Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 8, 2015

Original Article

ASSAY METHOD DEVELOPMENT AND VALIDATION OF DABIGATRN ETEXILATE IN CAPSULES BY UV SPECTROSCOPY

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Received: 20 Apr 2015 Revised and Accepted: 26 Jun 2015

ABSTRACT

Objective: To develop a simple and cheap UV spectrophotometric method for the quantitative estimation of Dabigatrn etexilate (150 mg) in capsules and validate as per ICH guidelines.

Methods: The optimized method uses 0.05N HCl as a solvent for the estimation of assay of Dabigatrn etexilate in capsules at a wavelength of 325 nm.

Results: The developed method resulted in Dabigatrn etexilate exhibiting linearity in the range 5-15µg/ml. The assay precision is exemplified by relative standard deviation of 1.4%. Percentage Mean recovery was found to be in the range of 98-102, during accuracy studies.

Conclusion: A simple and a cheap UV spectrophotometric method was developed and validated for the quantitative estimation of Dabigatrn etexilate in capsules as per ICH guidelines and hence it can be used for the routine analysis in various pharmaceutical industries.

Keywords: UV, Dabigatrn etexilate, 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 pro drug, 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 methanesulfonate [1] corresponding to the molecular formula $C_{35}H_{45}N_7O_8S$. Dabigatrn 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 Dabigatrn etexilate by UV [3], LC/MS [4] and UPLC MS/MS [5] in bulk and/or plasma. While two stability indicating assay methods are cited in the literature using HPLC in bulk [1, 6] and two methods in formulations [7, 8]. As there exists only one UV spectrophotometric assay method in capsule dosage form [3] using acetonitrile as solvent, an organic solvent which is costly, we here report a cheap and rapid UV spectrophotometric method using 0.05N HCl as solvent for the quantitative estimation of Dabigatrn etexilate in capsules and validate the method as per ICH guidelines.

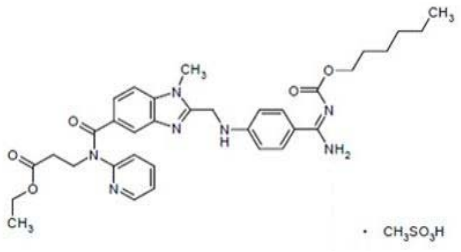

Fig. 1: Structure of Dabigatrn etexilate mesylate

MATERIALS AND METHODS

Materials

Instrument

A double beam UV-visible spectrophotometer (Shimadzu, model 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) and a sonicator (sonica, model 2200 MH) were used in this study.

Chemicals and reagents

Analytically pure sample of Dabigatrn etexilate with purities greater than 99% was obtained as gift sample from Chandra labs, Hyderabad, India and PRADAXA capsules was procured from APOLLO pharmacy, Hyderabad, India with labelled claim of 150 mg. Concentrated Hydrochloric acids (LR Grade) were obtained from SD Fine chemicals (Hyderabad, India).

Method

Solvent

4.25 ml of concentrated hydrochloric acid is made up to 1000 ml to get 0.05NHCl.

Selection of suitable detection wavelength

Suitable wavelength for the total experiment was determined by recording UV spectrum in the range of 200-400 nm for Dabigatrn etexilate and suitable wavelength selected was 325 nm (fig. 2).

Preparation of stock and working standard solution

10 mg of Dabigatrn etexilate was accurately weighed and taken in 100 ml clean and dry volumetric flask containing 80 ml of solvent and then the solution was made up to the mark using the solvent.

This is considered as the standard stock solution (100µg/ml). 1 ml of the stock solution was pipetted out and made up to 10 ml to get a concentration 10µg/ml, treated as working standard, 100% target concentration.

