LC: Analysis of Photodegradation Kinetics of Nitazoxanide in Pharmaceutical Formulations

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Abstract

Nitazoxanide is a new broad-spectrum, antiparasitic drug agent. The photodegradation of nitazoxanide was studied in order to investigate the degradation kinetics of this drug. The analyses of the degraded samples were performed by a stability-indicating liquid chromatographic method. The degradation was carried out in acetonitrile with coated tablets or oral suspension powder in quartz cells under UVC light at 254 nm. The kinetics parameters, such as order of reaction, rate constants, half-life ($t_{1/2}$), and the time when 90% of the drug original concentration was left, were determined. The photodegradation of nitazoxanide for both pharmaceutical formulations in acetonitrile solution shows a zero-order kinetics under the applied experimental conditions. The obtained results confirm the reliability of the chromatographic method for determining the kinetics run of nitazoxanide in the presence of its degradation products. The present study reveals the photolability of the drug in solution. Thus, appropriated photoprotection is recommended during the manipulation of the drug.

Introduction

Nitazoxanide (NTZ, Figure 1) is a new broad-spectrum, antiparasitic drug agent. According to the World Health Organization, intestinal parasitic infections are among the most common infections disease in humans worldwide and are a significant cause of morbidity and mortality. NTZ is a new synthetic nitrothiazole benzamide compound (2-acetyloxyl-N-(5-nitro-2-thiazolyl) benzamide) notable for its activity in treating both intestinal protozoal and helminthic infections (1–3). It was first described in 1975 by Jean Francois Rossignol and was initially developed as a veterinary antihelminthic with activity against intestinal nematodes, cestodes, and trematodes. The U.S. Food and Drug Administration (FDA) has approved this drug in 2002 for treatment of diarrhea caused by cryptosporidium parvum and giardia lambia in children 1–11 years of age (1–3). NTZ was been reported to be effective against a broad range of parasites, including entamoeba histolytica, cryptosporidium parvum, giardia lamblia, trichomonas vaginalis, isospora belli, ascaris lumbricoides, taenia saginata, and taenia solium (3–5). Its precise mechanism of action is unknown, but studies have shown that NTZ inhibits pyruvate ferredoxin oxireductase (PFOR) enzyme-dependent electron transfer reactions that are essential to anaerobic energy metabolism in these organisms (1,3).

NTZ is currently available in coated tablets (500 mg) and powder for oral suspension (100 mg/5 mL). An official method for determination of this drug in oral formulation has not been described yet. However, there are studies describing the determination of NTZ and metabolites in biological fluids by liquid chromatography (LC) (6) and LC–mass spectrometry (MS) (7). Recently, visible spectrophotometrics (8), high-performance thin-layer chromatography (HPTLC) (9), and stability-indicating LC methods for the estimation of NTZ in bulk and pharmaceuticals formulations were developed (10–12).

Forced degradation studies (hydrolysis, oxidation, and photolysis) of NTZ pure active pharmaceutical ingredient have been reported (10–12). However, there is no photodegradation kinetics described in the literature for NTZ in pharmaceutical formulations.

The effect of light is often considered an important factor in drug stability. Stability problems increase in shorter light wavelengths, particularly in the short visible and ultraviolet (13–15). Preliminary stability investigations realized by our research group revealed that NTZ undergo degradation upon exposure to light, and its photolability was established by forced degradation testing.

The purpose of this study was to determine the protodegradation kinetics of NTZ in acetonitrile prepared from coated tablets and oral suspension powder using a stability-indicating LC method.

Experimental

Chemicals and reagents

NTZ used as a reference substance (assigned purity, 99.53%) was kindly supplied by Shin Yang–Hangzhou Shinyang Samwoo

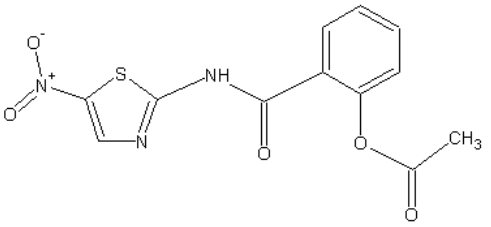

Figure 1. Chemical structure of nitazoxanide.

