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Validated stability-indicating high performance liquid chromatographic assay method for the determination of Dabigatran Etexilate Mesylate.

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ABSTRACT

Dabigatran Etexilate Mesylate was subjected to different ICH recommended stress conditions. Degradation of drug occurs almost in all conditions (hydrolytic, oxidative and photolytic) while mild degradation was seen with thermal stress. A validated stability-indicating HPLC method was developed for the analysis of drug in presence of its degradation products. The stressed samples of drug were analyzed using Kinetex C-8 column (5 μ , 250 \times 4.6 mm) column using a mobile phase composed of methanol: water, which was delivered initially in the ratio of 70:30 (v/v) for 1 min, then changed to 90:10 (v/v) for next 9 min and finally equilibrated back to initial composition 70:30 (v/v) from 11 to 20 min. The flow rate was maintained at 1.0 ml/min and detection was carried out at 230 nm using 996 PDA detector. The method was validated in terms of linearity, accuracy, precision specificity and selectivity.

Keywords: Dabigatran Etexilate Mesylate, HPLC, Stability-indicating assay, ICH.

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INTRODUCTION

According to ICH (International Conference on Harmonization) drug stability test guidelines Q1A (R2), the stability-indicating assay method (SIAM) is employed for the analysis of stability samples [1,2]. The analysis of stability test samples should be done by using validated SIAM after subjecting a drug to a variety of stress conditions such as hydrolysis, oxidation, photostability and thermal degradation [3]. The ICH guidelines Q6A provides guidance on specifications [4] and also the requirement of stability-indicating assays under Universal Tests/Criteria for drug substances and drug products. Apart from ICH, the United states-Food and Drug Administration (US-FDA) draft guidelines of 1998 also provides guidance on stability testing of drug substances and drug products [5]. The requirement of stability testing of well-established or existing drug substances and products is also provided in World Health Organization (WHO) guidelines [6].

The aim of the present study was to study degradation behavior of Dabigatran Etexilate Mesylate (DEM) under a variety of ICH recommended stress conditions and to develop a validated stability-indicating HPLC method. Dabigatran Etexilate Mesylate is chemically β -Alanine, N-[[2-[[[4-[[[(hexyloxy) carbonyl]amino]iminomethyl] phenyl]amino] methyl]-1-methyl-1H-benzimidazol-5-yl]carbonyl]-N-2-pyridinyl, ethyl ester, methane sulfonate. Dabigatran can be used for the prevention of stroke in patients with atrial fibrillation. It is orally active competitive and reversible direct thrombin inhibitor. There are few analytical methods reported in literatures for analysis of DEM which includes HPLC [7], UPLC MS/MS [8] in human plasma, LC/MS [9] based metabolite identification and semi-quantitative estimation approach in the investigation of in vitro dabigatran etexilate metabolism. However the present work was aimed to develop new and economical method for determination of Dabigatran Etexilate Mesylate according to ICH recommended stress conditions. The structure of DEM is shown in Figure1.

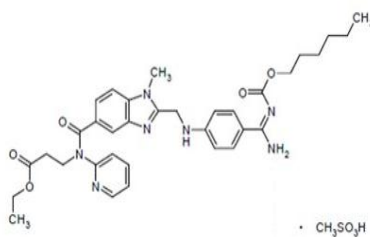


Figure 1: Structure of Dabigatran Etexilate Mesylate

MATERIALS AND METHODS

Materials

Dabigatran Etexilate Mesylate was kindly obtained from Dr. Reddy's Lab. and was used without further purification. HPLC grade methanol was used for the study. Buffer materials and other chemicals were of analytical reagent grade. Deionized water was used throughout the experiment.

Instrumentation

For the separation of Dabigatran Etxilate Mesylate and its degradation products the HPLC system (Waters, Milford, USA) consisted of a 600E pump, a 996 PDA (photo-diode array) detector, Waters HPLC autosampler/Injector, Waters™ 600 controller and Waters in-line degasser AF module was used. The data were acquired and processed using EMPOWER Build 1154 software. The separations were achieved on a Kinetex C-8 column (5 μ , 250 \times 4.6 mm).

EXPERIMENTAL

Preparation of stock and standard solutions

The stock solution of Dabigatran Etxilate Mesylate (1000 μ g/ml) was prepared by dissolving 25mg in 25ml methanol. The working solution of was prepared by further dilution of stock solution with methanol to get final concentration of 100 μ g/ml.

