Acta Scientific Pharmaceutical Sciences (ISSN: 2581-5423)

Volume 3 Issue 1 January 2019

Research Article

UPLC-MS Stability-Indicating Method for Determination of Edoxaban and its Acid Degradation Products

Basima Arous* and Mhd Amer Al-Mardini

Department of Pharmaceutical Chemistry and Quality Control, Faculty of Pharmacy, Damascus University, Damascus, Syria

***Corresponding Author:** Basima Arous, Department of Pharmaceutical Chemistry and Quality Control, Faculty of Pharmacy, Damascus University, Damascus, Syria.

Received: November 16, 2018; **Published**: December 28, 2018

Abstract

Edoxaban is a novel anticoagulant drug acting as a direct, selective and reversible inhibitor of the coagulation factor Xa. Forced degradation under acid condition was carried out in order to establish its stability towards acid hydrolysis. Edoxaban was subjected to acid hydrolysis as per International Conference on Harmonisation ICH guidelines and found susceptible to acid. A linear gradient and validated ultra-performance liquid chromatographic method was applied for the identification and characterization of acid degradation products of Edoxaban in active pharmaceutical ingredient (API). Six major degradation products were detected, well separated and determined by LC-MS, then three of them were isolated by semi-preparative HPLC and subjected to NMR spectroscopy and FT-IR to characterize and elucidation of their most possible structures.

Keywords: Edoxaban; LC-MS; Stability Indicating; Acid Degradation Products; NMR, FT-IR.

Introduction

Edoxaban (Figure 1), chemically known as N'-(5-chloropyridine-2-yl)-N-[(1S,2R,4S)-4-(dimethylcarbamoyl)-2-[(5-methyl-6,7-dihydro-4H-[1,3]thiazolo[5,4-c]pyridine-2-carbonyl)amino] cyclohexyl] oxamide, is a novel anticoagulant drug invented and manufactured by Daiichi Sankyo, acting as a direct, selective and reversible inhibitor of the coagulation factor Xa. It is prescribed as treatment of venous thromboembolism, which includes deep vein thrombosis and pulmonary embolism, and is also used after hip or knee replacement surgery to prevent a type of blood clot called deep vein thrombosis (DVT) [1-4].

Molecular Formula: $C_{24}H_{30}ClN_7O_4S$. Molecular Weight: 548.059

Figure 1: Structure of the Edoxaban [5,6].

As the (API) may undergo degradation, leading to the drug activity loss or to the occurrence of adverse effects associated with degradation products, thorough knowledge of API's stability profile is required [7]. Stability testing provides evidence of the quality of an API when exposed to the influence of environmental factors such as pH, temperature, humidity, and light. The data from such studies enables storage conditions, re-test periods and shelf lives to be established. Stress testing helps to determine the intrinsic stability of the molecule by establishing the degradation pathways [7-9]. And since Edoxaban has an oxamide bond which is susceptible to acid hydrolysis there was an importance to study its stability towards the acid condition.

According to the literature survey there wasn't any study dealing with Edoxaban's stability profile or impurity profile, there were only a few studies for Edoxaban measurement in plasma [10] or determination in bulk and tablet Dosage Form by using RP-HPLC [11], or measurment of Edoxaban, Apixaban, Rivaroxaban and Dabigatran in human plasma using turbulent flow liquid chromatography with high-resolution mass spectrometry [12], also there was a study for determination of Rivaroxaban, Apixaban and Edoxaban in rat plasma by UPLC–MS/MS method [13]. In addition, Edoxaban is not yet official in any of the pharmacopeias [14-16]. So, the aim of this research was to develop a stability indicating method for determination of Edoxaban, identification, isolation, and characterization of its acid degradation products (DPs) in order to identify its major degradation pathway in the acid condition, by using techniques of LC-MS, semi-preparative HPLC, NMR spectroscopy and FT-IR spectral data.

Materials and Methods

Chemicals and reagents

Edoxaban standard and APIs were purchased from Beijing Eagle Sky Pharmatech Co., Ltd. HPLC acetonitrile, hydrochloric acid, dimethylsulphoxide-d6 (DMSO-d6) were purchased from SIGMA-ALDRICH®. HPLC water was obtained from Siemens Water Technologies LaboStar.