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Fine Chemical CO. (Ningbo, China). According to ICH guidelines (16) and US Pharmacopeia (17), the use of reference substances with checked purity for quality control is mandatory. The obtained NTZ standard was analyzed by analytical techniques such as: LC–MS, differential scanning calorimetric (DSC), infrared absorption spectroscopy, and ^{1}H and ^{13}C nuclear magnetic ressonance spectroscopy. No impurities were found.

Nixoran (Roemmers, Buenos Aires, Argentina) coated tablets for oral administration (500 mg per tablet, excipients: maize starch, pregelatinized starch, hydroxypropyl methylcellulose, sodium starch glycollate, talc, magnesium stearate, triacetin, iron oxide yellow, titanium dioxide, polyethylene glycol 6000) and Alinia (Romark Laboratories, Tampa, FL) powder for oral suspension (100 mg/5 mL, excipients: sodium benzoate, sucrose, xanthan gum, microcrystalline cellulose and carboxymethylcellulose sodium, anhydrous citric acid, sodium citrate dihydrate, acacia gum, sugar syrup, FD&C Red #40 and natural strawberry flavoring) were purchased. All chemicals were of pharmaceutical- or analytical-grade.

LC apparatus and conditions

The LC system consisted of a Shimadzu LC-10ADVP pump, an SPD-M10AVP diode array detector, a SCL-10AVP system controller, CTO-10ACVP column oven, SIL-10ADVP auto injector, and a degasser module DGU-14A. Data were acquired and processed by Shimadzu Class-VP V 6.14 software program (Kyoto, Japan). The LC method was previously developed and validated by our research group (12). The column utilized was a Phenomenex (Torrance, CA) Synergi Fusion C_{18} column (250 mm × 4.6 mm, i.d., 4 µm particle size) coupled to a C_{18} guard column (4.0 mm × 3.0 mm, i.d., 4 µm). The Shimadzu LC system was operated isocratically at 25°C using a mobile phase of *o*-phosphoric acid 0.1% (v/v) (pH 6.0 adjusted by addition of triethylamine)–acetonitrile (45:55, v/v) run at a flow rate of 1.0 mL/min and using PDA detection at 240 nm. The injection volume was 20 µL of a solution containing 20 µg/mL for the reference substance and for the samples. The quantitation was performed using the absolute area of the peak.

Photodegradation studies

The effect of light was studied exposing the pharmaceutical formulations solutions in 1 cm quartz cells. The light source was an UVC – 254 nm 30 W lamp (Philips, Amsterdam, Holland) fixed to a chamber in a horizontal position. The chamber was internally coated with mirrors (0.16 × 0.16 × 1 m – width × height × length) for distributing the light uniformly. The distance between the lamp and the samples was 10 cm. The temperature was controlled in the chamber.

Preparation of sample stock solutions

Twenty tablets were weighed and crushed to a fine powder. An accurately weighed amount of tablet powder or oral suspension powder equivalent to 20 mg of NTZ was transferred to a 100-mL volumetric flask with 50 mL of acetonitrile and sonicated for 10 min, followed by the addition of the same solvent to complete the volume in order to give a final concentration of 200 µg/mL. The solutions were filtered using 0.45-µm nylon membranes.

Kinetics determination

The photodegradation kinetics of NTZ in pharmaceutical formulations was evaluated in acetonitrile under the same conditions described earlier. Stock solutions of each pharmaceutical formulation were prepared, and the stress degradation study was performed in the chamber exposing closed quartz cells containing the solutions. The samples were positioned horizontally to provide maximum area of exposure to the light source. The pharmaceutical formulations solutions were exposed to the UVC irradiation in the following time intervals: 0, 60, 120, 150, 180, 210, 240, and 270 min. Three samples were analyzed for each time interval. The influence of the temperature was evaluated through analyzed protected samples, wrapped in aluminum foil, which were the dark controls. After the required time, aliquots of 1 mL were transferred to a 10-mL volumetric flask and diluted with acetonitrile to 20 µg/mL for the LC determination. The standard solution was prepared in acetonitrile at the concentration of 20 µg/mL for the quantitation of the drug. Placebo solutions were prepared at the same way for both pharmaceutical formulations to verify the influence of the excipients in the degradation process. All solutions were injected in triplicate. Peak purity tests were performed by the photodiode array

