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Stability indicating RP-HPLC method for quantification of Edoxaban tosylate

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ABSTRACT

Edoxaban tosylate is a member of the novel oral anti-coagulants (NOACs) class of drugs, and is a rapidly acting, oral, selective factor Xa inhibitor. The main objective of present study is to develop a stability indicating RP-HPLC method for quantification of edoxaban tosylate. The separation of edoxaban tosylate was achieved on LC-GC Qualisil gold C_{18} (250 X 4.6 mm i.d., 5µ) using a mobile phase of 65:35 *% v/v* acetonitrile and 0.2% TEA in water (pH adjusted to 3 with OPA) with 1ml/min flow rate at a wavelength of 260 nm. The linearity of the proposed method was in the range of 2-25 µg/ml with R^2 value of 0.9996. The LOD and LOQ was found to be 0.46 µg/ml and 1.40 µg/ml for edoxaban tosylate respectively. The developed method was able to separate all the degradants from each other and also from API. The developed methods were validated for various parameters like Specificity, Linearity, Precision, Accuracy, Limit of detection (LOD), Limit of quantification (LOQ) and Robustness and is in accordance with ICH guidelines.

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INTRODUCTION

Edoxaban tosylate (EDXBN) is chemically 4-methylbenzene-1-sulfonic acid; N'-(5-chloropyridin-2-yl)-N-[(1S,2R,4S)-4-(dimethylcarbamoyl)-2-{5-methyl-4H,5H,6H,7H-[1,3]thiazolo[5,4-c]pyridine-2-amido}cyclohexyl]ethanediamide (pubchem). Edoxaban is a direct oral anticoagulant ,which exerts its effects through inhibition of factor Xa. Based on results from the ENGAGE AF-TIMI 48 and Hokusai-VTE trials (Giugliano et al., 2013; Buller et al., 2013), it was approved by the European Medicines Agency (EMA) regulatory for two main indications 1) The prevention of stroke/systemic embolism in patients with non-valvular atrial fibrillation (NVAF) with one or more risk factor. 2) Ttreatment/secondary prevention of acute VTE (DVT and/or PE) in adults (Lixiana, 2017). Arterial injury activates the coagulation cascade and generates factor Xa (FXa) and thrombin (Fredenburgh et al., 2017). Literature survey reveals that there are limited techniques for the estimation of edoxaban using UV Spectrophotometric (Ravisankar et al., 2018), HPLC methods (Satyanarayana reddy et al. 2016; Zhiwei et al., 2017), LC-MS (Gous et al., 2014). Pasam Satyanarayana Reddy et al., reported RP-HPLC stability method that stress conditions adopted were not appropriate thus the method did not reveal stress specific impurities. Another HPLC method related related substances. Hence, the proposed HPLC method was designed to reveal all possible degradation products by using forced degradation studies.

EXPERIMENTAL

Chemicals and Reagents

Sample of EDXBN was kindly provided by S.R Chemicals & Pharmaceuticals, Surat, India. HPLC grade solvents were procured from Merck Pvt Ltd, India, and *Ortho* phosphoric acid (OPA), Triethylamine (TEA) was obtained from Qualigens, Mumbai (India). Calibrated class A type glassware (Borosilicate) were used. Agilent LC 1200 system with EZ chrome elite software, photodiode array detector (PDA), rheodyne manual injector (20µL) was used in this study. Calibrated Axis LC GC balance was used for weighing chemicals. Systronics Digital pH meter 802 was used for measuring pH of samples.