Degradation Studies

All the reactions were carried out at a drug concentration of 1mg/ml. For degradation study in water, the drug solution was exposed at 80 $^{\circ}$ C for 2h. For degradation study in 0.1M HCl and 0.01M NaOH, the drug solution was exposed at 80 $^{\circ}$ C for 2h and 1h respectively. Oxidative studies were carried out in 3% H₂O₂ at room temperature for 1h. Thermal degradation of the drug powder was studied by exposing it to 60 $^{\circ}$ C in an oven for 48h. Photostability studies were carried out by exposing the drug powder to light in a UV chamber for 3h. Samples were withdrawn periodically and analyzed by HPLC after suitable dilutions.

RESULTS AND DISCUSSION

HPLC method development and optimization

A gradient elution was necessary for optimizing separation of degradation products formed under variety of stressed conditions. Among the various trials for separation of degradation products by gradient programming, the best resolution was achieved with initial run of methanol: water in the ratio of 70:30 (v/v) for 1 min. then changed to 90:10 (v/v) for next 9 min and finally equilibrated back to initial composition 70:30 (v/v) from 11 to 20 min. The Injection volume was 20 μ l and mobile phase flow rate was 1.0 ml/min. The column was maintained at ambient temperature and detection wavelength was 230 nm. The PDA scans were simultaneously recorded between 210 and 400 nm.

Degradation behavior of DEM

HPLC analysis of stressed samples of Dabigatran Etxilate Mesylate under variety of stress conditions using methanol: water as the mobile phase suggested the following degradation behaviour.

Hydrolytic studies

DEM shows sufficient degradation in water and in 2h. The degradation product appeared at RTs 3.132 min. (NDP I) and 5.311 min. (NDP II) as shown in Figure 2. Similarly in 0.1M HCl, the degradation products appeared at RT 5.354 min. (HDP I) in 2h as shown in Figure 3. The drug was found to be highly labile to alkaline hydrolysis in 0.01M NaOH and most of the decomposition occurs within 1h. The major degradation product appeared at RT 7.052 min. (DAB A-I) as shown in Figure 4.

