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Stability Indicating RP-UPLC Photo-Diode Array based Method for Determination of Edoxaban Tosylate

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ABSTRACT

The objective of the current study was to develop a specific, precise, accurate and robust gradient stability indicating reversed-phase ultra performance liquid chromatography (RP-UPLC-PDA) assay method and validated for determination of edoxaban tosylate in API. Gradient separation was achieved on an acquity UPLC BEH C18 column (50 mm, 2.1 mm and 1.7 µm) column using mobile phase of acetoitrile:20 mM potassium dihydrogen phosphate, pH 3.0 ± 0.05 adjust with OPA at flow rate of 0.6 mL/min, the injection volume was 1 µL and the detection was carried out of 289 nm by using photo-diode array detector. The drug was subjected to oxidation, hydrolysis, photolysis, and heat to apply stress condition. The method was linear in the drug concentration range of 100-300 µg/mL with correlation coefficient of 0.999. Degradation products produced as a result of stress studies did not interfere with detection of edoxaban tosylate and the assay, thus developed stability indicating method can be used for routine analysis in pharmaceutical industry.

KEYWORDS

Edoxaban tosylate, Stability indicating assay, RP-UPLC method, Photo-diode array.

INTRODUCTION

Edoxaban tosylate is a selective inhibitor of factor Xa (activated factor X) a serine endopeptidase of clotting cascade required for cleavage of prothrombin into thrombin. Edoxaban tosylate is chemically known as 4-methylbenzene-1-sulfonic acid N′-(5-chloropyridin-2-yl)-N-[(1*S*,2*R*,4*S*)-4-(dimethyl carbamoyl)-2-{5-methyl-4*H*,5*H*,6*H*,7*H* [1,3]thiazolo[5,4-*c*]-pyridine-2-amido}cyclohexyl]ethane diamide hydrate (Fig. 1) belongs to a oral anticoagulant drugs [1].

Forced degradation studies can be a useful tool to predict the stability of a drug substance or a drug product with effects on purity, potency and safety under various environmental conditions [2,3]. It involves the exposure of representative samples of drug substance or drug product to the relevant stress conditions of acid/base hydrolysis, oxidation, photolytic and thermal [4,5]. Stability indicating assay method can be developed by generating degraded samples for testing selectivity of method, determining limit of quantification threshold for degraded product according to ICH Q3B [5].

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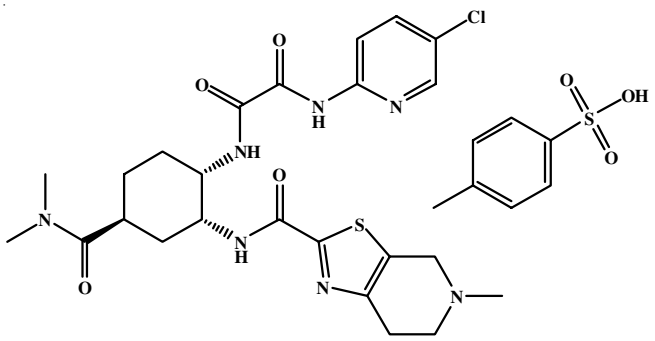

Fig. 1. Structure of edoxaban tosylate

Stability study was carried out in various force degradation conditions like acid hydrolysis, base hydrolysis, oxidative, thermolytic and photolytic by applying stress. The developed RP-UPLC PDA method was validated as per ICH Q2(R1) guideline [6].

The literature survey reveals that the edoxaban tosylate is not official in any pharmacopoeia. Methods reported so for edoxaban tosylate is UV spectroscopy [7], HPLC [8] and UPLC-MS/MS [9,10]. None of the analytical method shows stability indicating studies. Thus, it is worthwhile to develop stability indicating RP-UPLC-PDA method for edoxaban tosylate. This work describes forced degradation studies of edoxaban tosylate under stress conditions like acid hydrolysis, base hydrolysis and oxidation, thermal and photolytic stress. Proposed method can be used for routine analysis by pharma industry.

EXPERIMENTAL

Edoxaban tosylate standard was provided by Piramal Healthcare Ltd., Ahmedabad, India. Water and acetonitrile used were of HPLC-grade and potassium dihydrogen phosphate, Orthophosphoric acid used were of Analytical grade (Merck Pvt. Ltd.).

