IJBPAS, April, 2019, 8(4): 749-757

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

METHOD DEVELOPMENT AND VALIDATION OF DABIGATRAN ETIXILATE MESYLATE BY RP-HPLC METHOD AND IT' S DEGRADATION STUDIES

BORSE SL[1*], CHHABRA GS[1], BORSE LB[2] AND JADHAV AG[3]

1: Department of Pharmaceutical Chemistry, Sandip Institute of Pharmaceutical Sciences, Nasik, Maharashtra, India

2: Department of Pharmacology, Sandip Institute of Pharmaceutical Sciences, Nasik, Maharashtra, India

3: Department of Pharmacognosy, Sandip Institute of Pharmaceutical Sciences, Nasik, Maharashtra, India

***Corresponding Author: E Mail: sandhya.borse@sandippharmacy.org; sandhyaborse27@rediffmail.com; Mob. – 8007122655**

Received 20th Nov. 2018; Revised 11th Dec. 2018; Accepted 25th Dec. 2018; Available online 1st April 2019

https://doi.org/10.31032/IJBPAS/2019/8.4.4689

ABSTRACT

The objective of research was to develop the simple and sensitive reverse phase high performance liquid chromatographic method for determination of Debigatran etexilate mesylate in bulk & capsule dosage form and to validate the developed methods according to ICH guidelines. Quantitative HPLC was performed with HPLC Binary Gradient System using software HPLC workstation, model no. HPLC 3000 series, and company Analytical Technologies Ltd. with UV-Visible- 3000 m detector using column grace C18 ×4.6ID, particle size 5 micron. The mobile phase used in study was methanol: water (90:10). The conditions optimized were flow rate of 0.8 ml/min. Column temperature was maintained at room temperature. The developed method was evaluated in the assay of commercially available capsule Paradaxa containing Dabigatran Etexilate Mesylate. The robustness of the method was studied. The stability studies of drug were also carried out. Retention time was found to be 4.8 min. The linearity was found to be in the concentration range of 10- 50 µg/ml. The amount of drug in capsule was found to be 75 mg. Results of analysis were validated

statistically and by recovery studies. The recovery studies 99 % were indicative of the accuracy of proposed method. The interday and intraday precision was found to be 0.08 % and 0.07 %. The limit of detection and limit of quantitation was found to be 0.51 µg/ml and 1.54 µg/ml. The proposed method was found to be simple, reproducible and sensitive and can be used routinely to determine Dabigatran etexilate mesylate in pharmaceutical formulations.

Keywords: Debigatran etexilate mesylate, HPLC, method validation, precision, stability studies

INTRODUCTION

It is important to locate the content of each medication either in bulk or single or combined dosage forms for purity testing. It is additionally basic to know the concentration of the medication and it's metabolites in biological fluids after taking the dosage form for treatment. The extent of development and validation of an analytical method is to ensure a specific method for a specific analyte more precise, specific, and accurate. The fundamental goal is to enhance the conditions and parameters, which ought to be followed in the development and validation. As per the review, it was discovered that couple of analytical techniques, for example, (RP-HPLC Spectrophotometric) were accounted for the determination of Dabigatran Etexilate Mesylate. Likewise a couple of LC-MS and UPLC/MS were accounted for the measurement of drug in human plasma. The goal of the proposed technique is to create simple and precise strategies for the estimation of the drug by RP-HPLC method in pharmaceutical dosage forms & its stability indicative studies.

Dabigatran etexilate mesylate is chemically β-Alanine, N-[[2-[[[4-[[[(hexyloxy) carbonyl] amino] phenyl] amino] methyl]-1-methyl-1H-benzimidazol-5yl] carbonyl]-N pyridinyl- ethyl ester, methane sulfonate. The empirical formula$C_{34}H_{41}N_7O_5.CH_4O_3S$ and the molecular mass of mesylate salt is 723.86 and that of free base is 627.75. It is exceptionally dissolvable in methanol and moderately in water. Dabigatran etexilate mesylate is a whitish yellow powder. A saturated solution prepared in pure water has a solubility of 1.80 mg/ml. In methanol, it is highly soluble; slightly in ethanol and sparingly in isopropanol. It is an inactive pro-drug which is converted to active Dabigatran. It is the major active constituent of plasma having rapid-acting competitive and reversible direct inhibition of thrombin.

