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Multi-technique approach for qualitative and quantitative characterization of furazidin degradation kinetics under alkaline conditions

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ABSTRACT

Degradation of drug furazidin was studied under different conditions of environmental pH (11–13) and temperature (30–60 °C). The novel approach of hybrid hard- and soft-multivariate curve resolution-alternating least squares (HS-MCR-ALS) method was applied to UV–vis spectral data to determine a valid kinetic model and kinetic parameters of the degradation process. The system was found to be comprised of three main species and best characterized by two consecutive first-order reactions. Furazidin degradation rate was found to be highly dependent on the applied environmental conditions, showing more prominent differences between both degradation steps towards higher pH and temperature. Complimentary qualitative analysis of the degradation process was carried out using HPLC-DAD-TOF-MS. Based on the obtained chromatographic and mass spectrometric results, as well as additional computational analysis of the species (theoretical UV–vis spectra calculations utilizing TD-DFT methodology), the operating degradation mechanism was proposed to include formation of a 5-hydroxyfuran derivative, followed by complete hydrolysis of furazidin hydantoin ring.

1. Introduction

Chemical stability of API molecules is a matter of a great concern as it affects the safety, efficiency and purity of the drug product [1]. The International Conference on Harmonization (ICH) guidelines require to carry out stress tests to understand how the quality of the drug substance changes with time under influence of various environmental factors – temperature, relative humidity, pH, light radiation and others [2]. These tests also aid to describe the nature of the degradation products and the operating degradation mechanisms, and reveal useful information necessary for improving manufacturing processes, validating expiration dates and selecting proper packagings [3,4].

For drugs sparingly soluble in aqueous medium, stress testing at various environmental pH conditions is particularly important. During API manufacturing, adjustment of pH is a widely accepted strategy for improving drug solubility [5]. However, this can also lead to API degradation, typically through hydrolysis, and depending on the environmental pH, different degradation products may even form [6].

Nowadays many different analytical methods are used to investigate the drug degradation process. The spectrophotometric methods, however, are still favored due to the ease of spectral data acquisition, handling and interpretation. In addition, they are highly sensitive and thus very suitable for studying chemical reactions in solutions [7]. Other significant advantages for spectrophotometric methods include amped selectivity (simultaneous measurement and evaluation of the absorbance with the reaction time) and possibility to avoid interference from colored and opaque samples [8].

Alongside spectrophotometric techniques, chromatographic analysis combined with a high-resolution mass spectrometry has long served as an industry standard for insuring the quality of the manufactured drug product [9]. Time-of-flight mass spectrometry (TOF-MS), in particular, is commonly selected for stress testing purposes, as it is known to be a very reliable and accurate technique, to determine the exact chemical structure of an unknown species.

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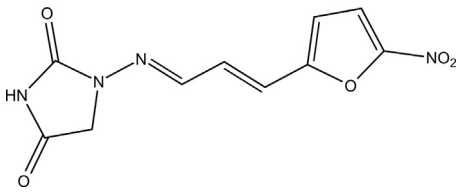

Fig. 1. Chemical structure of furazidin (FUR).

Additionally, with advances in computing power, computational chemistry techniques are becoming more appealing for characterizing chemical entities, including speculative degradation products. Although, the descriptive accuracy of these methods can often be challenged, they can still provide relevant data to support results obtained by experimental studies.

Furazidin (FUR, Fig. 1), also known under generic name furagin ($C_{10}H_8N_4O_5$, 1-[(*E*)-[(*E*)-3-(5-nitrofuran-2-yl)prop-2-enylidene]amino]imidazolidine-2,4-dione) belongs to a group of 5-nitrofuran derivatives, that are well known and widely used due to their exceptional antibacterial properties [10]. Furazidin is commonly used in the treatment of acute and chronic urinary tract infections (among others in prostatitis and cystitis) and in long-term prevention the recurrence of these infections [11]. However, due to the limited solubility, only furazidin potassium, sodium, calcium and magnesium salts are currently used in pharmaceutical practice. The synthesis of a particular salt is usually carried out under alkaline conditions by using according metal hydroxide aqueous solution as a reaction medium.

To the best of our knowledge, the degradation process of furazidin has not been previously studied. The degradation mechanism was investigated using HPLC-DAD-TOF-MS (complimented by computational analysis) and was proposed to include the formation of 5-hydroxyfuran derivative followed by hydrolysis of the hydantoin ring. Both pH (11–13) and temperature (30–60 °C) influence on the degradation process was evaluated by kinetic analysis of UV–vis data using novel hybrid hard- and soft-multivariate curve resolution-alternating least squares (HS-MCR-ALS) method.

