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Stability-indicating spectrophotometric methods for determination of the anticoagulant drug apixaban in the presence of its hydrolytic degradation product

Mahmoud A. Tantawy [a,*], Nariman A. El-Ragehy [a], Nagiba Y. Hassan [a], Mohamed Abdelkawy [b]

[a] Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr el Aini Street, 11562 Cairo, Egypt
[b] Analytical Chemistry Department, Faculty of Pharmacy, Future University, end of 90th St., Fifth Settlement, New Cairo, Egypt

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ABSTRACT

Apixaban (a novel anticoagulant agent) was subjected to a stress stability study including acid, alkali, oxidative, photolytic, and thermal degradation. The drug was found to be only liable to acidic and alkaline hydrolysis. The degradation product was then isolated and identified by IR and GC–mass spectrometry. Four spectrophotometric methods, namely; first derivative (D^1), derivative ratio (DR), ratio difference (RD) and mean centering of ratio spectra (MCR), have been suggested for the determination of apixaban in presence of its hydrolytic degradation product. The proposed methods do not require any preliminary separation step. The accuracy, precision and linearity ranges of the proposed methods were determined, and the methods were validated as per ICH guidelines and the specificity was assessed by analyzing synthetic mixtures containing different percentages of the degradation product with the drug. The developed methods were successfully applied for the determination of apixaban in bulk powder and its tablet dosage form.

1. Introduction

Apixaban is chemically designated as1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl)-phenyl]-4,5 dihydropyrazolo[5,4-c]pyridine-3-carboxamide. It is a novel anticoagulant drug which acts as a direct, selective and reversible inhibitor of the coagulation factor Xa. It is prescribed for the treatment of venous thromboembolism [1]. Apixaban is not officially reported in any pharmacopeia (USP, BP and EP) up to date. It is recently approved by FDA in December 2012.

There are some methods for estimation of apixaban in human plasma by different liquid chromatographic techniques [2–5]. Some stability-indicating chromatographic methods for its determination were also reported [6–8]. Prabhune et al. [6] and Landge et al. [7] stated that the drug is only liable to acid and alkali hydrolysis and only small percentage of the drug is hydrolyzed giving rise to different unknown degradation products. Secrétan et al. [8] identified seven degradation products (generated for about 15% of drug degradation) resulting from acid and alkali hydrolysis by HPLC coupled with mass spectrometry technique. They managed to use 0.1 M HCl or 0.1 M NaOH for acid or alkali hydrolysis, respectively.

The conditions required for complete degradation (leading to formation of a new degradation product) have not been reported yet. No spectrophotometric methods have been reported for the determination of apixaban in presence of its degradation products or impurities. The aim of this work was to specify the conditions required for complete degradation of apixaban, isolation and identification of the degradation product and finally, to develop and validate stability-indicating spectrophotometric methods for its determination.

2. Experimental

2.1. Instruments

Spectrophotometric measurements were carried out on a dual beam Shimadzu (Kyoto, Japan) UV–Vis spectrophotometer, model UV-1601 PC. The bundle software, UV PC personal spectroscopy software version 3.7 (Shimadzu, Kyoto, Japan) was used to process absorption and derivative spectra, scans were carried out in the range from 200 nm to 400 nm at 0.1 nm intervals using 1.00 cm quartz cells.

HPLC system consists of: Agilent pump with different flow rates (model 1100 series, Agilent, Germany), equipped with a variable wavelength detector and a 20 μL injection loop. Zorbax ODS (5 μm, 250 mm × 4.6 mm i.d.) column was used as a stationary phase. The samples were injected with 50 μL Hamilton analytical syringe. Mobile phase of pH 5.5 phosphate buffer: acetonitrile: triethylamine (53:47:0.03, by volume) and flow rate of 1 mL min^{-1} were applied.

* Corresponding author.
E-mail address: matantawy@hotmail.com (M.A. Tantawy).

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IR (Infrared) spectrophotometer, Shimadzu 435, Kyoto, Japan.

GC–MS (gas chromatography–mass spectrometry), Shimadzu GC–MS-QP2010 with a Shimadzu AOC-20i autosampler. A 30 m, HP-5 MS column [(5%-Phenyl)-methylpolysiloxane] was employed for separation, with a 0.253 mm i.d. and 0.50 μm film thickness (Agilent, Palo Alto, California).

2.2. Materials and reagents

2.2.1. Pure standard

Apixaban was kindly supplied by Bristol-Myers Squibb. Its purity was checked and found to be 100.62% according to a reported HPLC method [6].

2.2.2. Pharmaceutical dosage form

Eliquis® (5 mg), lot no. 3 L73079, labeled to contain 5 mg apixaban per tablet, manufactured by Bristol-Myers Squibb-pfizer and obtained from Australian pharmacies by personal communication.

2.2.3. Degradation product

Degradation product was prepared as will be described later under procedures.

2.2.4. Chemicals and reagents

All chemicals used throughout the work were of analytical grade and solvents were of HPLC grade.

Methanol (Merck, Germany), acetonitrile (Merck, Germany), hydrochloric acid (Merck, Germany); 3 M and 5 M aqueous solutions, sodium hydroxide (Merck, Germany); 5 M aqueous and methanolic solutions, phosphate buffer solution pH 5.5 (750 μL phosphoric acid to 530 mL double distilled deionized water and pH was adjusted to 5.5 using 10% potassium hydroxide) [9], double distilled deionized water (Otsuka, Cairo, Egypt), triethylamine (Sigma-Aldrich, Belgium).