MATERIALS AND METHODS

Instruments and Reagents: The chromatographic separation was performed

by using HPLC Binary Gradient System model no: HPLC 3000 Series (Analytical Technologies Ltd), UV-Visible Detector (UV-3000-M), a Pump: P- 3000- M Reciprocating (40 MPa), Column: Grace C 18 (250nm × 4.6ID, Particle size: 5 µ). Using Spin Chrome software & Hamilton injector, chromatographic data was collected. Analytical balance (Wenser), Ultra Sonicator (Wenser) has been used in the work. Pure drug was procured from SR Laboratories, Hyderabad, India. Paradaxa capsules were produced from local market (75mg, Mfg by: Boehringer Ingelheim Pvt. Ltd.). HPLC Grade methanol, acetonitrile and water were obtained from Merck.

Optimized chromatographic conditions: The mobile phase of methanol: water = 90:10 were used in this study. The conditions optimized were flow rate (0.8 ml/min), wavelength (227 nm) and run time was 10 min. Temperature of the column was kept at room temperature. Retention time was observed to be 6.198 min. Analytical Column Grace C^{18} (250mm × 4.6ID, Particle size; 5 micron) was used as stationary phase in the study.

Preparation of Mobile phase: Methanol and water were mixed in the proportion of 90:10 and filtered through membrane filter of 0.2 µm and degassed for 10 minutes in a sonicator.

Preparation of Standard solution: An appropriate amount of analyte (10mg) was dissolved in methanol (100ml) which was sonicated and equilibrated to room temperature.

Preparation of Test solution: An appropriate amount of capsule content (28.65mg) was dissolved in methanol (100ml) which was sonicated and equilibrated to room temperature.

Method Validation: As per ICH guidelines, the parameters of method validation to be inspected were linearity, accuracy, precision, limit of detection, limit of quantification.

Linearity and Range: The linearity of the technique was detected by preparing different concentrations of solutions in the range from the stock solution (10-50 µg/ml) and calibration curve was plotted with peak area versus concentration of solution. The drug was found to give linear response with correlation coefficient value of 0.999.

Accuracy: To study the accuracy, 10 capsules content was removed and analysis of the same was carried out. Recovery studied were carried out by standard additional method by adding the known amount of Dabigatran etixilate mesylate to the preanalyzed sample at three different concentration level that is 50, 100 and 150% of assay concentration and % recoveries were calculated.

Precision: The precision of an analytical method was studied by performing intra-day and inter- day precision.

1. Intra-day Precision: Intra-day precision was calculated by evaluating the standard solution of Dabigatran etixilate mesylate (30 µg/ml) at three different time intervals on same day (Evening).

2. Inter-day Precision: Inter- day precision was determined by analyzing the standard solution of Dabigatran etixilate mesylate (30 µg/ml) on three consecutive days (Afternoon).

LOD and LOQ: Detection limit and quantitation limit were determined on the basis of standard deviation of y -intercepts of five calibration curves and average slope of six calibration curves as mentioned.

Method Robustness: Standard solution of Dabigatran etixilate mesylate (10 µg/ml) were prepared and analyzed at different Wavelength.

Forced degradation studies:

Forced degradation of Dabigatran etexilate mesylate API was strictly followed as per protocol. At different stress conditions, the API was subjected to examine the rate and degree of degradation likely to occur during storage or after administration. This accelerated stability studies helps to determine the fate of the drug, which commonly occur after long storage, in a minute time compared to the real-time or long-term stability test. The different pathways of degradation to be investigated are acid hydrolysis, basic hydrolysis, oxidative, photolytic and thermolytic degradation.