Instruments

The chromatographic analysis was performed with SHIMADZU LC prominence system (Shimadzu, Japan) provided with UV-Vis Detector SPD 20A, MS Detector 2020, two pumps A and B: LC/20AD, column oven CTO-20A, manual injector and with Shim-pack XR-ODS II (100 x 3.0 mm, 2.2µm particle size), System control and data analysis were carried out using LabSolutions CS (Shimadzu, Japan). KNAUER HPLC Smartline system with PDA detector (Germany). Jasco PU-2080 plus Semipreparative-HPLC (Jasco, Japan). Sartorius sensitive analytical balance (sensitivity of 10-4g). JEKEN Digital Ultrasonic Cleaner. Bechers, Volumetric flasks, Micropipettes, and Glassware of different volumes from Marienfeld Company. Filters PVDF 0.45µm for HPLC purchased from TEKNOKROMA. NMR BRUKER 400 MHz Ultra shield TM instrument. FTIR Nicolet 6700 with the Detector DTGS Operating software (OMNIC version 7.3 Thermo Nicolet USA).

Chromatographic conditions

The chromatographic separation was performed on Shim-pack XR- ODS II (100 x 3.0 mm, 2.2µm particle size) at a column temperature of 25°C, The mobile phase A was water with 0.1% of formic acid, while the mobile phase B was acetonitrile with formic acid (pH=5), the gradient program of the mobile phase was set as [Time(min)/Pump B Value(%), 0.01/15, 23/100 and 25/15], The mobile phase was filtered using 0.45 µm disposable filter, and degassed by ultrasonic vibration prior to use. The flow rate was 0.15 ml/min. The injection volume was 10 µL and the detection was carried out at 291nm. Water and acetonitrile 50:50 (v/v) were used as a diluent. The analysis was performed in positive electro-spray/positive ionization mode ESI+, the ion source voltage was 5000 V, the source temperature was 450°C, and the curtain gas flow was 15 psi. This LC-MS method was successfully developed and validated as per ICH guidelines [17] and according to USP 38 guideline recommendations [14].

Semi-preparative HPLC conditions

A Zorbax Eclipse-C18 (250 x 4.6 mm, 5µm particle size) column with a mobile phase consisting of water and acetonitrile, the gradient program of the mobile phase was set as [Time (min)/ Pump B Value (%)] [0.01/60, 15/65, 25/40,30/30 and 55/60] at a flow rate of 1.2 ml/min, and the detection was carried out at 291 nm.

NMR H^1 and C^{13} spectroscopy

H^1 and C^{13} NMR spectra were recorded in DMSO-d6 at 25°C. The NMR chemical shift values were reported on the δ scale in ppm, relative to TMS (δ = 0.00) as an internal standard.

FT-IR spectroscopy

The IR spectra for Edoxaban and acid degradation products were recorded in the solid state as KBr dispersion, with Range 400-4000nm, the resolution was 4 cm^{-1}, scans were 32.

Degradation protocols

The acid hydrolysis was performed as per ICH recommendation with 1 N HCI for 24h, degradation samples were prepared by diluting Edoxaban stock standard solution (2 mg/ml) in diluents to obtain a final concentration of (1 mg/ml), all of the stressed samples were quantified against the Edoxaban reference standard. Each experiment was performed in triplicate and the working solutions were allocated in 10 ml hermetically sealed glass vials.

Results

Method development and validation

The main target of the chromatographic method is to achieve the separation of acid degradation products from Edoxaban in APIs after acid hydrolysis employed for degradation studies. The described LC-MS method was successfully developed and validated as per ICH and USP 38 guidelines. It was suitable for the separation of Edoxaban from acid degradation products and the retention time for Edoxaban A was 3.728min and for Edoxaban B was 7.432min (Figure 2). Since Edoxaban has three asymmetric carbons (chiral carbons) it leads to eight stereoisomers which appear as two peaks in the chromatographic analysis (Edoxaban A and Edoxaban B). The acid degradation products produced in the forced degradation were well-separated (Resolution > 2.0) from Edoxaban, the tailing factor for Edoxaban A was 0.855 and for Edoxaban B was 1.249, and the theoretical plates were 16430 and 26814 for A and B: respectively. The method was proved to be linear over the calibration range 100–2000 µg/ml and the correlation coefficient was > 0.999 for both Edoxaban A and B, and its accuracy, precision, repeatability, and robustness were checked and the recovery was for Edoxaban (A and B) (103.55% and 106.20%), (101.40% and 102.01%), (100.90% and 99.20) and (101.65% and 104.22%) respectively while the RSD was 1.54 for Edoxaban A and 1.62 for Edoxaban B . The Detection limits (DL) and Quantification limits (QL) for Edoxaban (A and B) were (0.050 and 0.072) µg/ml and (0.168 and 0.195) µg/ml respectively.