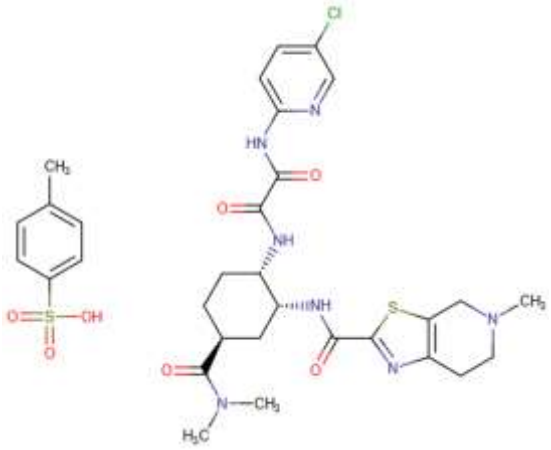

Figure 1: Structure of EDXBN

Instrumentation and chromatographic conditions

HPLC analysis was performed by Agilent-1200 binary pump with photo diode-array detector (PDA) and manual sampler. The output signal was monitored by using Ezchrome Elite software. Compounds were separated on a 250 mm-4.6 mm(id), 5µm particle size, Agilent C18 column with acetonitrile and 0.2% TEA (65:35 v/v) and pH of aqueous phase was adjusted to 3.0 with ortho phosphoric acid (OPA) as mobile phase. The injection volume was 20 µL and eluents detected at 260 nm with the flow rate 1.0 ml/min.

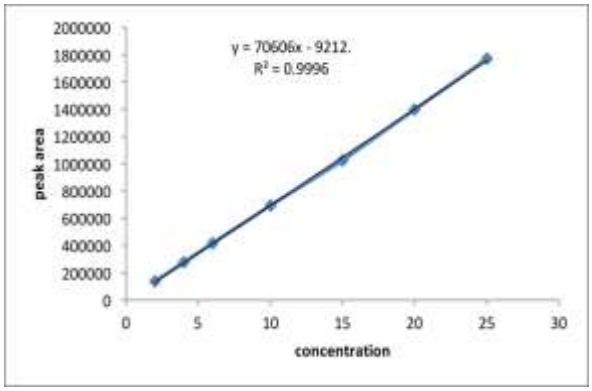

Figure 2: Linearity of EDXBN

Preparation of Standard Solution

Accurately weighed quantity of 10 mg of the edoxaban standard was transferred into 10ml volumetric flask and dissolved in Acetonitrile. The volume was made upto the mark with Acetonitrile to give concentration of 1,000 µg/ml. Later 1 ml of the above solution was diluted to 10ml with mobile phase to give a concentration of 100 µg/ml.

ANALYTICAL METHOD VALIDATION

Method was validated as per ICH (Q2) guidelines with respect to specificity, linearity and range, accuracy, precision, robustness, limit of detection (LOD) and limit of quantification (LOQ) (ICH Q2) (ICH, 2005].

Specificity

Stress degradation studies were performed on edoxaban to support the specificity of the stability-indicating method, and to reveal possible degradation products. The degradation of was performed by exposing edoxaban to Acid (1N HCl), base hydrolysis (0.1 N NaOH), oxidation (3% H_2O_2) sun light, heat, water hydrolysis. The above stress samples were analysed at 10 µg/ml. The specificity of the method was assessed by peak purity.

Linearity and Range

Based on response of analyte in the chromatogram, the linearity test was performed in between 2 to 25 µg/ml at seven different concentrations. The drug samples of concentrations used were 2, 4, 6, 10, 15, 20 and 25 µg/ml. These solutions were prepared in triplicate and injected into the column through the rheodyne injector to determine the response and were shown in table 3.

Accuracy

Accuracy was assessed by spiking of standard drug in laboratory excipient mixture. Three test concentrations of edoxaban (8, 10 ,12 µg/ml) were spiked in three 10 ml volumetric flasks containing of 1ml of 10% lactose solution. These samples were analysed and compared with three standard concentrations of edoxaban (8, 10 ,12 µg/ml) . % RSD and % recovery for this study were calculated and shown in table 5.

Precision

The precision study was carried out at the concentration of 10 µg/ml (n=6). The precision of the proposed method variations in the peak areas of the drugs solutions were calculated in terms of % RSD. Precision was proven by inter and intraday precision studies which were shown in table 4.