RESULTS AND DISSCUSION

Drug was analyzed at different chromatographic conditions to develop a linear, precise, suitable & specific stability showing RP-HPLC method & the observed results were presented. Isocratic elution is easy, requires one pump only & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here Analytical Column Grace C^{18} (250mm × 4.6ID, Particle size; 5 micron) was favored because peak shape, resolution and absorbance were good by using this column. Mobile phase & diluents for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (acetonitrile, methanol, 1M NaOH, IM HCl, water). The drug was exceptionally dissolved in methanol and moderately in water. Using these solvents with appropriate composition, newer methods can be developed and validated. The result indicates that the developed method is an accurate method for stability and assay

studies that can help to analyze the drug in various formulations.

Intraday and interday variation studies have shown the precision of the method. For intraday studies, the drug was injected into the HPLC system in triplicate and for interday studies, the drug was injected into the HPLC system in triplicate for three days. Statistically the data was subjected for the calculation of SD and % RSD. The % RSD value of drug for intraday studies was found to be 0.07. For interday studies, values were 0.087 and 1.57 respectively. This shows that values do not exceed 2%, indicating that the developed method is precise. The LOQ and LOD were found to be 1.5435µg/ml and 0.509 µg/ml respectively. The robustness of method was studied for the minute changes in chromatographic condition like changes in flow rates and wavelength and %RSD found to be less than 2. The developed method was found to be specific from the results of the forced degradation studies. The drug was found to be degraded only in 1N HCl, 1N NaOH, 3% H_2O_2, thermal and photolytic conditions.

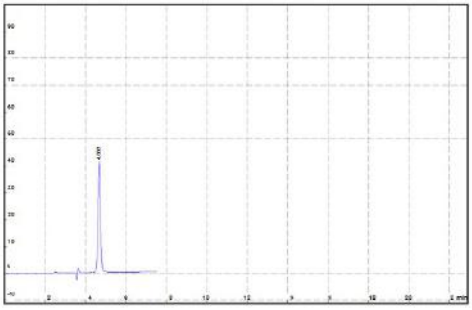
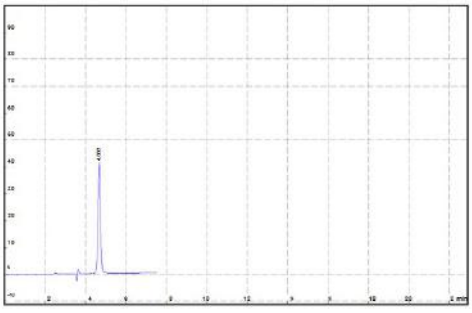


Fig. 1: Chromatogram of sample Dabigetran under optimized conditions

Table 1: Linearity calculation	
Concentration	**Area**
10	**1169014**
20	**2341385**
30	**3525522**
40	**4541880**
50	**5723150**



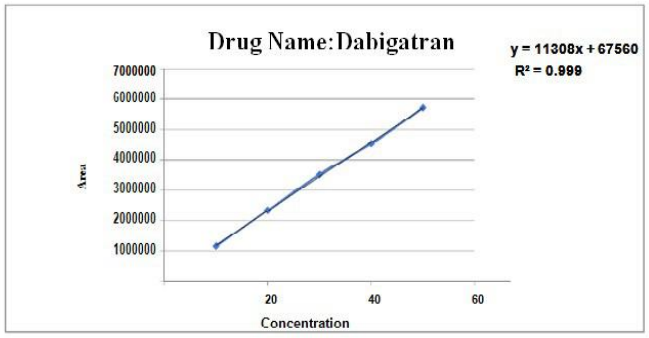


Fig. 2: Linearity graph

Table 2: Accuracy calculation

S. No	Concentration	Area	Standard Deviation		Accuracy	Precision
			Mean	SD	% SD	%RSD
1	10 10 10	1169014 1172591 1170373	1170659.333	1805.608577	0.1542386	0.154238601
2	30 30 30	3525522 3522464 3521983	3523323	1919.515824	0.0544802	0.054480268
3	50 50 50	5723150 5737425 5731236	5730603.667	7158.476817	0.1249166	0.124916627

