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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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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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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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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

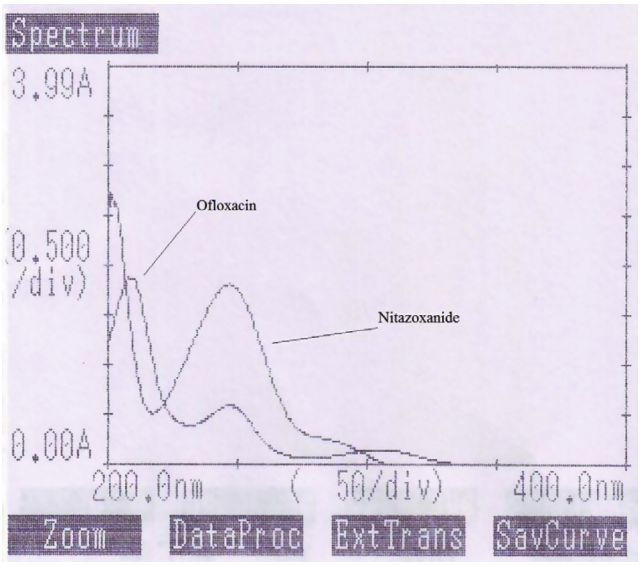

Figure 1 Overlain spectra Nitazoxanide and Ofloxacin.

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

and 20 μg/ml of Ofloxacin were scanned separately in the range of 200–400 nm to determine the wavelength of maximum absorption for both drugs. They were scanned in the wavelength range of 400–200 nm and the overlain spectrum was obtained. Two wavelengths 221.8 nm (λ_{max} of Nitazoxanide) and 244.3 nm (λ_{max} of Ofloxacin) were selected for the formation of simultaneous equation. The calibration curves were found to be linear in the concentration range of 5–25 μg/ml, for each drug. The absorptivity coefficients of each drug at both wavelengths were determined. The concentration of two drugs in the mixture was calculated using equations (Beckett and Stanlake, 1997).

$$C_{\text{Nitazoxanide}} = A_2 ay_1 - A_1 ay_2 / ax_2 ay_1 - ax_1 ay_2 \quad (1)$$

$$C_{\text{Ofloxacin}} = A_1 ax_2 - A_2 ax_2 / ax_2 ay_1 - ax_1 ay_2 \quad (2)$$

where, A_1 and A_2 are absorbance of mixture at 221.8 nm and 244.3 nm; ax_1 and ax_2, absorptivities of Nitazoxanide at 221.8 nm and 244.3 nm, respectively; ay_1 and ay_2 absorptivities of Ofloxacin at 266.8 nm and 244.3 nm, respectively. $C_{\text{Nitazoxanide}}$ and $C_{\text{Ofloxacin}}$ are concentration of Nitazoxanide and Ofloxacin in mixture. The absorptivities reported are the mean of six independent determinations.

In First order derivative spectroscopy method Nitazoxanide (50 μg/ml) and Ofloxacin (20 μg/ml), were prepared separately by appropriate dilution of standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. The absorption spectra thus obtained were derivatized from first to fourth order. First order derivative spectra were selected for the analysis of both drugs. From the overlain spectra of both drugs wavelengths selected for quantitation were 263.6 nm for Nitazoxanide (zero cross for Ofloxacin) and 269.2 nm for Ofloxacin (zero cross for Nitazoxanide). The calibration curves for Nitazoxanide and Ofloxacin were plotted in the concentration range of 5–50 μg/ml at wavelength 263.6 nm and 269.2 nm, respectively.

The methods were validated with respect to linearity, accuracy, precision and selectivity (ICH,1996). To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels 80%, 100% and 120%. The mean percent recovery for Nitazoxanide and Ofloxacin by all the three methods was found in the range of 97.66–101.11%. The linearity of measurement was evaluated by analyzing different concentrations of the standard solution of Nitazoxanide and Ofloxacin. For simultaneous equation and area under the curve method, the Beer–Lambert's concentration range was found to be 5–25 μg/ml and for derivative spectrophotometer 5–50 μg/ml for both Nitazoxanide and Ofloxacin. The reproducibility of the proposed method was determined by performing tablet assay at different time intervals (morning, afternoon and evening) on same day (Intraday assay precision) and on three different days (Interday precision). Result of intraday and interday precision is expressed in % RSD (Table 1). Percent RSD for Intraday assay precision was found to be 0.0783 (for Nitazoxanide) and 0.1863 (for Ofloxacin) in simultaneous equation method; 0.1392 (for Nitazoxanide) and 0.1174 (for Ofloxacin) in first derivative spectrophotometric method. Interday assay precision was found to be 0.263 (for Nitazoxanide) and 0.131 (for Ofloxacin) in simultaneous equation method; 0.029 (for Nitazoxanide) and 0.048 (for Ofloxacin) in first derivative spectrophotometric method (Table 2).

