EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 7
Issue: 4
336-339
Year: 2020

DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING RP-HPLC METHOD FOR ESTIMATION OF DABIGATRAN ETEXILATE MESYLATE

Yeragodala Narendra Reddy and Jadi Sreeramulu*

Department of chemistry, Sri Krishnadevaraya University, Ananthapuramu-515003 A.P, India.

***Corresponding Author: Dr. Jadi Sreeramulu**
Department of chemistry, Sri Krishnadevaraya University, Ananthapuramu-515003 A.P, India.

Article Received on 28/01/2019 **Article Revised on 02/03/2019** **Article Accepted on 12/05/2019**

ABSTRACT
The main objective of the study was to develop RP-HPLC method development and validation of the Dabigatran Etexilate Mesylate in the pharmaceutical dosage form. The technique was developed and thoroughly validated based on ICH (International Conference on Harmonization) guidelines. The validation process was done by precision, linearity, robustness studies, accuracy, Limit of Detection, Limit of quantification and degradation studies. The robustness studies were observed by making variations in the mobile phase, temperature, and flow rate. The developed RP-HPLC method achieved good precision and accuracy. The developed and validated method was suitable for analysis of Dabigatran Etexilate Mesylate.

KEYWORDS: Dabigatran Etexilate Mesylate, RP-HPLC, Method Development and Validation.

1. INTRODUCTION

Dabigatran Etexilate Mesylate is chemically Ethyl 3-{[(2-{[(4-{*N*'-hexyloxy carbonyl carbamimidoyl} phenyl) amino] methyl}-1-methyl-1*H*-benzimidazol-5-yl) carbonyl] (pyridin-2-amino) propanoate. Dabigatran Etexilate Mesylate is an oral anticoagulant[1] Dabigatran Etexilate Mesylate may be used to decrease the risk of venous thromboembolic events in patients in whom anticoagulation therapy is indicated.[2]

Literature search reveals following methods reported viz., DOAC-Stop Procedure by LC-MS/MS Assays [3] HPTLC Method[4] method for determination of Dabigatran Etexilate Mesylate, UV- spectrophotometric method[5] LC-MSMS assay[6] method for Stability Indicating RP-HPLC Method[7] for quantification of Dabigatran Etexilate Mesylate. There is a need to develop newer RP-HPLC method. The planned method is economical, simple, precise, stability-indicating and appropriate for routine investigation.

The guidelines entitled "Stability testing of novel drug samples and products" by The International Conference on Harmonization (ICH) need that stress testing be performed to clarify the natural stability distinctiveness of the dynamic substance.[8] The method was effectively validated in pursuance of ICH guidelines.[9-10]

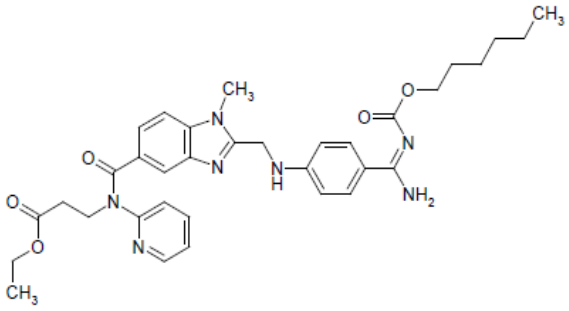

Fig. 1: Structure of Dabigatran Etexilate Mesylate.

2. MATERIALS AND METHODS

2.1 Instrumentation and Reagents

Agilent Make High performance liquid chromatography with photo diode array detector was adopted for the present study. Ammonium formate and Gradient grade acetonitrile reagents were used to prepare mobile phase. Zorbox SB Aq C8 250 x 4.6 mm, 5 µm as a stationary phase.

2.3 Chromatographic Conditions

The chromatographic separations achieved on column Zorbox SB Aq C8 250 x 4.6 mm, 5 µm as a stationary phase. The mobile phase was composed of Ammonium formate buffer and HPLC grade acetonitrile (1:1) at a flow rate was maintained 1.0 ml/min. The total run time was 12 min, and all establishments were performed at 30°C and the drugs were detected at 255 nm.

2.4 Standard solution

Accurately weigh and transfer about 25.0 mg of Dabigatran Etexilate Mesylate reference standard in a 100 ml volumetric flask, dissolve and make up to the volume with diluent.

2.5 Test solution

Accurately weigh and transfer about 25 mg of sample into a 100 ml volumetric flask, dissolve and dilute to the volume with diluents.