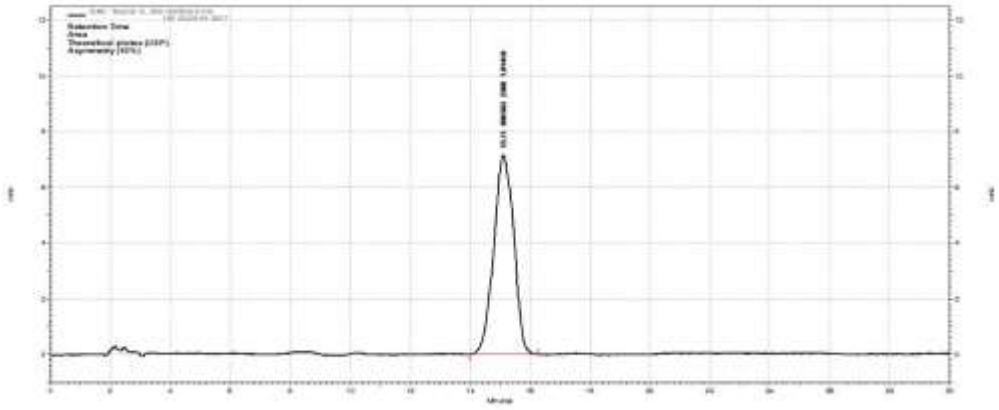

Figure 3: Optimized chromatogram of EDXBN

Table 1: Different trails of RP- HPLC Method optimization for EDXBN on c18 column

Trail no.	Mobile phase(% v/v),	Retention time (min)	Theoretical Plates	Tailing factor
1	ACN: Water 80:20	9.8	5406	2.23
2	ACN: Water 70:30	13.9	4358	2.17
3	ACN: Water 60:40	20.6	5485	2.35
4	ACN : 0.2% TEA (pH 3.0 with OPA) 65:35	15.1	2360	1.54

Table 2: System suitability Parameters for EDXBN

S.No	Parameters	Results
1.	Detection wavelength	260 nm
2.	Retention time (t_R)	15.1 ± 0.2 min
3.	Tailing factor	1.54
4.	Theoretical plates	2360
5.	Repeatability (% RSD)	0.92
6.	Impurity Retention minutes	*D1-4.2, D2-4.7, D3-5.0, D4-6.1, D5-8.7, D6-12.3, D7-24.2*
7.	Resolution EDXBN from impurities	>2

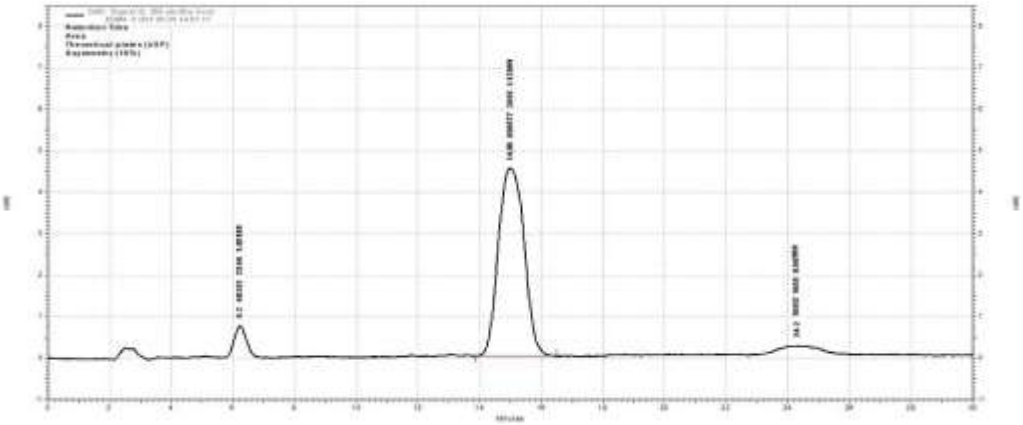

Figure 4: Acid degradation chromatogram for EDXBN

Limits of Detection (LOD) and Quantitation (LOQ)

The LOD and LOQ was calculated based on the formula as per ICH Q2 guidelines.

Robustness

The robustness of the developed method was determined by analyzing the samples under deliberate change in method parameters, such as change in flow rate (±0.1 ml/min), pH (±0.2) of the buffer and organic phase (±2%). The method was robustness assessed for USP tailing, USP plate count and % RSD. The results were shown in table 6.