Instrumentation and chromatographicconditions: The Waters Acquity UPLC H-class system (Waters, Milford, USA) consisting of a quaternary gradient pump, an inbuilt auto sampler, Photo-diode array detector (DAD) and column oven connected to a multi instrument data acquisition and processing system with Empower 3 version, analytical balance (Mettler Toledo, XP 504) and pH meter (Mettler Toledo). The optimized chromatographic and gradient conditions are shown in Tables 1 and 2, respectively.

Preparation of standard solution: Standard stock solution of edoxaban tosylate (1000 µg/mL) was prepared in CH_3CN: water (50:50% v/v) at appropriate dilution were finally used to prepare edoxaban tosylate 200 µg/mL from the stock solution.

Degradation study: The hydrolysis degradation study was performed at room temperature using 0.1N HCl (15 min), 0.1N NaOH (15 min) then the samples were neutralized and then appropriately dilute and injected into system. For oxidative degradation study 10% H_2O_2 (15 min) was used. For thermal and photolytic degradation, samples were kept at 105 ºC (1 h) and 1 ICH cycle in photolytic chamber, respectively.

TABLE-1
OPTIMIZED CHROMATOGRAPHIC CONDITION

Parameter	Condition
Column	Acquity UPLC BEH C18 column (50 mm, 2.1 mm, and 1.7 µm)
Flow rate	0.6 mL/min
Injection volume	1 µL
Column temperature	35 °C
Detection wavelength	289 nm

TABLE-2
GRADIENT USING PHOSPHATE BUFFER: ACN (GRADIENT)

Time	A (%)	B (%)
0	100	0
0.5	100	0
1.2	80	20
3.0	80	20
3.1	100	0
5.0	100	0

Method validation

Specificity: The specificity of the method was determined by checking the interference of placebo with analyte, % interference was recorded.

Linearity: Five points calibration curves were obtained in a concentration range from 100-300 µg/mL for edoxaban tosylate.

Precision: The precision of the method was evaluated in terms of repeatability by carrying out six independent of test sample preparation and intra-day and inter-day precision of the method were determined by analyzing these ample solution in 3 sets each of 50, 100 and 150% level in the same day and 3 different days at 150, 200 and 250 µg/mL for edoxaban tosylate.

Accuracy: The accuracy was carried out by spiking three concentration levels 50, 100 and 150% of the targeted concentration of analytes to placebo. At each level, three determinations were performed and the results were recorded. The accuracy was expressed as percent analyte recovered by the proposed method.

Limit of detection and limit ofquantification: Limit of detection (LOD) and limit of quantitation (LOQ) were calculated based on standard deviation and slope method.

Robustness: The robustness of the proposed method was determined by carrying out the analysis, during which change in flow rate ± 0.05 mL/min and temperature ± 1 ºC (*i.e.* 34 and 36 ºC) were altered and the peak areas, retention times and number of theoretical plates were noted.

RESULTS AND DISCUSSION

Optimized UPLC conditions: The chromatographic conditions of stability indicating RP-UPLC method were optimized with respect to suitability parameters. Acceptable separation of both drug with short run time was achieved on acquity UPLC BEH C18 column (50 mm, 2.1 mm and 1.7 µm). Various proportions of acetonitrile, trifluroacetic acid, potassium dihydrogen phosphate and orthophosphoric acid in mobile phase at different composition, pH and different flow rate were investigated. After trials, adequate result was obtained using gradient mobile phase consisting of phosphate buffer and acetonitrile an eluted compound were monitored at 289 nm by using PDA detector. The retention time of edoxaban tosylate were found to be 2.811 min, respectively (Fig. 2). The analyte peaks were well resolved and were free from tailing (< 2 for both analytes).

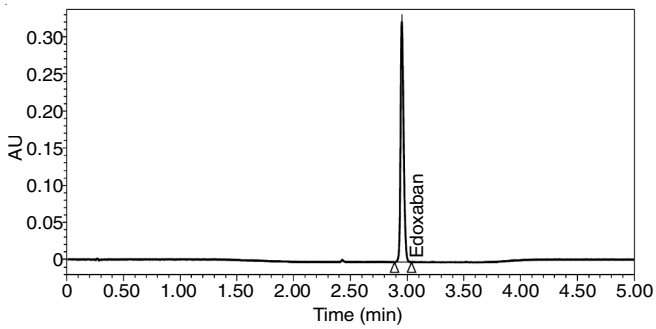

Fig. 2. Optimized chromatogram of edoxaban tosylate

System suitability: For all system suitability parameter, tailing was less than 2, theoretical plates were greater than 2000. System suitability parameter are shown in Table-3.