3. METHOD DEVELOPMENT AND OPTIMIZATION

The technique was developed by using the Mobile phase containing ammonium formate as buffer solution and acetonitrile used in the ratio 1:1 and Zorbox SB Aq C8 250 x 4.6 mm, 5 µm as a stationary phase at a flow rate of 1.0 mL/min. The temperature was maintained throughout the method at 30°C and the optimized wavelength chosen was 255 nm.

3.1 System Suitability Results

Six repeat injections of standard solution of Dabigatran Etexilate Mesylate were injected and the chromatograms be recorded. The system was appropriate for examination of the % relative standard deviation (%RSD) must be not more than 2.0%. USP tailing factor for Dabigatran Etexilate Mesylate peak should be not more than 2.0. Theoretical plates more than 10000 show the efficiency of the column. The results are revealed in **Table 1**.

Table 1: System suitability.

Analyte	Plate count	Tailing Factor	Retention Time
Dabigatran Etexilate Mesylate	13293	1.2	8.498

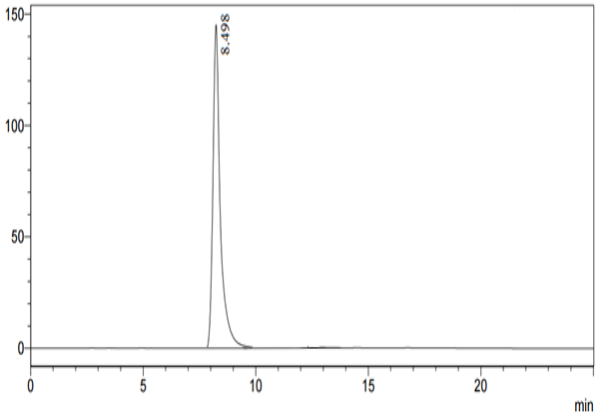

Fig. 2: Standard chromatogram of Dabigatran Etexilate Mesylate.

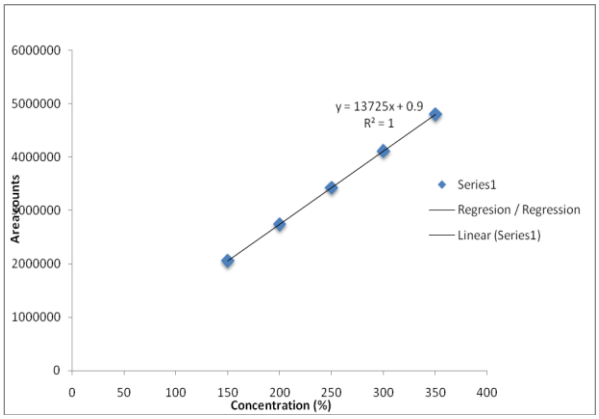

Fig. 3: Calibration curve for Dabigatran Etexilate Mesylate.

3.2 Linearity and Range

Linearity was tested for the range of concentrations from 60% to 140% of the test concentration level. Each sample in five replicates was analyzed and peak areas were recorded. Area was plotted against the corresponding concentrations to obtain the calibration curve. It shall be considered as linear if the correlation coefficient is 1.0000. The results are revealed in **Table 2**.

Table 2: Linearity results.

Dabigatran etexilate mesylate		
Level	**Concentration (µg/mL)**	**Area counts**
60%	150.00	2058713
80%	200.00	2744951
100%	250.00	3431189
120%	300.00	4117426
140%	350.00	4803663
	Slope	13724.75
	Correlation coefficient	1.0000
	Y-Intercept	0.90

3.3 Accuracy results

To check accuracy of the method recovery studies were carried out by adding standard solution to sample solution at three different levels 60%, 100% and 140% Dabigatran Etexilate Mesylate standard solution. These solutions were injected to obtain the chromatogram. The Acceptance criteria for the accuracy are 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 revealed in **Table 3**.

Table 3: Accuracy results

Preparation	60 % Level	100 % Level	140 % Level
	% Recovery	% Recovery	% Recovery
Preparation-1	96.5	98.0	99.4
Preparation-2	97.2	98.4	99.0
Preparation-3	96.0	98.8	99.2
Mean Recovery	96.5%	98.4%	99.2%

3.4 Precision method

The precision of the method was determined by analyzing a test sample of Dabigatran Etexilate Mesylate with five different preparations. The relative standard deviation was obtained within the limits.

Table 4: Precision results.