RESULTS AND DISCUSSION

HPLC method development and optimization

Preliminary trails were performed by using acetonitrile and water at different compositions. Peak

Table 3: Linearity of EDXBN

S.No.	Concentration (µg/ml)	Mean Peak Area± SD (n = 3)
1	2	138652 ± 2384
2	4	276535± 3401
3	6	416607± 4832
4	10	696452 ± 6408
5	15	1026266 ± 15599
6	20	1398185 ± 26146
7	25	1772433 ± 25345

Table 4: Precision for EDXBN

S. No	Amount (µg/ml)	Intra-day Precision		Inter-day Precision	
		Peak Area Mean ± SD (n=6)	% RSD	Peak Area ± SD Mean ± SD (n=6)	% RSD
1	10	696871 ± 697.87	0.10	696111 ± 905.94	0.13
2	10	695931 ± 766.52	0.11	695825 ± 1044.73	0.15
3	10	697127 ± 767.83	0.11	695972 ± 1184.15	0.17

Table 5: Recovery studies for EDXBN

Sample	Conc. (µg/ml)	Lactose Quantity spiked	Area Mean ± SD (n=3)	% Recovery	% RSD
Recovery sample	8	1ml	560383 ± 10872	100.14	1.94
	10	1ml	694346 ± 7638	99.58	1.10
	12	1ml	833243 ± 12666	99.65	1.52
Std	8	0ml	559553 ± 1083	100	0.19
	10	0ml	697215 ± 1880	100	0.26
	12	0ml	836113 ± 3233	100	0.38

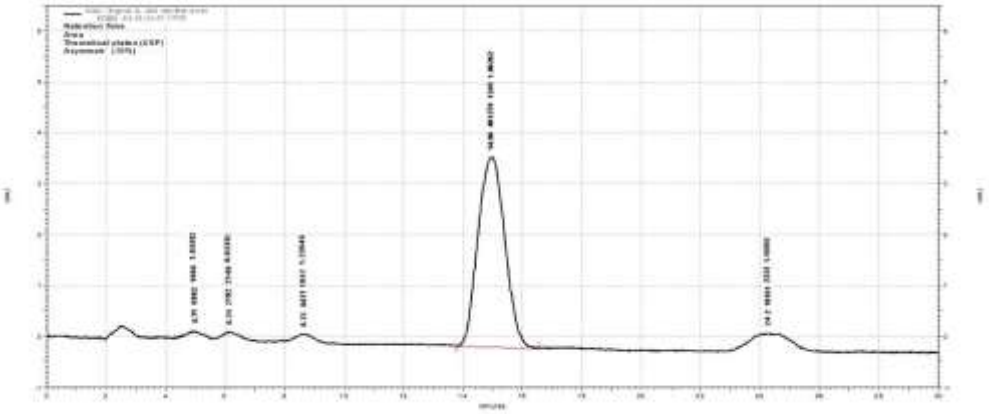

Figure 5: Base degradation chromatogram for EDXBN

shapes were not good. To eliminate the tailing effect, a trial with acetonitrile and 0.2% TEA in the ratio of 65:35 (pH 3 with OPA) at a flow rate of 1ml/min yields good peak shape and afforded a suitable method as per USP-SST. Drug was eluted at retention time of 15.1± 0.2 min. UV detection was carried out at 260 nm (photo diode detector). Various experimental trials carried for optimization of edoxaban were shown in table 1. The system suitability parameters (SSP) of the developed method are shown in table 2.

Method Validation

Method was validated as per ICH (Q2) guidelines with respect to specificity, linearity, precision, accuracy, robustness, limit of detection(LOD) and limit of quantification(LOQ). (ICH Q2) (ICH, 2005).

Specificity

The study employed the degradation protocol by exposing to sun light for 4 hr, heat at 100 °C for 2 hr, acid hydrolysis at 1 N HCl (RT) for 1 hr, base hydrolysis at 0.1 N NaOH (RT) for 30 min, water hydrolysis with pH 7 kept at RT for 8 days and oxidation at 3% H_2O_2 kept at RT for 3 hr. All degradants adequately separated from edoxaban, thus the specificity of the method was proven. The peak purity was assessed based on purity angle and purity threshold in which both degraded and control samples chromatogram showed peak purity in the range of 0.9981-0.9998.

