Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 145 (2015) 360–367

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Two different spectrophotometric determinations of potential anticancer drug and its toxic metabolite

Nehal F. Farid *, Nada S. Abdelwahab

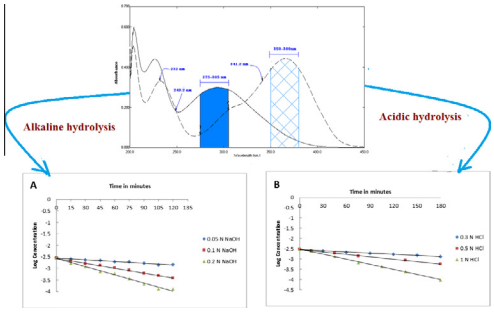
Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Benisuef University, Benisuef, Egypt

HIGHLIGHTS

- No kinetic spectrophotometric study was published for assay of flutamide.
- Developed methods were the first developed stability indicating ones for flutamide.
- Degradation identification by IR and mass spectra analyses.
- The drug degradation followed pseudo first order kinetics.
- Degradation rate was highly sensitive to temperature and pH.

GRAPHICAL ABSTRACT

Flutamide was highly sensitive to hydrolysis. Reaction kinetic was studied using AUC method and found to follow pseudo first order kinetics. Reaction rate constant (K) was found to be pH and temperature dependant.



ARTICLE INFO

Article history:
Received 5 November 2014
Received in revised form 23 February 2015
Accepted 1 March 2015
Available online 6 March 2015

Keywords:
Flutamide
Area under curve
Isoabsorptive
Dual wavelength
Kinetic study
Degradation

ABSTRACT

Flutamide is a hormone therapy used for men with advanced prostate cancer. Flutamide is highly susceptible to hydrolysis with the production of 3-(trifluoromethyl)aniline, which is reported to be one of its toxic metabolites, impurities and related substances according to BP and USP. Flutamide was found to be stable when exposed to oxidation by 30% hydrogen peroxide and direct sunlight for up to 4 h. Two accurate and sensitive spectrophotometric methods were used for determination of flutamide in bulk and in pharmaceutical formulations.

Method (I) is the area under curve (AUC) spectrophotometric method that depends on measuring the AUC in the wavelength ranges of 275–305 nm and 350–380 nm and using Cramer's rule. The linearity range was found to be 1–35 μg/mL and 0.5–16 μg/mL for the drug and the degradate, respectively. In method (II), combination of the isoabsorptive and dual wavelength spectrophotometric methods was used for resolving the binary mixture. The absorbance at 249.2 nm (λ_{iso}) was used for determination of total mixture concentration, while the difference in absorbance between 232 nm and 341.2 nm was used for measuring the drug concentration. By subtraction, the degradate concentration was obtained. Beer's law was obeyed in the range of 2–35 μg/mL and 0.5–20 μg/mL for the drug and its degradate, respectively.

The two methods were validated according to USP guidelines and were applied for determination of the drug in its pharmaceutical dosage form. Moreover AUC method was used for the kinetic study of the hydrolytic degradation of flutamide. The kinetic degradation of flutamide was found to follow pseudo-first order kinetics and is pH and temperature dependent. Activation energy, kinetic rate constants and $t_{1/2}$ at different temperatures and pH values were calculated.

* Corresponding author. Tel.: +20 1277950994.
E-mail address: nehalfayek@gmail.com (N.F. Farid).

http://dx.doi.org/10.1016/j.saa.2015.03.009
1386-1425/

1. Introduction

Flutamide is chemically known as 2-methyl-N[4-nitro-3-(trifluoromethyl)phenyl]propamide [1]. It is a nonsteroidal component with anti-adrenergic properties used for treatment of prostatic carcinoma [2]. Flutamide is susceptible to hydrolytic degradation with the production of 3-(trifluoromethyl)aniline which is stated to be one of the impurities and related substance of flutamide according to BP [3] and USP [4]. The resulted degradate was also reported to be one of the main toxic metabolites of flutamide [5].

Flutamide was determined in BP [3] by UV spectrophotometric method through measuring the absorbance at 295 nm of methanolic solution. On the other hand USP [4] determined the drug in pure form and in capsules by RP-HPLC method using acetonitrile: water (1:1, v/v) as a mobile phase.

Several methods were reported for analysis of the studied drug including electrochemical methods [6], different spectrophotometric methods [6–11] and chromatographic methods [6,12–15].