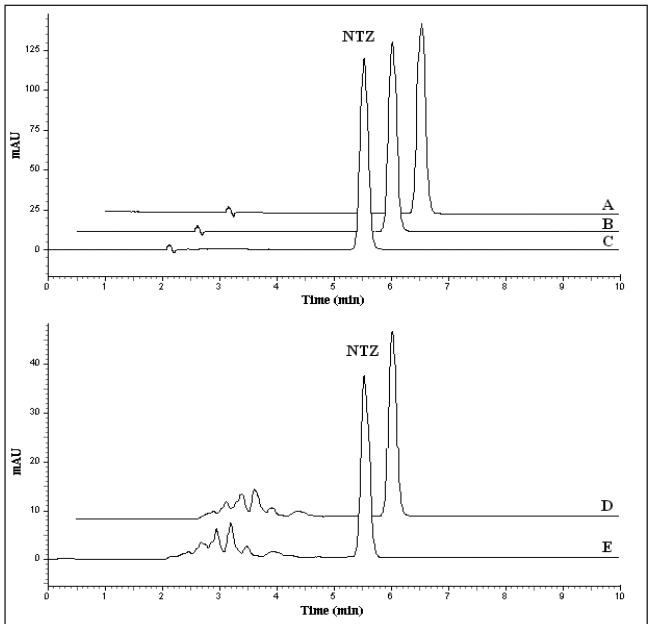

Figure 2. Typical chromatogram for 20.0 µg/mL nitazoxanide standard solution (A); Chromatograms showing the nitazoxanide intact peak for oral suspension powder (B) and coated tablets (C) during the time 0 (zero). The degradation after 270 min of light exposure for oral suspension powder (D) and coated tablets (E) in acetonitrile solution.

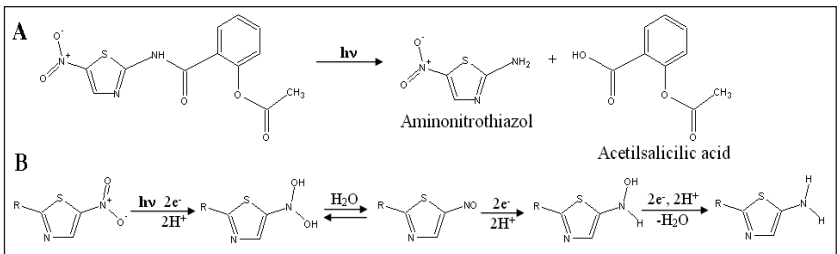

Figure 3. The suggested mechanism of amide degradation (A) and reductive nitro compounds (B) by photo irradiation process (254 nm).

detector, and it was useful to show that the analyte chromatographic peak did not contain more than one substance.

The concentration of the remaining NTZ determined at the different time intervals were used in the plots. The plots were: values of concentration versus time (zero-order reaction) (a), log of concentration versus time (first-order reaction)(b), and reciprocal of concentration versus time (second-order reaction)(c). The regression coefficients (r) were obtained, and the best fit observed indicates the reaction order. The kinetics parameters like apparent order degradation rate constant (k), half-life ($t_{1/2}$) and t_{90} (time where 90% of original concentration of the drug is left) were obtained. The kinetics models can be represented as:

Zero-order reaction:		
$C = C_0 - kt$	$t_{90\%} = 0.1C_0 / k$	$t_{1/2} = C_0 / 2k$
First-order reaction:		
$\ln C = \ln C_0 - kt$	$t_{90\%} = 0.106 / k$	$t_{1/2} = \ln 2 / k$
Second-order reaction:		
$1 / C = 1 / C_0 + kt$	$t_{90\%} = 1 / 9kC_0$	$t_{1/2} = 1 / kC_0$

where C_0 is the concentration of the reactants under consideration at time zero, C is the concentration after reaction time (t) and k is the reaction rate constant.