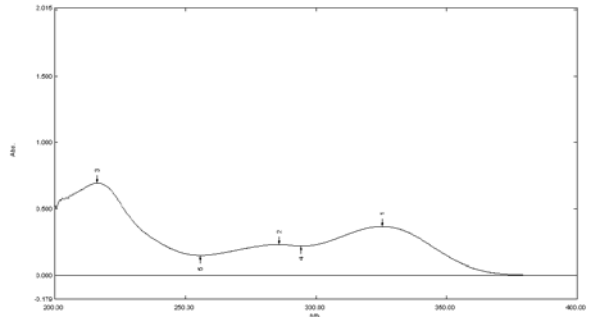

Fig. 2: UV spectrum of standard

Preparation of stock and working sample solution

Not less than 20 capsules were taken, emptied and test stock solution of Dabigatrn etexilate mesylate (750µg/ml) was prepared by transferring weight equivalent to 37.5 mg of Dabigatrn etexilate mesylate to 40 ml of solvent which is sonicated and shaked intermittently for 8 min and later made up to 50 ml with solvent. This solution was filtered using 0.22micron syringe filter. From the above stock solution 1 ml was pipetted out and made up to 10 ml and from this 1.33 ml was pipetted out and made up to 10 ml to get working sample solution concentration equivalent to 10µg/ml, 100% target concentration.

RESULTS AND DISCUSSION

Method development

Various solvents were explored including water, Hydrochloric acid at 0.1N and 0.05N and sodium hydroxide at 0.1N and 0.05N. Dabigatrn etexilate was found to be soluble and stable for the minimum of 1 hour at room temperature using 0.05N Hydrochloric acid and hence this solvent was initiated for the determination of suitable detection wavelength and working concentration of standard. In order to test the applicability of the developed method to a commercial formulation, assay of PRADAXA capsules were studied at working concentration. Assay for working concentration of the sample at 325 nm was in acceptance limits (95-105%) using the solvent via intermittent shaking and sonication method for 8 minutes fig. 3 illustrates UV spectrum for the sample. Hence the method is optimized.

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. UV spectrophotometric method developed was validated according to International Conference on Harmonization (ICH) guidelines [9] for validation of analytical procedures.

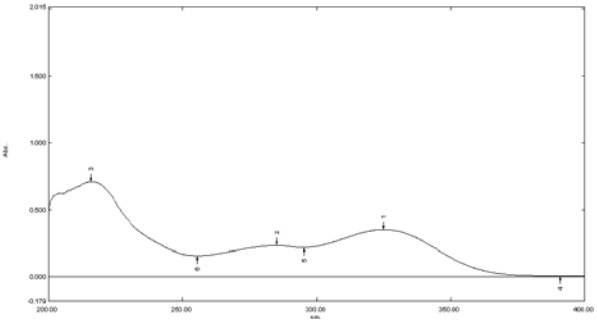

Fig. 3: UV spectrum of sample

The method was validated for the parameters like linearity, accuracy, system precision, intra-day precision, inter-day precision/ intermediate precision/ruggedness, limit of detection (LOD) and limit of quantitation (LOQ).

Precision

System precision

Six replicate recording of absorbancse at 325 nm of the standard solution at the working concentration showed % RSD (Relative Standard Deviation) less than 2, which indicates acceptable reproducibility and thereby the precision of the system. System precision results are tabulated in table 1.

Method precision

Method precision was determined by performing assay of the sample under the tests of (i) repeatability (Intra day precision) and (ii) Intermediate precision (Inter day precision or ruggedness) performed during 2 consecutive days by two different analysts, at working concentration.

Table 1: System precision results of dabigatrn etexilate.

n	Absorbance
1	0.336
2	0.338
3	0.339
4	0.338
5	0.339
6	0.343
Average	0.338
SD	0.002
% RSD	0.59

Repeatability (Intraday precision)

Six consecutive recording of absorbance at 325 nm of the sample from the same homogeneous mixture at working concentration showed % RSD less than 2 concerning % assay, 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 2).

Table 2: Intraday precision results of dabigatrn etexilate

n	Sample absorbance	% Assay
1	0.333	98.02
2	0.342	100.67
3	0.341	100.38
4	0.344	101.26
5	0.343	100.97
6	0.334	98.32
Average	0.339	99.93
S. D.	0.0047	1.4
% RSD	1.17	1.4

Intermediate precision (Inter day precision/Ruggedness)

Assay precision between two consecutive days performed by different analysts of the sample showed % RSD less than 2, which indicate the method developed is inter day precise/ rugged (table 3).