Figure 2: Chromatogram of the standard solution of Edoxaban.

Acid degradation of Edoxaban along with the formation of Degradation Products

The forced degradation was carried on Edoxaban, and significant degradation of Edoxaban was observed in acidic hydrolytic stress conditions. In total, six DPs were observed as it is shown in (Figure 3) and table 1. The degradation products are named "DPn", where n accounts for the elution order.

As shown in Table 1, loss of 76% of Edoxaban was observed after 24 hours in acidic conditions. The identification of Edoxaban and its acid degradation products was confirmed by ESI-MS using scan mode from 50-900 mu to produce spectra of molecular weight as shown in Table 2.

Most possible structures were proposed for DPs by comparing their fragmentation patterns with that of Edoxaban. The ESI-MS spectra of Edoxaban and DPs are shown in (Figure 4).

DP2, Dp3, and DP4 were isolated by semi-preparative HPLC and subjected to NMR and FT-IR studies for structure elucidation using conditions as mentioned in Materials and Methods section. Whereas structural elucidation of DPs has been accomplished from NMR, FT-IR and mass spectral data.

FT-IR spectra of Edoxaban, DP2, DP3 and DP4 are shown in (Figure 5).

Proton H^1 and carbon C^{13}, NMR spectra of Degradation Products are shown in (Figure 6).

Figure 3: Chromatograms of Acid Hydrolysis Degradation Products of Edoxaban.

Stress condition	Time	Assay of Edoxaban A (% w/w)	Assay of Edoxaban B (% w/w)	Total degradants (% w/w)	Mass balance	Commentaries
Edoxaban before degradation	-	75.709	24.241	0.032	99.982	-
Acid hydrolysis (1 N HCl)	24 hours	2.987	18.786	76.984	98.757	Degradation accompanied by the appearance of DP1, DP2, DP3, DP4, DP5 and DP6
Mass balance = assay %+ sum of all degredants%.						

Table 1: Summary of acid degradation studies of Edoxaban.

Stress condition	Peak name	Retention time RT (min)	$[M+H]^+$ m/z
Acid hydrolysis	DP1	2.105	200
	DP2	2.827	438
	Edoxaban A	3.743	548
	Edoxaban B	7.399	548
	DP3	7.796	521
	DP4	8.029	564
	DP5	12.698	498
	DP6	13.463	299

Table 2: $[M+H]^+$ m/z of Edoxaban and its Acid Degradation Products.

Figure 4: ESI-MS spectrums of Edoxaban and its Acid Degradation Products.

Figure 5: FT-IR spectra of Edoxaban and its Acid Degradation Products.

Figure 6: NMR H^1andC^{13} spectra of Edoxaban and its Acid Degradation Products.

Discussion

Under influence of acid stress conditions as per ICH guidelines, it was clear that Edoxaban was susceptible to hydrolysis condition, it degraded under stress acid hydrolytic degradation giving rise to new degradation products. The six acid degradation products were successfully characterized by the use of mass spectrometry, three of them were isolated by semi-preparative HPLC and subjected to NMR and FT-IR studies, and the structural elucidation of DPs has been accomplished from NMR, FT-IR and mass spectral data.

As shown in (Figure 3) the chromatographic method was able to separate all the acid degradation products not only from Edoxaban but also from one another in a stressed sample. The method thus proved to be selective and stability-indicating.

As shown in (Figure 3) the chromatographic method was able to separate all the acid degradation products not only from Edoxaban but also from one another in a stressed sample. The method thus proved to be selective and stability-indicating.