Reaction kinetics is the study of chemical change and the way by which this change is influenced by variable conditions such as concentration of reactant, the present chemicals and temperature. It provides information that permits a rational approach to the stabilization of drug products, prediction of shelf life and optimization of storage conditions [16–19]. Many environmental conditions such as heat, light, hydrolysis or oxidation can take part in pharmaceutical stability [20]. Hydrolysis is one of the most common degradation routes, since water exists for most drugs and excipients as a solvent or moisture in the air [21].

Most reported spectrophotometric methods included the formation of colored products that suffered from various drawbacks and tedious sample preparation. On the other hand, none of the reported methods described the degradation behavior or mechanism of flutamide. The work in this manuscript aimed to study the kinetic of the hydrolytic degradation of the anticancer flutamide under acidic and alkaline conditions using different temperatures through the development of stability indicating spectrophotometric methods. Characterization of the produced degradate (flutamide toxic metabolite) was carried out by IR and mass spectroscopic analyses. Other degradation pathways as oxidation and exposure to direct sunlight were tried and the drug was found to be stable under these conditions. The developed methods are easy to be applied, need simple sample preparation and data manipulation, hence they can be used in quality control and stability studies of the proposed drug.

2. Experimental

2.1. Instruments

- A double beam UV–Visible spectrophotometer (SHIMADZU, Japan), model UV-1601 PC with 1 cm path length quartz cell is used and it is connected to IBM compatible computer. The software was UVPC personal spectroscopy software version 3.7.
- UV lamp with short wavelength 254 nm (USA).

2.2. Materials

2.2.1. Pure samples

Flutamide sample was purchased from Sigma–Aldrich chemie GmbH., Germany, its purity was found to be 99.25%.

2.2.2. Pharmaceutical formulation

Cytomed® tablets is labeled to contain 250 mg flutamide and manufactured by CIPLA Ltd. India.

2.2.3. Chemicals and reagents

All chemicals and reagents used throughout this work were of analytical grade and were used without further purification.

Methanol and glacial acetic acid [(Sigma–Aldrich, Chromasolv®, Germany).

Sodium hydroxide (0.05 N, 0.1 N and 0.2 N aqueous solution), HCl (0.3 N, 0.5 N and 1 N aqueous solution), hexane, ethylacetate (El-Nasr Pharmaceutical Chemicals Co., Abu-Zabaal, Cairo, Egypt).

2.3. Preparation of flutamide degradation product

0.2 g of flutamide was dissolved into 10 mL methanol and then refluxed at 80 °C with 40 mL of either 1 N HCl or 0.1 N NaOH or 30% hydrogen peroxide for 3 h. The drug solution was also left for 4 h in direct sunlight. The degradation process was followed by TLC using a developing system consisting of hexane:ethyl acetate:glacial acetic acid (8:3:0.1, by volume). After complete degradation, the produced yellow solution was extracted with chloroform (3×10 mL) and the extracted degradate was dried at 60 °C and then identified by IR and mass spectroscopic analyses.

2.4. Solutions

2.4.1. Stock solutions of flutamide and degradate

(1 mg/mL) were prepared by accurately weighing 0.1 g flutamide and the prepared degradate in two separate 100-mL volumetric flasks and dissolving in methanol.

2.4.2. Working solution of flutamide and degradate

(0.1 mg/mL) were prepared by transferring 10 mL from their respective stock solutions (1 mg/mL) into two separate 100-mL volumetric flasks and then diluting with methanol.

2.4.3. For kinetic investigations, standard solutions of flutamide

(2.9×10^{-3} and 4.3×10^{-3} M) were prepared by dissolving the corresponding amount of flutamide in 10 mL methanol and then completing the volume to 25-mL with either HCl (0.3, 0.5 or 1 N) or NaOH (0.05, 0.1 or 0.2 N).

2.5. Preparation of laboratory prepared mixtures

Different aliquots of flutamide and its degradation product working solutions (0.1 mg/mL) were transferred to a set of 10-mL volumetric flasks and the volume was completed with methanol to prepare laboratory prepared mixtures containing from 5% to 90% of the degradation product.

2.6. Application to pharmaceutical formulation

Ten tablets of Cytomed® were grinded well and then an accurately weighted amount was transferred into 100-mL volumetric flask. 75 mL methanol was added and the solution was ultrasonicated for 30 min, filtered and then the volume was completed with the same solvent to prepare sample stock solution (1 mg/mL). Sample working solution (0.1 mg/mL) was then prepared.

2.7. Procedure

2.7.1. Spectral characteristics of flutamide and its degradation product

Zero order absorption spectra of 10 μg/mL each of flutamide and its degradation product were recorded in the range of 200–450 nm using methanol as a blank.

2.7.2. Construction of calibration curves

Concentrations of flutamide in the range of 1–35 μg/mL and of the degradation product in the range of 0.5–20 μg/mL were