TABLE-3
RESULTS OF SYSTEM SUITABILITY TEST

Parameters (n = 5)	Edoxaban tosylate	Specification
Repeatability	0.4	RSD ≤ 1%
Tailing factor	1.1	T ≤ 2
Theoretical plates	47683	N ≥ 2000

Force degradation: Major degradation was found in the alkali degradation condition and acidic degradation condition that product was degraded up to 15-16%. The major impurity peaks were found at 1.70 min (Fig. 3). In oxidative degradation condition and photolytic degradation condition, it was found that around 11-12% of the drug degraded and in thermal degradationcondition around 8-9% of the drug degraded. Edoxaban tosylate was found to be degraded in all degradation condition, which was eluted by checking the peak purity of edoxaban tosylate during the force degradation study. The peak purity of edoxaban tosylate was found satisfactory under different stress conditions. Results revealed that developed method is stability indicating assay method. The results of peak purity and force degradation study are shown in Table-4.

TABLE-4
STABILITY DATA OF EDOXABAN TOSYLATE

Condition	Retention time (min)	Purity angle	Purity threshold	Degradation (%)
Acidic	2.739	0.403	0.405	15.06
Alkali	2.881	0.499	0.524	15.34
Oxidative	2.740	0.333	0.387	11.95
Thermal	2.682	0.217	0.356	8.12
Photolytic	2.681	0.226	0.356	10.55

Method validation: The proposed method was validated with respect to various parameter including linearity, accuracy, precision, specificity and robustness according to ICH Q2 (R1) guideline.

Specificity: There was no interference of any peak of degradation products with the drug peak, which confirmed that method is specific.