2.3. Solutions

2.3.1. Stock standard solutions

Stock standard solutions of apixaban and its degradation product (8 mg mL^{-1}) were prepared in methanol.

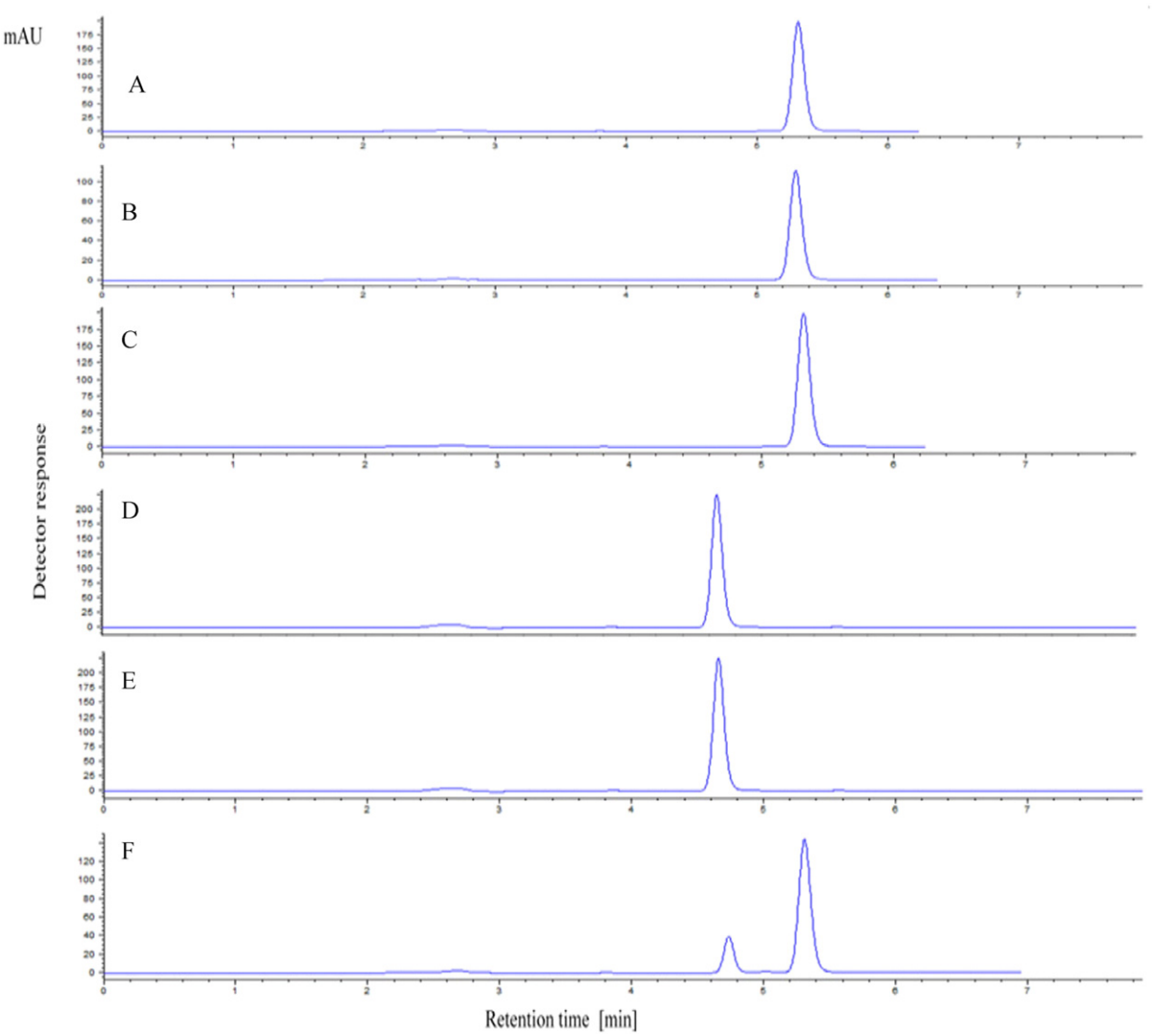

Fig. 1. HPLC chromatograms of the stability of apixaban [retention time (t_R) = 5.307 min] in different stress hydrolytic conditions, showing the disappearance of its peak after acid or alkali hydrolysis and the appearance of a new peak corresponding to its degradation product at t_R = 4.700 min: oxidative degradation of apixaban,70 μg/mL in 30% H_2O_2 (A); thermally treated apixaban, 40 μg/mL in 120 °C oven (B); photo treated apixaban,70 μg/mL subjected to a UV lamp (C) alkaline hydrolysis of apixaban, 70 μg/mL in 5 M NaOH at 120 °C for 7 h (D); acid hydrolysis of apixaban, 70 μg/mL in 5 M HCl at 100 °C for 5 h (E); a synthetic mixture of apixaban (50 μg/mL) and its degradation product (15 μg/mL) (F); all chromatograms obtained by using a Zorbax ODS column (5 μm, 250 × 4.6 mm i.d.), mobile phase of pH 5.5 phosphate buffer: acetonitrile: triethylamine (53:47:0.03, by volume), flow rate of 1 mL min^{-1} and detection at 275 nm.