Arabian Journal of Chemistry (2012) **xxx**, xxx–xxx

King Saud University

Arabian Journal of Chemistry

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ORIGINAL ARTICLE

Simultaneous determination of Nitazoxanide and Ofloxacin in pharmaceutical preparations using UV-spectrophotometric and high performance thin layer chromatography methods

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Received 4 October 2011; accepted 14 July 2012

KEYWORDS

Nitazoxanide;
Ofloxacin;
HPTLC;
Spectrophotometric method;
ICH

Abstract Simple, precise, and accurate UV-Spectrophotometric and high-performance thin-layer chromatography (HPTLC) methods for the simultaneous determination of Nitazoxanide and Ofloxacin in pharmaceutical preparations have been developed and validated. The method was developed using aluminum plates pre-coated with silica gel 60 F_{254} HPTLC plates as a stationary phase with toluene:chloroform:carbon tetra chloride:toluene:glacial acetic acid solutions in the proportion of (10:5:3:0.5 v/v/v/v) as mobile phase. Densitometric quantification was performed at 241 nm. Well-resolved bands were obtained with R_F values 0.36, 0.57 and 0.63 for Rosiglitazone maleate, Nitazoxanide, and Ofloxacin, respectively. Rosiglitazone maleate was used as an internal standard. The calibration curves were linear within the concentration range of 5–25 μg/ml for each drug. Two simple spectrophotometric methods have been developed for simultaneous estimation of Nitazoxanide, and Ofloxacin from tablet dosage form. The first method, simultaneous equation method, involves the measurement of absorbances at two wavelengths 221.8 nm (λ_{max} of Nitazoxanide) and 244.3 nm (λ_{max} of Ofloxacin), and the second method is First order derivative spectroscopy, wavelengths selected for quantitation were 263.6 nm for Nitazoxanide and 269.2 nm for Ofloxacin. The proposed method gave good validation results and the statistical analysis performed proved that the method is precise, accurate and reproducible, and hence can be employed for routine analysis of Nitazoxanide and Ofloxacin in bulk and commercial formulations.

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Peer review under responsibility of King Saud University.

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1. Introduction

Ofloxacin (OFL) is chemically 9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-Oxo-7*H*-pyrido (1,2,3-di)-1,4-benzoxazine carboxylic acid. It is a fluoroquinolone derivative. It is used mainly as an antibacterial. It is official in the United

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http://dx.doi.org/10.1016/j.arabjc.2012.07.009

State Pharmacopoeia, 2006. Literature survey reveals that spectrophotometric, HPLC, RP-HPLC, and HPTLC (Rane et al., 2008; Kasture et al., 2004; Gopu et al., 2007; Kraas and Hirrle, 1986; Gandhimathi et al., 2006) methods are available for the determination of Ofloxacin from pharmaceutical preparations and biological formulation. Nitazoxanide (NT), N-(5-nitro-2-thiazolyl) salicylamide acetate (O'Neil, 2001; Reynolds, 2002) is a Nitrothiazole derivative. Its chemical structure is related to metronidazole. It is a broad spectrum antiprotozoal. Nitazoxanide is an antiamebic and anthelmintic agent. It is indicated for amebiasis, helminthiasis, giardiasis, fascioliasis, trichomoniasis and cryptosporidiosis, including those with AIDS or HIV infections (Yoshimasa et al., 1996; Cavier, 1978). Literature survey reveals that, spectrophotometric and RP-HPLC (Kapse et al., 2006; Narayana et al., 2006) methods are available for the estimation of Nitazoxanide in single dosage form. A survey of the literature reveals of these combination that a variety of spectrophotometric and chromatographic methods (Singh et al., 2011; Game and Sakarkar, 2011; Lalitha et al., 2009; Mahaparale et al., 2009). An Ofloxacin and Nitazoxanide combination is indicated to have antibacterial and antiprotozoal activities. The combination of Nitazoxanide and Ofloxacin is antiparasitic and antibacterial which is effective against a wide variety of protozoa, helminthes and Gram-negative organisms. Oral bioavailability is good and well tolerated, with mild gastrointestinal side effects. Used in Giardia intestinal is induced diarrhea in patients (Guerrant et al., 2005). A combination of 200 mg of ofloxacin and 500 mg of Nitazoxanide is available commercially as tablets (Nitazete-O). The aim of this paper was to explore the possibility of techniques of simultaneous estimation using UV spectrophotometric, first derivative and HPTLC methods for quantifying Ofloxacin and Nitazoxanide simultaneously in their mixture forms. The proposed methods are simple, convenient, precise, accurate, and economical than the reported method. All chemicals and reagents used are of analytical grade and were purchased from Merck Chemicals, India. All dilutions were performed in standard volumetric flasks. Pure and tablet dosage form, Nitazete-O (claim: 500 mg NT and 200 mgOF) was procured from the local market (see. Fig. 1).