Table 6: Robustness for EDXBN

Parameter changed	Tailing ± SD	Plate count ± SD	%RSD
Flow rate(±0.1 ml/min)	1.19 ± 0.015	2542 ± 34.31	1.02
% Organic phase (±2 %)	1.27 ± 0.021	2475 ± 47.27	1.47
pH (±0.2 units)	1.33 ± 0.023	2817 ± 37.18	1.82

Table 7: Degradation data of EDXBN

Stress Condition	Duration	Rt (min) of Degradation products (% area)							% degradation of EDXBN	% Assay	Mass balance
		4.2 D1	*4.7 D2*	*5.1 D3*	*6.1 D4*	*8.7 D5*	*12.3 D6*	*24.2 D7*			
Acid (1 N HCL)	1 h	--	--	--	5.7	--	--	2.2	20.96	79.04	89.96
Base (0.1N NaOH)	30 min	--	--	1.0	1.03	1.24	--	2.69	29.48	70.52	76.48
Oxidation 3% H_2O_2	3h	--	1.09	0.57	1.47	1.60	--	1.56	36.30	63.70	69.99
Dry heat (100°C)	2h	1.1	--	--	--	0.9	--	--	40.92	84.72	61.08
Photolysis (Sun light)	4h	--	--	1.09	3.43	--	1.52	3.07	15.28	59.08	93.83

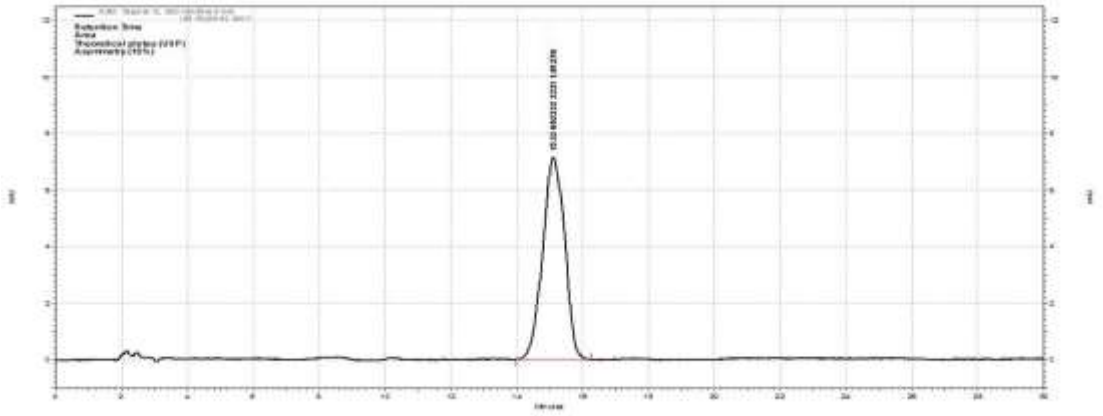

Figure 6: Neutral degradation chromatogram for EDXBN

Linearity and Range

The linearity of the method was proven at concentrations of edoxaban in the range 2- 25 µg/ml figure 2. The correlation coefficient (r^2 value) obtained was 0.9996 as shown in table 3.

Precision

The % RSD for interday and intraday precision studies were obtained from six replicate analyses of edoxaban at 10 µg/ml. The % RSD values for interday and intraday precision were below 2.0 % (acceptance < 2 %) indicated that the method was sufficiently precise, as shown in table 4.

Recovery Studies

Samples at 80,100,120 % of test concentrations were analysed. % recovery was calculated by comparing the peak area of test with standard. % RSD (<2) and % recovery (98-102%) values for edoxaban are within limits indicated that the method is accurate as shown in table 5.

Limit of Detection and Limit of Quantification

LOD and LOQ was found to be 0.46 µg /ml and 1.40µg/ml respectively.

Robustness

The % RSD for change in flow rate ±0.1 ml/min, organic phase ratio ±2 %, pH ±0.2 of the buffer, was found to be 1.02, 1.47 and 1.82 respectively. The method was found to be robust for all the parameters tested for USP tailing, USP plate count and % RSD (Assay) as shown in table 6.