Results and Discussion

The purpose of photostability testing is to provide evidence on how the quality of a drug varies with the time under the influence of the light. In this study, the photodecomposition of NTZ was carried out through the employment of a stress condition during different periods of time. Many factors can affect the stability of a pharmaceutical product, such as the stability of the active ingredient, the manufacturing process, the environmental conditions (heat, light, and moisture during storage), as well as some chemical reactions like oxidation, reduction, hydrolysis, and racemization that might occur (13–15,18). Photochemical degradation is an important factor of the pharmaceuticals stability. Because UV radiation has high energy, it can be the cause of many degradation reactions (13–15). Usually, the photodegradation of a chemical compound is caused by oxidation or by the breakdown of certain weak chemical bond. Both phenomena are energy related, and consequently, they are be preceded by photons with specified wavelengths. On the other hand, the rate of photodegradation reaction is dependent on light intensity, so higher light intensity provides faster reaction rates. The presence of chromophores groups in the chemical structure of NTZ, such as conjugated double bonds, nitro and amide, suggests that NTZ is susceptible to UV radiation (254 nm). The International Conference on Harmonization (ICH) guideline requires that stress testing should be carried out to elucidate the inherent stability characteristics of the active substance in a pharmaceutical preparation. The light testing should be an integral part of stress testing (19,20).

LC has become the most used method in drug analysis due to its advantage in being a separating tool, and it is appropriate for stability studies. The stability-indicating LC method was previously developed and validated for NTZ quantitation in pharmaceutical formulations, and it was applied in this study (12).

The NTZ photodegradation profile was evaluated at different time intervals under the same conditions described previously. Approximately 67% and 72% of drug degradation were observed during 270 min of light exposure for tablets and oral suspension powder solutions in acetonitrile. The solutions developed a slightly yellow color that decreased along the exposure time until it became colorless. The temperature, which was controlled in the chamber, was maintained below 25ºC. Figure 2 shows the modification in the samples chromatographic profile after 270 min comparing to time 0 (zero). Five majority photodegradation products eluated at 2.64, 2.95, 3.20, 3.45, and 3.93 min, resulting from degradation of each pharmaceutical formulation solution in acetonitrile. The shorter retention times of the photodegradation products compared to the NTZ retention time are due to more polar features of the degradation products. The presence of chromophores groups in the chemical structure of NTZ, such as nitro and amide, suggests the formation of some products (Figure 3), but the identification of these products was not performed yet. The isolation of those degradation products are been avaliated. Broekhuysen et al. (7) observed the formation of aminonitrothiazole as a drug metabolite in human plasma.

The placebo chromatograms solutions didn't show any peak in the retention time of NTZ nor in the photodegradation products. So, there is no influence of the excipients in the determination of the photodegradation kinetics of this drug. The software chromatographic peak purity tool was applied to verify NTZ peak, showing that it was 100% pure in all cases. Thus, it was suggested that there was no interference of any other substance in the retention time of the drug.

The photodegradation kinetics was calculed for both pharmaceutical formulations through the decrease in drug concentration by time. The concentration, log, and reciprocal concentration values of the remaining drug versus time are

Table I. Photodegradation Kinetics of NTZ Pharmaceutical Formulations Solutions Exposed to UVC Lamp

Time (min)	Concentration* RSD (%), µg/mL	Log of Concentration	1/Concentration
Dosage form (Tablets)			
0	98.60, 1.6	19.39	1.2877	0.05156
60	84.27, 0.8	16.58	1.2195	0.06033
120	69.91, 1.4	13.75	1.1383	0.07272
150	60.81, 0.3	11.96	1.0778	0.08360
180	54.37, 2.6	10.69	1.0291	0.09351
210	45.76, 2.1	9.00	0.9543	0.11109
240	40.28, 1.1	7.92	0.8989	0.12622
270	33.14, 2.8	6.52	0.8141	0.15342
Dosage form (Powder for oral suspension)			
0	98.92, 0.6	19.78	1.2963	0.05055
60	83.11, 1.0	16.62	1.2207	0.06016
120	68.06, 0.9	13.61	1.1339	0.07346
150	54.69, 0.8	10.94	1.0389	0.09143
180	50.67, 1.8	10.13	1.0058	0.09867
210	42.38, 2.5	8.48	0.9281	0.11799
240	34.63, 2.6	6.93	0.8405	0.14439
270	27.76, 2.3	5.55	0.7444	0.18015