Preparation	Sample weight	Peak area of sample
1	25.12	3432266
2	25.22	3442555
3	25.32	3462244
4	24.99	3429999
5	24.85	3425555
AVG		3438524
STD DEV		14653.67
%RSD		**0.43**

3.5 Limit of detection and quantification (LOD and LOQ)

From the linearity data the limit of Detection and Quantification was calculated, using the following formula. LOD= 3.3 σ/ S and LOQ = 10 σ/ S σ = standard deviation of the response S = slope of the calibration curve of the analyte.

3.6 Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase ratio, flow rate, pH ware altered and the effects on the peak area were noted.

4. FORCED DEGRADATION STUDY

Degradation studies were performed to demonstrate stability indicating nature of the method. Dabigatran Etexilate Mesylate test sample was exposed to various stress conditions like thermal and photolytic, acid hydrolysis, base hydrolysis and oxidative stress. RP-HPLC results showed that the Dabigatran Etexilate Mesylate purity angle was less than the purity threshold and hence the proposed method was the specific and revealed its stability-indicating power.

Table 5: Degradation results.

Stress condition	Conc. µg/mL	Purity angle	Purity Threshold	Mass balance
Non stressed	250	0.07	5.21	100.0
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
Photo stability	255	0.07	2.45	98.5

5. CONCLUSIONS

The current study describes new and simple, reliable, economic elution RP-HPLC method for the Dabigatran Etexilate Mesylate. The forced degradation studies were conducted for the three drugs by using several degradation conditions like oxidation, acidic, alkali, thermal, and photolytic conditions and proposed method was effectively employed from the resolution of employed samples peaks. To the best of our knowledge, no such detailed and stability indicating method has been presented for the assay of this drug. The developed method finished use of HPLC as a tool for peak integrity and purity confirmation. Therefore the proposed study method can be used for quantification Dabigatran Etexilate Mesylate in bulk and pharmaceutical dosage form. Finally, this method was carefully validated; as a result, it can be suggested for routine analysis and for testing quality through stability studies of the drug.

6. ACKNOWLEDGEMENT

The authors thankful to Spectrum Pharma Pvt. Ltd., Hyderabad, India for providing gift samples and also to the Department of chemistry, S. K. University, Ananthapuramu, India for their encouragement.

7. REFERENCES

1. www.en.m.wikipedia.org/wiki/Dabigatran.com (accessed on 15/5/2014).
2. https://www.drugbank.ca/drugs/DB06695
3. L. Slavik , J. Jacova , D. Friedecky , J. Ulehlova , Z. Tauber , J. Prochazkova , A. Hlusi , and M. Palova, Evaluation of the DOAC-Stop Procedure by LC-MS/MS Assays for Determining the Residual Activity of Dabigatran, Rivaroxaban, and Apixaban. Clinical and Applied Thrombosis/Hemostasis, 2019; 25: 1-6
4. Pintu B. Prajapati, Arti J. Rakholiya, Kunjan B. Bodiwala, Bhavin P. Marolia and Shailesh A. Shah.

Stability Indicating HPTLC Method for Estimation of Dabigatran Etexilate Mesylate in its Pharmaceutical Dosage Form. Eurasian Journal of Analytical Chemistry, 2017; 12(2): 75-86.
5. Harini U., N. Madhavi latha, A.K.M Pawar. "Development and validation of UV-spectrophotometric method for the estimation of dabigatran etexilate mesylate (dem). J. of Pharmacy and Analytical Research, 2016; 5(1): 218-223.
6. Nouman EG, MA Al-Ghobashy, HM Lotfy. Development and validation of LC-MSMS assay for the determination of the prodrug dabigatran etexilate and its active metabolites in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci., 2015; 989(1): 37-45.
7. Dare M, R Jain and A Pandey, Method Validation for Stability Indicating Method of Related Substance in Active Pharmaceutical Ingredients Dabigatran Etexilate Mesylate by Reverse Phase Chromatography. J Chromatogr Sep Tech, 2015; 6(2).
8. ICH, Q1A, Stability Testing of New Drug Substances and Products, in: Proceedings of the International Conference on Harmonisation, Geneva, October, 1993.
9. ICH, Q2A, Hamonised Tripartite Guideline, Test On Validation of Analytical Procedures, IFPMA, in: Proceedings of the International Conference on Harmonization, Geneva, March, 1994.
10. ICH, Q2B, Hamonised Tripartite Guideline, Validation of Analytical Procedure: Methodology, IFPMA, in: Proceedings of the International Conference on Harmonization, Geneva, March 1996.