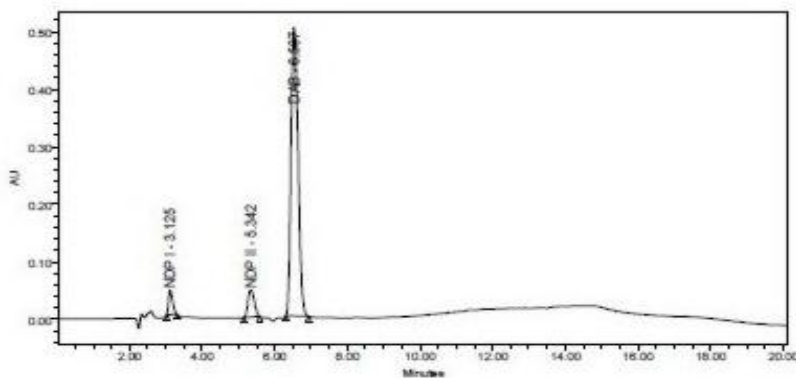


Figure 2: Typical HPLC chromatogram of neutral hydrolysis degraded DEM

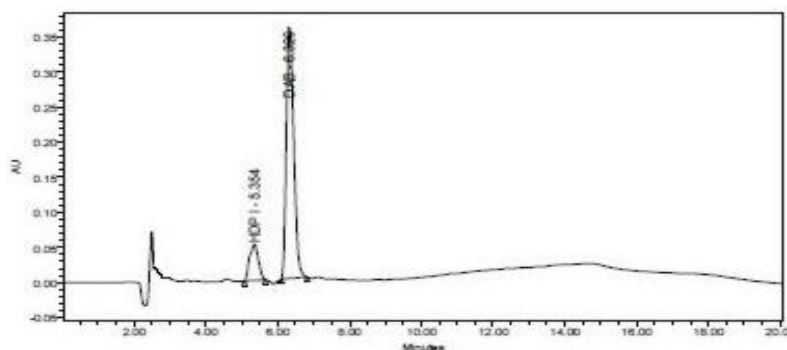


Figure 3: Typical HPLC chromatogram of acid hydrolysis degraded DEM

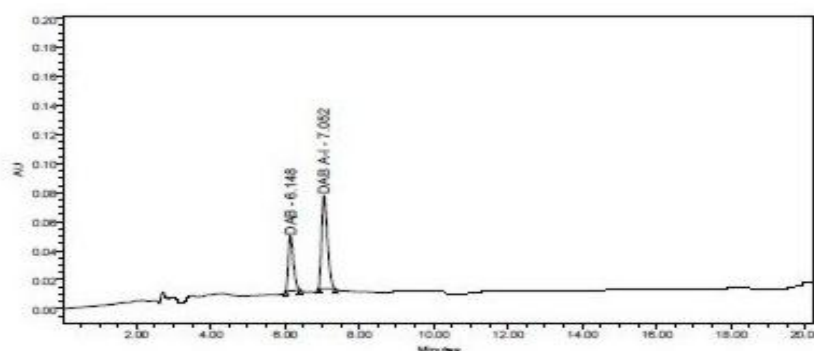


Figure 4: Typical HPLC chromatogram of base hydrolysis degraded DEM

Oxidative studies

The drug was found to be very susceptible to oxidative degradation. The sufficient degradation of drug occurs in 3 % H₂O₂ in 1h. The degradation product appeared at RT 3.569 min. (PDP I) and is shown in Figure 5.

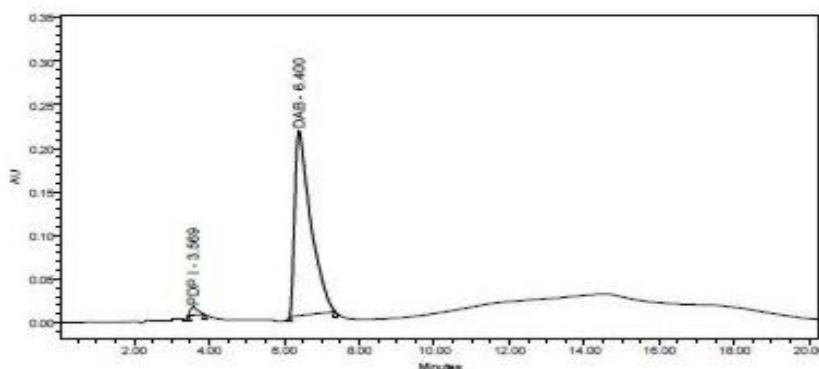


Figure 5: Typical HPLC chromatogram of oxidative degraded DEM

Photostability

Dabigatran Etexilate Mesylate rapidly degraded in presence of UV light. Almost 98% drug of the drug degraded in 3 hr and the major degradation products appeared at RTs 2.757 min. (DUV-I) and 7.547 min. (DUV-II) as shown in Figure 6.

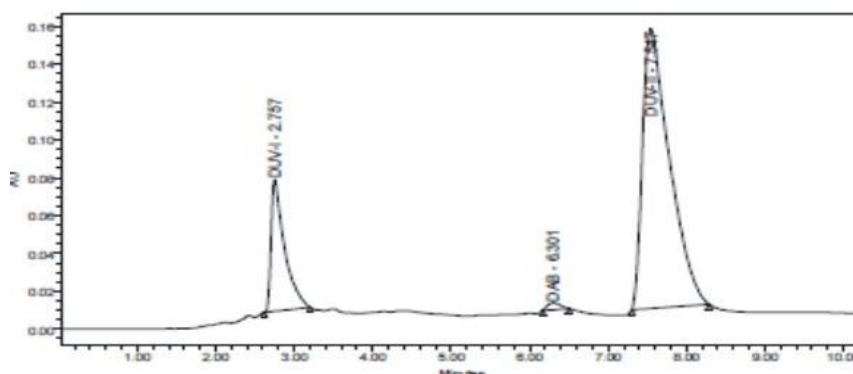


Figure 6: Typical HPLC chromatogram of photolytic degraded DEM

Thermal studies

The drug was found to be thermally stable. Negligible degradation was seen after exposing the drug powder to dry heat in an oven at 60°C for 48h. Typical chromatogram of DAB after subjecting it to above condition is shown in Figure 7.