2. Experimental

2.1. Instrumentation and chromatographic conditions

Chromatography was performed on 10 cm × 10 cm precoated silica gel 60 F_{254} HPTLC plates. The chromatographic plates were prewashed with methanol and dried in an oven at 120 °C for 2 h before use. The samples were applied onto the plates as a band with 6 mm width using Camag 100 µl sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland). Linear ascending development was carried out in a twin trough glass chamber (for 10 × 10 cm). Densitometric scanning was performed using Camag TLC scanner 3 in the range of 400–1500 ng/spot and operated by winCATS software (V 1.4.2, Camag). The chromatography estimation was performed using the following conditions: stationary phase was precoated with silica gel 60 F_{254} aluminum sheets and the mobile phase used was chloroform:carbon tetra chloride:toluene:glacial acetic acid (10:5:3:0.5 v/v). The source of radiation was a deuterium lamp. Slit dimensions were

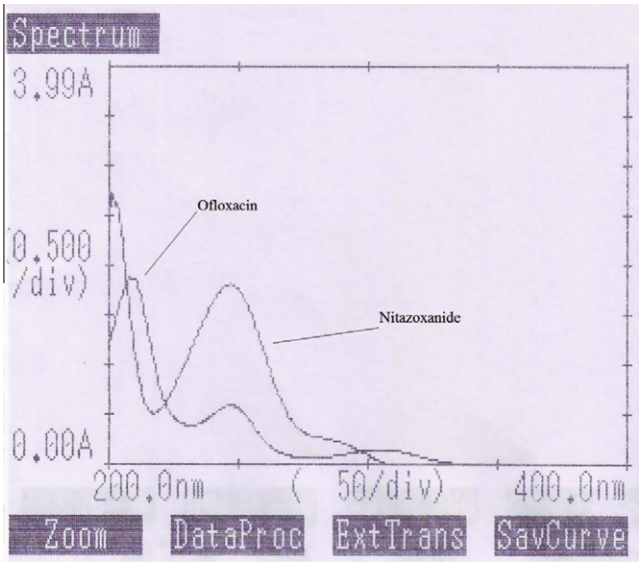

Figure 1 Overlain spectra Nitazoxanide and Ofloxacin.

6 mm × 0.45 mm and the scanning speed 20 mm/s. Plates were evaluated densitometrically at 289 nm with a CAMAG Scanner III, in conjunction with the winCATS software for quantitation.

2.2. Preparation of standard and sample solution of Nitazoxanide and Ofloxacin

Accurately weighed 50 mg of Nitazoxanide was taken and transferred to a 10 ml volumetric flask, dissolved in methanol and diluted up to mark to obtain stock solution of 5000 µg/ml. From this solution, 0.5 ml was diluted to 20 ml to get 2500 µg/ml standard Nitazoxanide solutions. Accurately about 10 mg of Ofloxacin was weighed and transferred to a 10 ml amber colored volumetric flask and dissolved in methanol and volume was made up to mark to get stock solution of 1000 µg/ml. From this solution, 0.75 ml was further diluted to 10 ml to get 75 µg/ml standard Ofloxacin solutions. Accurately weighed 5 mg of rosiglitazone maleate was taken in a 25.0 ml volumetric flask. This was dissolved in minimum quantity of acetonitrile and diluted up to the mark with acetonitrile to get 500 µg/ml of rosiglitazone maleate. Rosiglitazone maleate was used as an internal standard.

Twenty Nitazete-O tablets were weighed and finely powdered. An amount of the tablet powder equivalent to 500 mg Nitazoxanide and 200 mg of Ofloxacin was weighed and transferred into a 100.0 ml standard volumetric flask. Sixty milliliters of methanol was added to the flask and was sonicated for 30 min. The solution was then cooled to room temperature and diluted up to the mark with methanol. The resultant solution was filtered through Whatman Grade I filter paper. The filtrate was used as sample solution.

2.3. UV–vis spectrophotometric

UV–vis spectrophotometer 1601 (Shimadzu, Japan) with spectral bandwidth of 2 nm and 10 mm matched quartz cells was used. By appropriate dilution of two standard drug solutions with methanol, solutions containing 50 µg/ml of Nitazoxanide

Please cite this article in press as: Sharma, S. et al., Simultaneous determination of Nitazoxanide and Ofloxacin in pharmaceutical preparations using UV-spectrophotometric and high performance thin layer chromatography methods. Arabian Journal of Chemistry (2012), http://dx.doi.org/10.1016/j.arabjc.2012.07.009