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Original Article

UV SPECTROPHOTOMETRIC ASSAY METHOD DEVELOPMENT AND VALIDATION OF DABIGATRAN ETEXILATE IN CAPSULES

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ABSTRACT

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

Methods: The optimized method uses triethyl ammonium phosphate aqueous solution (pH 2.5) as a solvent for the estimation of assay of Dabigatran etexilate in capsules at a wavelength of 325 nm.

Results: The developed method resulted in Dabigatran etexilate exhibiting linearity in the range 5-15µg/ml. The assay precision is exemplified by relative standard deviation of 1.07%. Percentage Mean recovery was found to be in the range of 98-102, during accuracy studies. Method was found to be robust with respect to wavelength and pH of the solvent.

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

Keywords: UV, Dabigatran etexilate, Method development, Validation.

INTRODUCTION

Dabigatran etexilate (DE) is the oral pro drug of the active moiety Dabigatran. 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 $C_{35}H_{45}N_7O_8S$. Dabigatran 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 Dabigatran 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 triethyl ammonium phosphate aqueous solution (pH 2.5) as solvent for the quantitative estimation of Dabigatran etexilate in capsules and validate the method as per ICH guidelines.

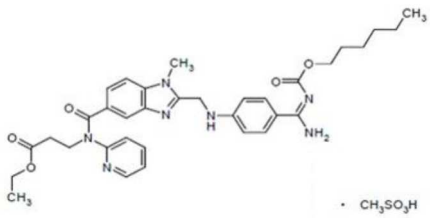

Fig. 1: Structure of dabigatran etexilate mesylate

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 Dabigatran etexilate with purities greater than 99% was obtained as gift sample from Chandra labs, Hyderabad, India and tablet formulation [PRADAXA] was procured from APOLLO pharmacy, Hyderabad, India with labelled claim of 150 mg. Triethylamine and ortho phosphoric acid were obtained from SD Fine chemicals (Hyderabad, India).

Method

Solvent

Solvent is prepared by adding 5 ml of triethylamine to 1000 ml of distilled water and later pH was adjusted to 2.5 using 30% v/v of ortho phosphoric acid in water.

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 Dabigatran etexilate and suitable wavelength selected was 325 nm (fig. 2).

Preparation of stock and working standard solution

10 mg of Dabigatran 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 pipette out and made up to 10 ml to get a concentration 10µg/ml, treated as the working standard, 100% target concentration.

Preparation of stock and working sample solution

Not less than 20 capsules were taken, emptied and test stock solution A of Dabigatran etexilate mesylate (750µg/ml) was prepared by transferring weight equivalent to 37.5 mg of Dabigatran etexilate mesylate to 40 ml of solvent which is sonicated and shaken 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, A 1 ml was pipetted out and made up to 10 ml to get stock solution B (75µg/ml). From stock solution B, 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.

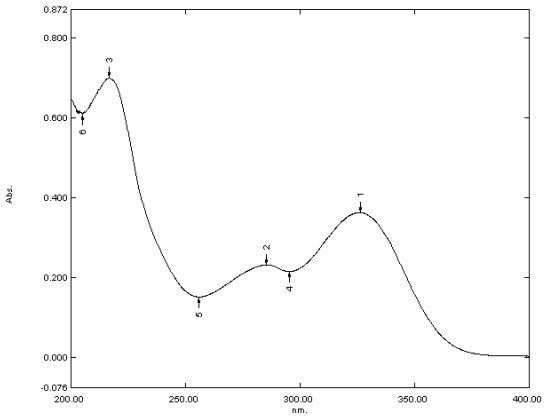

Fig. 2: UV spectrum of standard

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. Dabigatran etexilate was found to be soluble and stable for minimum of 1 hour at room temperature using triethyl ammonium phosphate aqueous solution (pH 2.5) 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 sample at 325 nm was in acceptance limits (95-105%) using the solvent via intermittent shaking and sonication method for 20 minutes. Hence the method is optimized. Fig. 3 illustrates UV spectrum for the sample.

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. The method was validated for the parameters like linearity, accuracy, system precision, intra-day precision, inter-day precision/ intermediate precision/ruggedness and robustness.

Precision

System precision

Six replicate recording of absorbance at 325 nm of 100% working concentration standard solution 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 sample under the tests of (i) repeatability (Intraday precision) and (ii) Intermediate precision (Inter day precision or ruggedness) performed during 2 consecutive days by two different analysts, at 100 % working concentration.

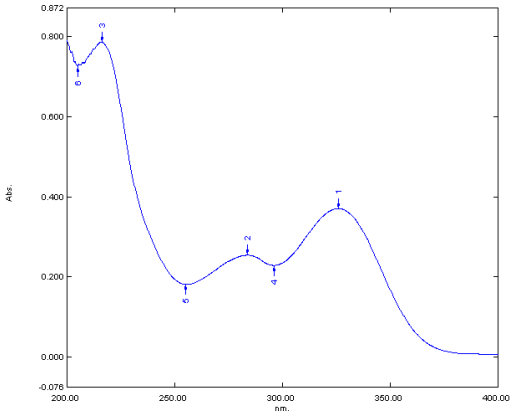

Fig. 3: UV spectrum of sample

Table 1: System precision results

n	Absorbance
1	0.353
2	0.350
3	0.350
4	0.351
5	0.351
6	0.351
Average	0.351
SD	0.001
% RSD	0.284

Repeatability (Intra day precision)

Six consecutive recording of absorbance at 325 nm of 100% working concentration of the sample from the same homogeneous mixture 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). % Assay was calculated by the standard formula using absorbance of the sample and standard, dilution factors for standard and sample, average weight of sample, label claim and potency.