DP2 appears at retention time 2.8min, with $[M+H]^+$ 438m/z. It could be clearly seen from NMR spectra of DP2 by comparing with that belong to Edoxaban, that there was a lose in protons (three protons) and carbons (five carbons), The NMR H^1 shows a loss in protons at δ = 7.088, δ = 7.107 and δ = 7.990 which belong to the chloropyridine, in addition to the loss of N-H proton at δ = 8.449 and replace it with O-H proton at δ = 10.283. Also, the NMR C^{13} shows a loss in absorptions of carbons at δ = 115.53, δ = 127.19, δ = 127.30, δ = 148.32, and δ = 149.09 which belong to the chloropyridine, in addition to the displacement of carbons absorption from δ = 158.88, and δ = 159.25 to δ = 161.30, and δ=169.73 respectively. FT-IR spectrum shows the disappearance of the absorption bands of pyridine at 1010 cm^{-1}, 1377 cm^{-1}, and 1616 cm^{-1} also a decrease in N-H band at 1501 cm^{-1}, and the appearance of wider OH band at 3349 cm^{-1}, thus and with the aid of MS spectrum it was suggested that DP2 has molecular formula: $C_{19}H_{27}N_5O_5S$, and that Edoxaban has lost chloropyridine and the OH group replaced the amine bond.

DP3 appears at retention time 7.796min, with $[M+H]^+$ 521m/z. It could be clearly seen from NMR spectra of DP3 by comparing with that belong to Edoxaban, that there was a lose in protons (five protons) and carbons (two carbons), The NMR H^1 shows a loss in 6 protons their absorption extend from δ = 2.789 to δ = 3.376 which belong to the dimethyl in the dimethyl carbamoyl group, in addition to the appearance of new absorption at δ =10.449 which belongs to the proton in O-H that replaced the amide bond in cyclohexane. Also, the NMR C^{13} shows a loss in absorptions of carbons at δ = 33.42 and δ = 35.44 which belong to the dimethyl carbamoyl group, in addition to the displacement of carbons absorption from δ = 42.29, and δ = 174.34 to δ = 42.99, and δ = 180.88 respectively which belong to carbons next to the dimethyl carbamoyl group. The FT-IR spectrum shows a decrease in alkane (CH_2-CH_2) band at 2928 $cm^{-1}$and in the C-N band at 1296 cm^{-1}, and the O-H broadband appears at 3349 $cm^{-1}$and 1433 cm^{-1}. It was clear that Edoxaban has lost the dimethylamine group and replace it with the hydroxyl group, and the molecular formula of DP3 could be $C_{22}H_{25}ClN_6O_5S$.

DP4 appears at retention time 8.0min, with $[M+H]^+$ 564m/z. It could be clearly seen from NMR spectra of DP4 by comparing with that belong to Edoxaban, that they have the same numbers of protons and carbons, The NMR H^1 shows a small displacement of three protons which is next to the nitrogen atoms in methyl dihydropyridine, chloropyridine and cyclohexyloxamide, while the NMR C^{13} was the same as that of Edoxaban. The FT-IR spectrum shows new absorption bands at 916 $cm^{-1}$and 1170 cm^{-1} which belong to the nitroso group. According to that, it was clear that Edoxaban has gained an oxygen producing a nitroso compound with a molecular formula $C_{24}H_{30}ClN_7O_5S$.

The above results allowed to Propose degradation pathways of Edoxaban, and the schematic representations of the mechanism of formation of the degradation products under acid hydrolytic stress are shown in (Figure 7).

Figure 7: Proposed degradation pathways of Edoxaban by acid hydrolysis.

Conclusion

The acid degradation behavior of Edoxaban was studied as per ICH prescribed guidelines. Six degradation products were formed under stress acid hydrolytic conditions as detected by LC-MS. ESI-MS studies were carried out to characterize structures of degradation products. Three of them were isolated and characterized also by NMR and FT-IR spectroscopy, and it was possible to predict the major degradation mechanisms. A simple, rapid and selective stability indicating LC method has been developed and validated for the determination of Edoxaban and its acid degradation products.

Acknowledgment

The authors are thankful to the colleagues at the central laboratory in the faculty of science and the colleagues at the Atomic Energy Commission for their cooperation in carrying out this work.

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