* Data expressed as mean of three determinations.

shown in Table I. The NTZ concentration remaining was calculated at each time interval for the three replicates and compared to mean drug concentration of the standard solution. The concentration, log, and reciprocal concentration plots of remaining drug versus time obtained during the kinetic studies are shown in Figure 4. According to the evaluation of the correlation coefficients, it can be concluded that the NTZ photodegradation for both pharmaceutical formulations in acetonitrile solution show a zero-order kinetics under the experimental conditions applied. After analyzing the straight lines slopes, it was possible to calculate the apparent zero-order degradation rate constant k, $t_{1/2}$, and t_{90} at each pharmaceutical formulation in acetonitrile solutions tested (Table II).

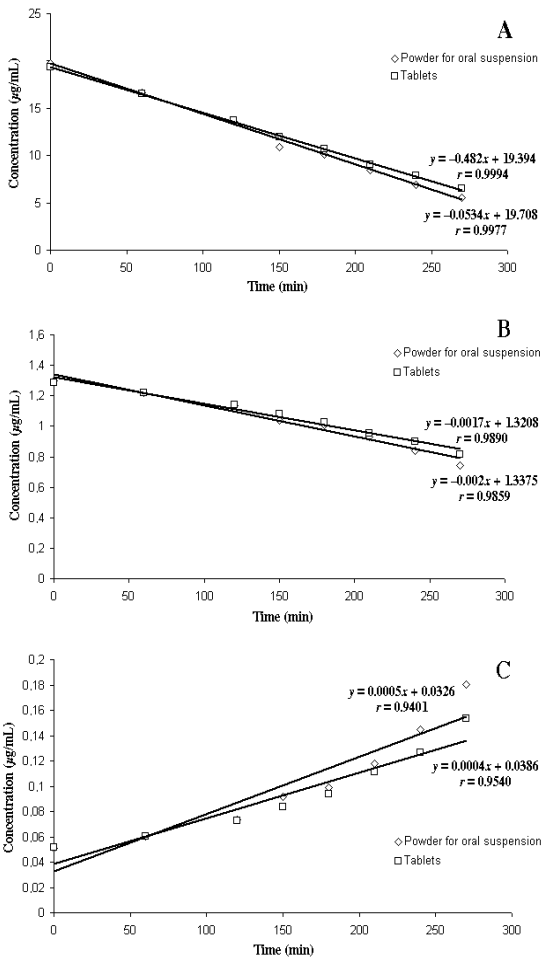

Figure 4. Plots of concentratiion [zero-order reaction (A)], log of concentration [first-order reaction (B)] and reciprocal of concentration [second-order reaction (C)] of remaining nitazoxanide versus time.

Table II. Degradation Rate Constant (k), Half-life ($t_{1/2}$), and t_{90} for NTZ in Pharmaceutical Formulations Solutions Submitted to Photodegradation and Determined by LC Method

Dosage forms	k/min	$t_{1/2}$ (min)	t_{90} (min)
Tablets	4.81×10^{-2}	201.56	40.30
Powder for oral suspension	5.38×10^{-2}	183.83	36.75

Conclusions

The NTZ photodegradation kinetics studies in acetonitrile solutions were determined in the tablets and oral powder suspension. The degradation of this drug during photodegradation follows zero-order reaction kinetics for both pharmaceutical formulations. The kinetics parameters of degradation rate constant, $t_{1/2}$, and t_{90} can be predicted. NTZ photolability showed by the present study indicates that it is necessary to avoid exposure of the drug from light effects. So, special care must be taken during the preparation, manufacture, and storage of this pharmaceutical drug.

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