Table 2: Intraday precision results

n	% Assay
1	100.41
2	100.68
3	100.14
4	101.20
5	99.05
6	102.30
Average	100.63
S. D.	1.08
% RSD	1.07

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

Different concentrations (5-15µg/ml) of Dabigatran etexilate standard were prepared by serial dilutions from the stock solution

100 µg/ml. Calibration curve (fig. 4) was constructed by plotting the concentration of drug versus absorbance at 325 nm. The results show an excellent correlation between absorbance and concentration 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.

Table 3: Inter day precision/Ruggedness results

n	Analyst 1	Analyst 2
1	100.41	100.40
2	100.68	100.15
3	100.14	102.43
4	101.20	98.14
5	99.05	102.30
6	102.30	101.86
Average	100.63	100.88
SD	1.08	1.65
% RSD	1.07	1.63

Table 4: Calibration curve

% Level	Concentration (µg/ml)	Absorbance
50	5.00	0.176
75	7.50	0.251
100	10.0	0.329
125	12.5	0.412
150	15.0	0.474
Regression equation		y=0.03028x+0.0256
Regression coefficient		0.998

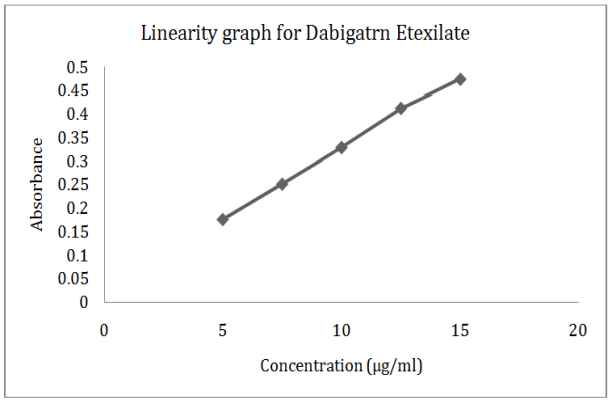

Fig. 4: Linearity graph of Dabigatran etexilate mesylate

Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample by percentage method at three different levels (50-150%, *viz* 5, 10 and 15µg/ml). 50 to 150% of the sample solutions were prepared as per the procedure given in the methods from the sample stock solution B (75µg/ml).

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.

Table 5: Accuracy studies

% Level	Absorbance	% recovery	Mean % recovery	% RSD
50	0.184	99.85		
50	0.185	100.42	101.05	1.22
50	0.186	100.97		
100	0.371	100.41		
100	0.370	100.14	100.41	0.26
100	0.372	100.68		
150	0.550	99.22		
150	0.560	101.02	100.18	0.89
150	0.556	100.3		

Robustness

The robustness of an analytical method 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. It is concluded that the method is robust as it is found that the % RSD is less than 2 for the drug concerning % assay despite deliberate variations done concerning pH±0.2 and detection wavelength±2 nm (table **6**). Table 7 summarizes the validation parameters.

Table 6: Robustness studies

Variation parameter	Variation	Sample absorbance	Standard absorbance	% assay	% RSD
pH	2.3	0.376		100.9	
(±0.2)		0.378	0.354	101.15	0.13
		0.376		100.9	
	2.7	0.359		100.3	
		0.356	0.34	99.47	0.42
		0.358		100.02	
Wave length	323	0.368		99.31	
(±0.2)		0.364	0.352	98.23	0.56
		0.367		99.04	
	327	0.368		98.47	
		0.371	0.355	99.28	0.78
		0.374		100.04	

Table 7: Optical characteristics and validation parameters of Dabigatran etexilate

Parameters	Results
Detection wavelength (nm)	325
Beer's Law limits (µg/ml)	5-15
Regression equation (y = mx+c)	y = 0.03028x+0.0256
Correlation coefficient (r^2)	0.998
Slope (m)	0.0328
Intercept (c)	0.0256
% Relative Standard Deviation (% RSD) System precision	0.284
(% RSD) Intra-day precision	1.07
(% RSD) Inter-day precision	≤ 2
Accuracy (% Mean Recovery)	
50 % Level	101.05
100 % Level	100.41
Robustness	
pH (±0.2) (% RSD)	≤ 2
Wavelength (±2 nm) (% RSD)	≤ 2

CONCLUSION

A cheap and a rapid UV spectrophotometric method was developed and validated for the quantitative estimation of Dabigatran etexilate in capsules as per ICH guidelines. The developed method resulted in Dabigatran etexilate exhibiting linearity in the range 5-15µg/ml. The precision is exemplified by relative standard deviation of 1.07%. 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 routine analysis of Dabigatran etexilate in tablets in various pharmaceutical industries.

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CONFLICT OF INTERESTS

Declared None.

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