Linearity

Standard solutions of Dabigatrn etexilate at different concentrations level (50%, 75%, 100%, 125% and 150%) were prepared. Calibration curve (fig. 4) was constructed by plotting the concentration level of drug versus absorbance at 325 nm.

The results show an excellent correlation between absorbance and concentration level of drug within the concentration range (5-15µg/ml) for the drug (table 4). The correlation coefficient was greater than 0.995, which meet the method validation acceptance

criteria and hence the method is said to be linear in the range of 5-15µg/ml.

Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample by standard addition method at three different levels (80-120%). At each level, three determinations were performed. Percent mean recovery was calculated as shown in table 5**.** 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.

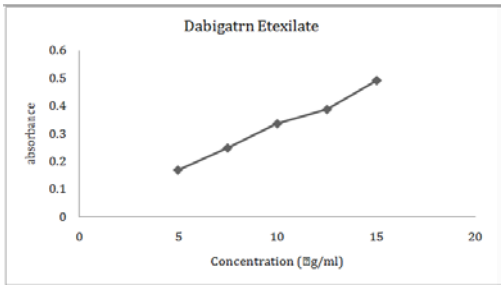

Fig. 4: Linearity graph of dabigatrn etexilate mesylate

Table 3: Inter day precision/Ruggedness results

No.	Analyst 1		Analyst 2	
	Absorbance	% Assay	Absorbance	% Assay
1	0.333	98.02	0.341	100.38
2	0.342	100.67	0.345	101.56
3	0.341	100.38	0.344	101.26
4	0.344	101.26	0.343	100.97
5	0.343	100.97	0.346	101.85
6	0.334	98.32	0.344	101.26
Average	0.339	99.93	0.346	101.21
SD	0.004	1.4	0.0028	0.506
% RSD	1.17	1.4	0.57	0.499

Table 4: Calibration data for Dabigatrn etexilate

% Level	Concentration (µg/ml)	Absorbance
50	5	0.17
75	7.5	0.249
100	10	0.336
125	12.5	0.398
150	15	0.491

Regression equation: y=0.03164+0.0124, Regression coefficient (r^2): 0.998

Table 5: Results of accuracy studies for dabigatrn etexilate

% Spiked	Absorbance	% Recovery	Mean % recovery	%RSD
80	0.35	100		
80	0.348	99.8	100.27	0.03
80	0.352	101.01		
100	0.441	101.84		
100	0.438	101.54	101.82	0.27
100	0.44	102.08		
120	0.51	101		
120	0.506	100.5	100.7	0.39
120	0.507	100.7		

Table 7: Optical characteristics and validation parameters of dabigatrn etexilate

Parameters	Results
Detection wavelength (nm)	325
Beer's Law limits (µg/ml)	5-15
Regression equation (y = mx+c)	y = 0.0316x+0.0124
Correlation coefficient (r^2)	0.998
Slope (m)	0.0316
Intercept (c)	0.0124
% Relative Standard Deviation (% RSD) System precision	0.59
(% RSD) Intra-day precision	1.4
(% RSD) Inter-day precision	≤ 2
Accuracy (% Mean Recovery)	
80 % Level	100.27
100 % Level	101.82
120 % Level	100.7

CONCLUSION

A cheap and a rapid UV spectrophotometric method was developed and validated for the quantitative estimation of Dabigatrn etexilate in capsules as per ICH guidelines. The developed method resulted in Dabigatrn etexilate exhibiting linearity in the range 5-15µg/ml. The precision is exemplified by relative standard deviation of 1.4%. Percentage Mean recovery was found to be in the range of 98 102, during accuracy studies. Accordingly it is concluded that the developed UV spectrophotometric method is accurate, precise, linear and rugged and therefore the method can be used for the routine analysis of Dabigatrn etexilate in tablets in various pharmaceutical industries.

ACKNOWLEDGEMENT

The authors would like to thank the management of Vijaya College of pharmacy (VJYH), Hyderabad, for providing the necessary facilities to carry out of this research work. The authors are grateful to Chandra labs, Hyderabad for providing drug in form of gift sample.

CONFLICT OF INTERESTS

Declared None.

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