3. Results and discussion

The proposed method was validated for precision, accuracy, specificity, linearity and range, limit of detection (LOD) and limit of quantitation (LOQ), and robustness. Validation of the proposed method was carried out in accordance with the International Conference on Harmonization (ICH, 1996) guidelines. The linearity of the calibration plots was confirmed by the high value of the correlation coefficients (r^2 = 0.9991 for Nitazoxanide and 0.9997 for Ofloxacin). The assay of Nitazoxanide and Ofloxacin was found to be 99.96% and 100.11%. From the recovery studies it was found that about 100.36% and 100.27% of Nitazoxanide and Ofloxacin respectively which indicates high accuracy of the method (Table 3). The minimum amounts detected under the chromatographic conditions used were estimated in terms of the LOD and was found to be 5.3 and 12.32 ng band^{-1} for Nitazoxanide and Ofloxacin respectively which indicates the method has sufficient sensitivity. The LOQ was determined experimentally by spotting six replicates of each drug at the LOQ concentration. By area, LOQ was found to be 26 and 6.5 ng band^{-1} for Nitazoxanide and Ofloxacin, respectively. Repeatability studies were performed by the analysis of three different concentrations (500, 1000, 2500 ng band^{-1}) of the drug, each concentration injected six times on the same day. Intermediate precision of the method was checked by repeating studies on three different days. In

Table 1 Regression analysis of calibration graph for Nitazoxanide and Ofloxacin.

Parameter	UV method-I		UV method-II		HPTLC	
	NTO	OFL	NTO	OFL	NTO	OFL
Repeatability	0.874	0.577	0.681	0.317	0.451	0.654
Precision (a) Intra-day	2.65	1.63	0.954	0.215	1.45	0.974
(b) Inter-day	1.64	0.48	1.21	0.62	0.154	0.654
R_F (SD)	—	—	—	—	0.57	0.63
Linearity and range (ng/spot)	—	—	—	—	400–2500	400–1200
Linearity detection (ng/spot)	—	—	—	—	112	150
Limit of quantification (ng/spot)	—	—	—	—	164	217
% Accuracy ± SD[a] (n = 6) (%)	101.04	99.96	99.98	100.08	99.94	99.85
LOD	0.021	0.065	0.144	0.165	5.3	6.5
LOQ	0.012	0.041	0.095	0.0.81	26.00	12.32

NTO = Nitazoxanide; OFL = Ofloxacin.
[a] SD = Standard deviation.

Table 2 Results of analysis of tablet formulation.

Method	Tablet content	Label claim (mg/tab)	Amount Found[a]		±SD[a]	RSD (%)[a]
			(in mg)	(In%)		
UV-I	NTO	500	499.974	99.994	0.041	0.18
	OFL	200	199.053	99.931	0.035	0.051
UV-II	NTO	500	500.03	100.05	0.143	0.217
	OFL	200	199.31	99.986	0.087	0.328
HPTLC	NTO	500	499.941	99.941	0.198	0.054
	OFL	200	200.31	100.31	0.142	0.083

NTO = Nitazoxanide; OFL = Ofloxacin.
[a] Denotes $n = 6$, average of six determinations.

Table 3 Results of recovery studies.

Level of recovery (%)	Drug	HPTLC		UV-Method I		UV-Method II	
		Recovery (%)*	±SD*	Recovery (%)*	±SD*	Recovery (%)*	±SD*
80	NTO	100.36	0.043	99.95	0.033	100.07	0.0321
	OFL	100.27	0.154	99.97	0.0761	100.21	0.0543
100	NTO	100.01	0.049	100.04	0.0207	99.96	0.0612
	OFL	99.93	0.0654	100.06	0.0381	100.09	0.0431
120	NTO	99.97	0.044	100.08	0.0549	99.90	0.0543
	OFL	100.12	0.219	99.94	0.0087	99.82	0.0451

simultaneous equation method, wavelengths selected for quantitation were 221.8 nm (λ_{max} of Nitazoxanide) and 244.3 nm (λ_{max} of Ofloxacin). In first order derivative spectroscopy, wavelengths selected for quantitation were 263.6 nm for Nitazoxanide (zero cross for Ofloxacin) and 269.2 nm for Ofloxacin (zero cross for Nitazoxanide). For UV spectrophotometric method, linearity was obtained in the concentration range of 5–25 μg/ml, for both drugs; with regression 0.9994 and 0.9997, intercept −0.0854 and −0.0328 and slope 0.0188 and 0.0742 for Nitazoxanide and Ofloxacin, respectively. For UV Spectrophotometric method LOD for Nitazoxanide and Ofloxacin was found to be 0.021 and 0.065 μg/ml respectively. LOQ for Nitazoxanide and Ofloxacin was found to be 0.012 and 0.041 μg/ml respectively. These data show that microgram quantity of both drugs can be accurately determined. Robustness of the method when small changes in the mobile phase composition (±0.1 ml for each component) were made and the effects on the results were examined.

4. Conclusion

The proposed HPTLC method was validated as per ICH guidelines. The standard deviation, %RSD and standard error calculated for the method are low, indicating a high degree of precision of the methods. The results of the recovery studies performed show a high degree of accuracy of the proposed methods. The proposed method is highly accurate, selective and precise hence can be used for a routine quality-control analysis and quantitative simultaneous determination of Nitazoxanide and Ofloxacin in pharmaceutical preparations. Moreover, the proposed method has the advantages of simplicity, convenience and quantification of Nitazoxanide and Ofloxacin in combination and can be used for the assay of their dosage form.

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