 7: Accuracy Results for DEM.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	54575.7	12.5	12.5	100.09	100.41
100%	109749.0	25	25.16	100.63	
150%-3	164433.7	37.5	37.69	100.52	

Robustness

Robustness of the method was checked by making slight deliberate changes in chromatographic conditions like mobile phase ratio (±10%), flow rate (±0.2ml/min). It was observed that there were no marked changes in system suitability parameters, which demonstrated that the developed RP-HPLC method is robust. The results were shown in Table-8,9.

Table 8: Robustness Results for DEM

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	1.05	2252.22
2	1	1.07	2334.25
3	1.1	1.06	2925.12

Table 9: Robustness Results for DEM.

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	1.15	3005.27
2	*Actual	1.07	2334.25
3	10% more	1.11	3384.13

Limit of detection and Limit of quantification

The limit of detection (LOD) and limit of quantitation (LOQ) of the method were determined by standard deviation of response and slope method.

Degradation Studies

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Dabigatran EMe using the proposed method. The results were shown in Table-10.

Preparation of stock

Accurately weigh 5 tablets crush in mortor and pestle and transfer equivalent to 25mg Dabigatran EM (marketed formulation=42.5mg of tablet Powder) sample into a 25ml clean dry volumetric flask add about 10 ml of Diluent and sonicate it up to 30 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.44 micron Injection filter (Stock solution).

Hydrolytic degradation under acidic condition

Pipette 0.3 ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

Hydrolytic degradation under alkaline condition

Pipette 0.3ml of above solution into a 10ml volumetric flask into a 10ml volumetric flask and add 3ml of 0.1N NaOH was added in 10ml of volumetric flask. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

Oxidative degradation

Pipette 0.3ml above stock solution 2 into a 10ml volumetric flask solution into a 10ml volumetric flask 1 ml of 3% w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

Thermal induced degradation

Dabigatran EM sample was taken in petridish and kept in Hot air oven at 110^0 C for 24 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analysed.

Photo degradation: Pipette 0.3 ml above stock solution into a 10ml volumetric flask and expose to sunlight for 24hrs and the volume was made up to the mark with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

Table 10: Degradation results for Dabigatran EM.

Sample Name	Dabigatran EM				
	Area	% Degraded	Purity Angle	Purity Threshold	Peak purity
Standard	108838.7				
Acid	106936	1.75	0.153	0.250	Passes
Base	106497	2.15	0.219	0.352	Passes
Peroxide	107083	1.61	0.162	0.296	Passes
Thermal	103735	4.69	0.191	0.315	Passes
Photo	105639	2.94	0.145	0.326	Passes

CONCLUSION

In the present work a new, accurate, precise and robust HPLC method was developed and validated for estimation of DEM in pharmaceutical dosage form in accordance with the ICH parameters. Linearity is observed in the concentration range of 10-50µg/ml at 334 nm. The results of the analysis of pharmaceutical formulation by the proposed method are highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be useful for the routine analysis of the DEM tablet dosage form without any interference of excipients.

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