Forced Degradation

After various stress trials, stress degradation was found in 1N HCl, 0.1N NaOH, 3% H_2O_2, at 100°C,

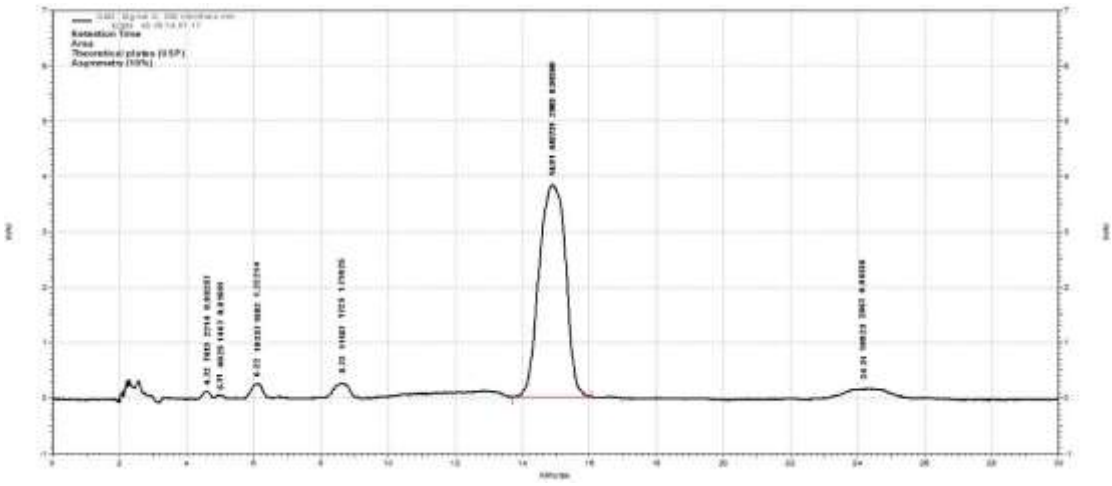

Figure 7: Oxidative degradation chromatogram for EDXBN

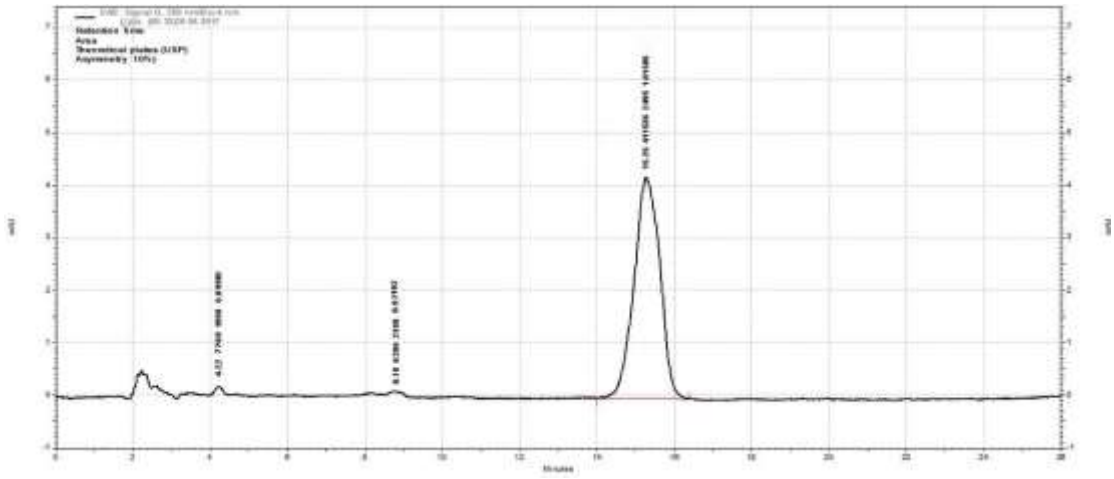

Figure 8: Thermal degradation chromatogram for EDXBN

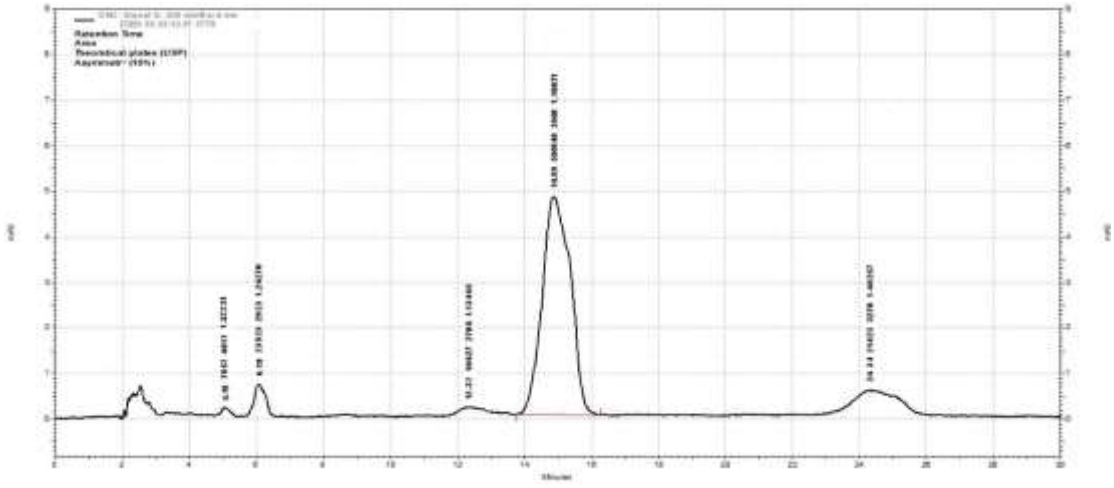

Figure 9: Photolytic degradation chromatogram for EDXBN

and sunlight. The chromatograms were analysed for peak separation, peak purity, number of degradants, % degradation of edoxaban. The results were discussed in table 7.

Acid induced degradation

The drug showed sufficient degradation with stress condition of 1N HCl at room temperature (RT) for 1h. Two degradant peaks were formed namely, D-4 and D-7 at retention times of 6.1 min and 24.2 min. The percentage degradation of edoxaban was 20.96% as shown in the figure 4. The % assay and mass balance were 79.04, 89.96 respectively.

Base induced degradation

The drug showed sufficient degradation with stress condition of 0.01N NaOH at room temperature. Four degradant peaks were formed, namely D-3, D-4, D-5 and D-7 at retention times of 5.1min, 6.1min, 8.7 min and 24.2 min respectively. The percentage degradation of edoxaban was 29.48% as shown in the figure 5. The % assay and mass balance were 70.52, 76.48 respectively.

Neutral degradation

The drug had showed negligible degradation at RT for 8 days as shown in the figure 6.

Oxidative degradation