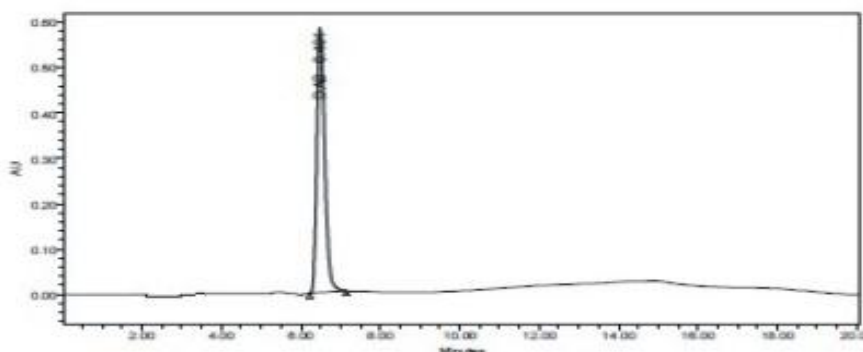


Figure 7: Typical HPLC chromatogram of thermally degraded DEM

Validation of the developed stability-indicating method

The developed optimized chromatographic method was validated for different parameters such as linearity, accuracy, precision, specificity and selectivity.

Linearity

The response of DEM was found to be linear ($r^2=0.996$) in the concentration range between 120-180 $\mu\text{g/ml}$ and each of this concentration was injected in triplicate into the HPLC column. The results of the linearity studies are shown in Table 1.

Table 1: Linearity study data

Concentration ($\mu\text{g/ml}$)	Mean peak area (n=3)
120	9850467
135	11148191
150	12505936
165	14102819
180	15809791

Recovery

The accuracy study was performed in terms of recovery using standard addition method. Excellent recoveries of the spiked drug were obtained at each added concentration and the method was found to be accurate. Percentage recovery was calculated from the amount found and actual amount added. The results of recovery studies are shown in Table 2.

Table 2: Recovery study data (n=3)

Actual Concentration ($\mu\text{g/ml}$)	Mean Concentration Found ($\mu\text{g/ml}$), % RSD	% Recovery
120	119.35 0.28	99.46
150	153.26 1.24	102.17
180	181.37 0.67	100.76

Precision

Precision studies were performed under different conditions Intra-day and Inter-day. Intra-day study was performed by analyzing three different concentrations of drugs for three times on the same day. Inter-day precision was performed by analyzing three different concentration of drug for three different days. The results of precision study are shown in Table 3.

Specificity and Selectivity

The specificity of the HPLC method was established through study of resolution factor of the drug peak from nearest resolving peak. Selectivity was established through

determination of purity of each peak using PDA detector. In purity plots, the purity angle of each peak was found to be less than purity threshold thus indicates method is selective. The purity plot of Dabigatran Etxilate Mesylate in presence of degradation products is shown in Figure 8.

Table 3: Precision study data

Concentration (µg/ml)	Intra-Day		Inter-Day	
	Mean (n=3)	% RSD	Mean (n=3)	% RSD
120	121.06	0.78	120.31	1.25
150	150.45	0.38	149.73	0.72
180	179.13	0.64	180.28	1.73

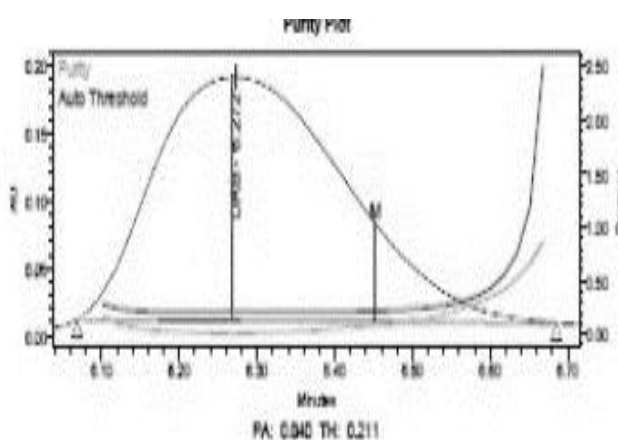


Figure 8: Purity plot of DEM in presence of degraded products

CONCLUSION

In this study, the degradation behavior of Dabigatran Etxilate Mesylate was established according to the ICH recommended stress conditions. The degradation of drug occurred extensively in hydrolytic, photolytic and oxidative conditions whereas mild degradation of it was seen to thermal stress. The results of the stress testing according to ICH guidelines reveal that method is selective and stability-indicating. The developed method is simple, accurate, precise, specific and is able to separate drug from degradation products. The method is proposed for the analysis of stability samples generated during stability studies on drug and its formulations.

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