2. Materials and methods

2.1. Chemicals

Furazidin (purity >99%) was provided by JSC Olainfarm (Olaine, Latvia) and used without further purification. Analytical grade potassium hydroxide was purchased from Enola Ltd. (Riga, Latvia). Deionized water (<0.1 μS) was freshly prepared by Adrona Crystal 5 water purification unit (Riga, Latvia). Eluents for HPLC-DAD analysis were water and acetonitrile (LiChrosolv® Hypergrade solvents for HPLC, >99.9%) purchased from Merck Millipore (Darmstadt, Germany).

2.2. Furazidin degradation in alkaline media

Twelve samples of furazidin (0.0010 g) were dissolved in 50 mL aliquots of 0.001, 0.01 and 0.1 M potassium hydroxide aqueous solutions and maintained at different temperatures – 30 °C, 40 °C, 50 °C and 60 °C. The pH of the prepared homogenous samples was specified using a Hanna Instruments HI1332B combined glass electrode (Woonsocket, RI, USA) and Adrona pH-meter AD 1405. Before measurements, the glass electrode was calibrated according to manufacturer instructions at 25 °C using three commercial reference solutions from Hanna Instruments – pH 4.01 ± 0.01, 7.01 ± 0.01 and 10.01 ± 0.01.

UV–vis absorption spectra were recorded on Shimadzu UV-2700 series spectrophotometer (Columbia, MD, USA) in the wavelength range between 215 and 550 nm using a 10 mm quartz cuvette (with a lid) for total of 20–1440 h (depending on the sample). Furazidin samples of 0.001 M and 0.01 M KOH solutions were kept at desired temperature using a Memmert Lab oven UFB 400 (Schwabach, Germany) and analyzed with at least 1 h interval. However, the samples prepared using 0.1 M KOH were monitored "in situ" and according temperature was maintained using Shimadzu TCC-100 Constant-Temperature Cell Holder accessory. The UV–vis data for these samples was collected every 20 min.

Furazidin sample of 0.01 M potassium hydroxide aqueous solution (maintained at 30 °C) was also analyzed by HPLC-DAD-TOF-MS upon preparation, as well as after 2, 5, 10, 20, 45, 80, 160, and 300 h.

2.3. High performance liquid chromatography – time of flight mass spectrometry (HPLC-DAD-TOF-MS)

An Agilent 1290 Infinity series instrument (Wilmington, DE, USA) equipped with an Agilent 1290 infinity DAD detector and Agilent 6320 TOF-MS mass-spectrometer was used for the HPLC-DAD-TOF-MS analysis.

Chromatographic separation was performed at 30 °C using a column oven, and Waters XTerra MS C18 column (2.1 × 150 mm, 3 μm) (Milford, MA, USA). The UV–vis spectra were recorded between 200 and 600 nm. The chromatographic profiles were registered at 268, 292, 306, 380 and 414 nm.

Analysis was performed with a gradient, and the mobile phase (98:2, v/v) was composed of Solvent A (HPLC grade water) and Solvent B (HPLC grade acetonitrile), gradually, within 8 min, it was changed to 5:95 and then maintained for additional 5 min. The injection volume was 15 μL and mobile phase flow rate was 0.3 mL/min.

Mass spectrometry operating conditions were as following: positive ionization mode, gas temperature of 325 °C, nitrogen flow rate of 10 L/min, nebulizer pressure 45 psi, capillary voltage 4000 V and applied fragmentor was 130 V.

Mass spectra were recorded across the range *m*/*z* 65–1500 with accurate mass measurement of all mass peaks. All the operation, acquisition and analysis of data were controlled by Agilent MassHunter® Workstation version B05.00. Accurate mass measurements of each peak from the total ion chromatogram (TIC) were obtained by means of an automated calibrate delivery system using ESI source that introduces a low flow (100 μL/min) of an Agilent calibrating solution A, which contains the internal reference masses at *m*/*z* 121.050873 and 922.009798.

2.4. Computational details

Theoretical absorption spectra of furazidin and our suggested alkaline degradation products were calculated using the time-dependent density functional theory (TD-DFT) methodology, which describes the excited states in terms of all possible single excitations from occupied to virtual orbital [12]. For this purpose, long range corrected CAM-B3LYP [13] functional with the 6-311++G basis set, augmented with polarization functions (d,p) was employed. The ground state geometries of all the species were first optimized in the gas phase with full relaxation on the potential energy surfaces at the same level of theory, and the resultant geometries were used as inputs for further calculations.

All computational calculations were executed using the Gaussian 09 software package [14] without any special adjustments of convergence criteria, atomic radii or other parameters which also take the default values. Visualization of the calculated results was performed using the GaussView software.