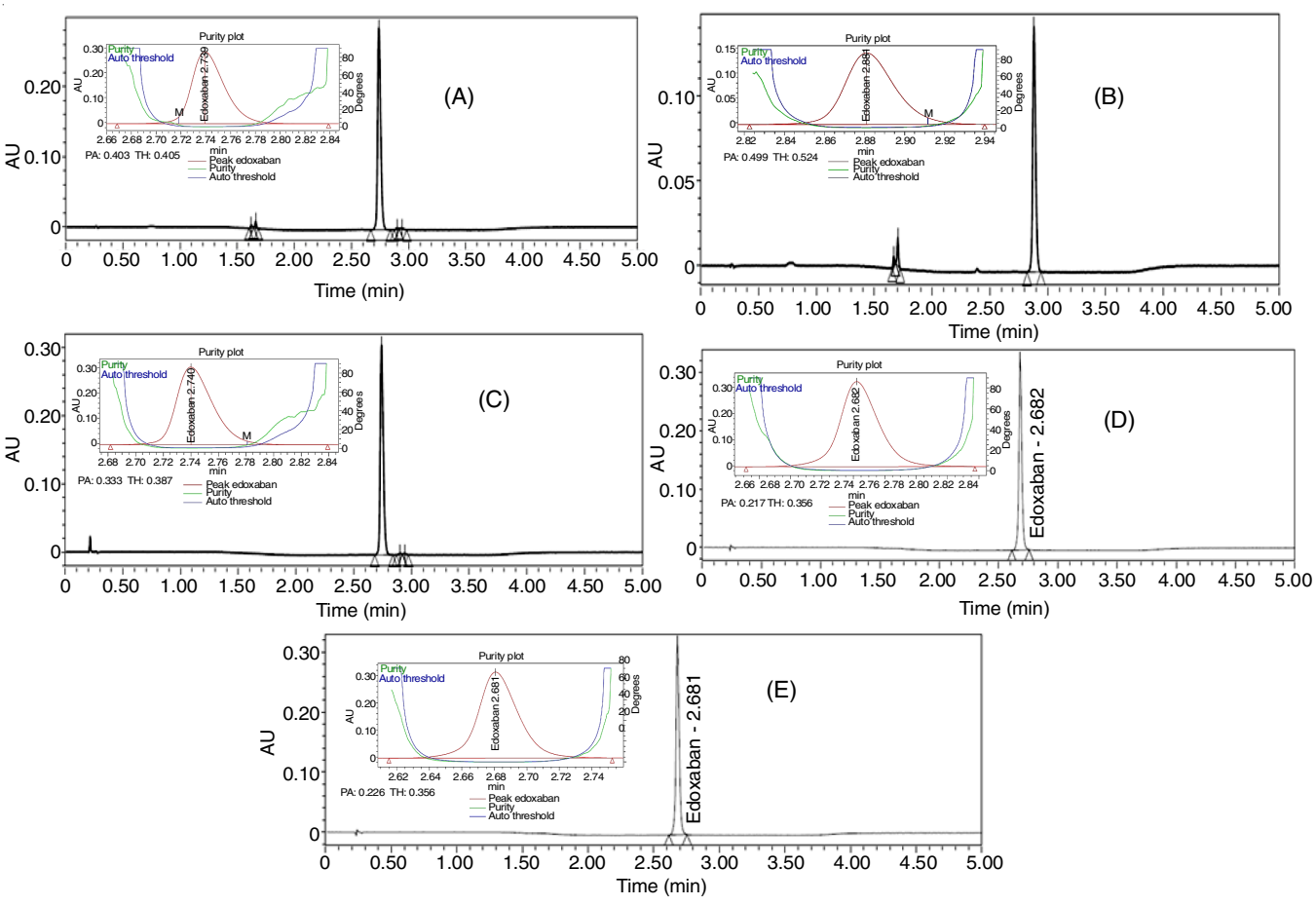

Fig. 3. Chromatogram and peak purity graphs of edoxaban tosylate in (A) acid degradation (B) alkali degradation (C) oxidative degradation (D) thermal degradation (E) photolytic degradation

Linearity: Linearity was obtained in a concentration range from 100-300 µg/mL for edoxaban tosylate (Fig. 4). Regression equation was y = 2803.1x + 6876.9 with correlation coefficient 0.999.

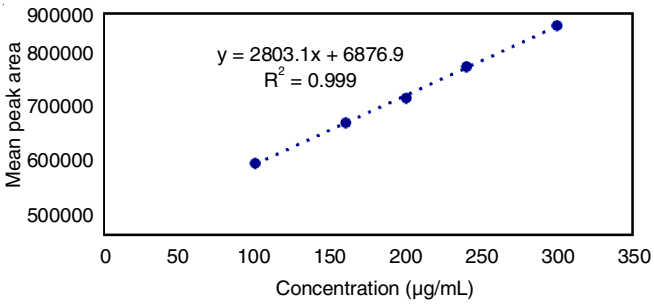

Fig. 4. Calibration curve of edoxaban tosylate

Precision: The developed method is precise as the RSD values for the repeatability and intermediate precision studies were < 0.4 and < 0.75, respectively. The results for precision are expressed in Table-5.

TABLE-5
PRECISION AND ACCURACY DATA
FOR EDOXABAN TOSYLATE

Precision		Intra-day precision	Inter-day precision	Accuracy
Drug	% Level (n = 3)	RSD	RSD	Recovery (%)
Edoxaban Tosylate	50	0.47	0.75	98.20
	100	0.26	0.22	99.58
	150	0.44	0.21	100.44

Accuracy: Method is accurate as percentage recovery for edoxaban tosylate was found to be within 98.20-100.44%. The results of accuracy is shown in Table-5.

Limit of detection (LOD) and limit of quantification (LOQ): LOD and LOQ for edoxaban tosylate were found as 12.54 and 38 µg/mL, respectively.

Robustness: The robustness of the suggested method is confirmed by performing the analysis with modifications to the flow rate and temperature. RSD of peak area were found to be less than 2% indicated that the method is robust. Results remained unaffected by small variations of these parameters.

Conclusion

A specific, accurate, precise and robust stability indicating RP-UPLC PDA detector method is developed and validated for determination of edoxaban tosylate in API. Degradation was carried out under oxidation, hydrolysis, photolysis and heat conditions. The method is good enough to separate the peaks of active pharmaceutical ingredients (API) from the degradation products (generated during stress condition) and peak purity was also passed. Order of stability for edoxaban tosylate is thermal > photolytic > oxidative > acidic > alkali. The range of 100-300 µg/mL (r^2 = 0.999) and the % accuracy was found to be in the range of 98.21-101.42. The precision shows RSD was less than 2. So, all these parameters convince to conclude that the assay method can be successfully used for stability studies inAPI.

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