On treatment with 3% H_2O_2at RT for 3 hrs at room temperature, five degradant peaks were formed namely, D2, D3, D4, D5, D7 at retention time of 4.7min, 5.0min, 6.1min, 8.7min and 24.2min respectively. The percentage degradation of edoxaban was 36.30 as shown in the figure 7. The % assay and mass balance were 63.70, 69.99 respectively.

Thermal degradation

The drug had shown sufficient degradation when exposed to dry heat in oven at 100°C for 2 hrs with the formation of two degradation products, namely D-1 and D-5 at retention times of 4.2 min and 8.7 min respectively The percentage degradation of edoxaban was 40.92 as shown in the figure 8. The % assay and mass balance were 84.72, 61.08 respectively.

Photolytic degradation

The drug in Acetonitrile (10 µg/ml)was subjected to photolytic conditions for 4 hrs under direct sunlight. The drug was degraded with the formation of four degradant peaks, D-3, D-4, D-6, D-7 at retention time of 5.1min, 6.1min,12.3min and 24.2min respectively. The percentage degradation of edoxaban was15.28 as shown in the figure 9. The % assay and mass balance were 59.8, 93.83 respectively.

CONCLUSION

The developed SIAM for edoxaban demonstrated accepatable accuracy, precision and specificity with economic reagents and solvents, such as acetonitrile and formate buffer. Thus, the method was less complex, more reliable for quantification of edoxaban in presence of 7 degradation products. The method fulfilled the ICH Q_2 requirements thus it can be concluded that the method can be successfully used for detect and identify any kind of degradants during stability studies of tablet dosage forms.

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