Table 3: Results of inter-day precision of Dabigatran

Inter day						Mean	%RSD
Day 1			Day 2				
3525522	3522464	3521983	3522313	3529134	3534395	3524395	0.08 %
1715156	1655265	1647770	1695102	1693321	1696587	1696587	1.57%

Table 4: Results of intra-day precision of Dabigatran

Intraday						Mean	%RSD
Morning			Evening				
3525522	3522464	3521983	3527544	3524719	3528323	3525093	0.07 %

Table 5: Result of chromatogram of robustness of Dabigatran

Variation in flow rate	Time	Area	Theoretical plate number	Asymmetry	Standard deviation		Accuracy
					Mean	SD	% SD
0.8 ml/min	5.472	2340669	6632	1.22	2342196	2056.74	0.08781251
1 ml/min	4.778	2341385	7995	1.23			
1.2 ml/min	4.293	2344535	7152	1.30			

Variation in wavelength	Time	Area	Theoretical plate number	Asymmetry	Standard deviation		Accuracy
					Mean	SD	% SD
225 nm	5.472	2340669	6632	1.22	2337852	7620.35	0.32595535
227 nm	4.778	2341385	7995	1.23			
229 nm	4.293	2344535	7152	1.30			

Table 6: Result of % recovery of Dabigatran

S. No	% Composition	Area of standard	Area of sample	% Recovery
1	50	3525522	3524691	99.9742902
2	100	4541880	4539894	99.95627361
3	150	5723150	5716981	99.89220971

Results of degradation studies:

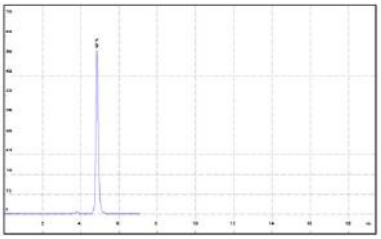


Figure: 3. Chromatogram of Acid degradation of Dabigatran

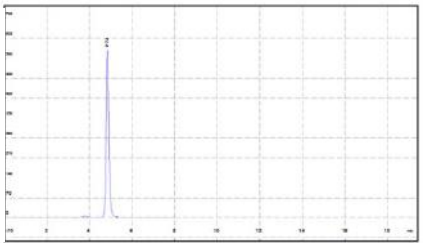


Figure: 4. Chromatogram of Base degradation of Dabigatran

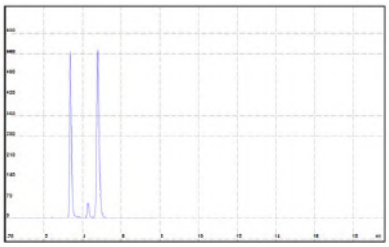


Figure: 5. Chromatogram of H_2O_2 degradation of Dabigatran

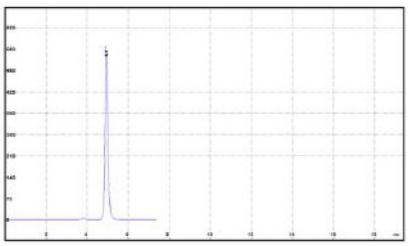


Figure: 6. Chromatogram of Photolytic degradation of Dabigatran

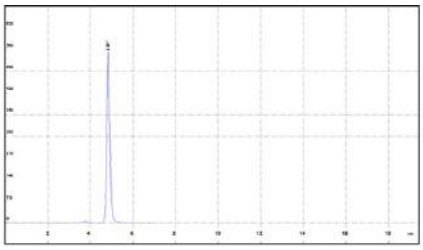


Figure: 7. Chromatogram of Thermolytic degradation of Dabigatran

Table 7: Result of forced degradation study of Dabigatran etexilate mesylate

S. No	Degradation	Area of standard	Area of degraded sample	Degraded upto%	Actual % degradation
1	Acid degradation	5723150	5479354	95.74017805	4.259821951
2	Base degradation	5723150	5504936	96.18716995	3.812830347
3	H_2O_2degradation	5723150	5227490	91.3398478	8.660615221
4	Photolytic degradation	5723150	5632716	98.4198562	1.580143802
5	Thermolytic degradation	5723150	5579810	97.49543521	2.504564794

CONCLUSION

The proposed method is simple, reproducible and sensitive and can therefore be used routinely to determine Dabigatran etexilate mesylate in bulk and in pharmaceutical formulations. The statistical analysis of the results revealing high precision and accuracy was carried out.

ACKNOWLEDGEMENT

The authors wish to acknowledge Sandip Institute of Pharmaceutical Sciences and School of Pharmaceutical Sciences, Sandip University, Nashik for their support and cooperation.

REFERENCES

[1] Valko K, Snyder LR, Glajch J: Retention in reversed-phase liquid chromatography as a function of mobile phase composition. Journal of Chromatography A 1993; 656 (1-2): 501–520.

[2] Snyder LR, Schunk TC: Retention mechanism and the role of the mobile phase in normal-phase separation on amino-bonded-phase columns.

Analytical Chemistry 1982; 54(11): 1764-72.

[3] William Kemp: Organic spectroscopy, Palgrave, New York 2005.

[4] Skoog DA, Holler FJ and Nieman TA: Principle of Instrumental Analysis. Saunders college publishing, Fifth Edition 1998.

[5] Loyd R S, John W D: High-performance gradient elution: the practical application of the linear-solvent-strength model. Wiley Interscience 2006.

[6] Willard HH, Merritt LL, Dean JA, Settel FA: Instrumental Method of Analysis. CBS Publishers Distributors, Seventh Edition 1986.

[7] Srinivas A, Sridhar Reddy K: Method development and validation of Dabigatran etixilate masylate by RP-HPLC method and its degradation studies, International Journal of Trends in Pharmacy and Life Sciences 2016; 2(1):769-777.

[8] Dare et al: Method validation for stability indicating method of related substances in active pharmaceutical ingredients dabigatran etixilate mesylate by reverse phase chromatography. Journal of Chromatography Sepember 2015; 6.2.

[9] A. John Adamovies: Chromatographic Analysis of Pharmaceutical. Marcel Dekker, New York, Second Edition.

[10] Behnam Davani: Pharmaceutical Analysis-7. USP 28-NF 23: 1160.

[11] Beckett HA, Stenlake BJ: Practical Pharmaceutical Chemistry. CBS Publications, New Dehli, Forth Edition 2001.

[12] Vijay Kumar D, Balaraju K, Ashok Kumar A: A rapid RP-HPLC method development and validation for the quantitative estimation of dabigatran etexilate mesylate in capsules. International Journal of Pharmacy and Pharmaceutical Science 2015; 7:10

[13] ICH Q2A: Validation of analytical procedures. International Conference on Harmonization Geneva October 1991; 1:5.

[14] Code Q2B: Validation of analytical procedures, methodology. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland Sixth November 1996; 1:8

[15] Pradaxa Full Prescribing Information Archived 2015-08-10 at the Wayback Machine, Boehringer Ingelheim, October 2010.

[16] Pollack Charles V et al (2015-08-06): Idarucizumab for Dabigatran reversa. The New England Journal of Medicine; 373 (6): 511– 520.

[17] Gomez-Outes A et al: Dabigatran, Rivaroxaban, or Apixaban versus

Warfarin in patients with nonvalvular atrial fibrillation: A systematic review and meta-analysis of subgroups. Thrombosis 2013; 640723.