International Journal of Research in Pharmacy and Pharmaceutical Sciences
ISSN: 2455-698X
Impact Factor: RJIF 5.22
www.pharmacyjournal.in
Volume 3; Issue 3; May 2018; Page No. 28-31

Simultaneous estimation of nitazoxanide and ofloxacin in tablet formulation by ratio spectra derivative spectroscopy

Dr. Santosh V Gandhi*, Parag L Khairnar, Aishwarya J Pate
AISSMS College of Pharmacy (Affiliated to Savitribai Phule Pune University), Kennedy Road, Near RTO, Pune, Maharashtra, India

Abstract
A simple, sensitive, rapid, accurate and precise method for simultaneous determination of Nitazoxanide and Ofloxacin in combined tablet dosage form has been developed. The method is based on ratio spectra derivative spectrophotometry. The amplitudes 269 nm and 236.5 nm in first derivative of the ratio spectra were selected to determine Nitazoxanide and Ofloxacin respectively in combined formulation. The developed method was showing linearity in concentration range of 5-30 µg/ml for Nitazoxanide and 2-12 µg/ml for Ofloxacin with correlation coefficient (R^2) 0.999 and 0.996; respectively. The percent assay was found to be 99.12 % and 100.69 % for Nitazoxanide and Ofloxacin, respectively. The method showed good linearity, precision and reproducibility. Results of analysis were validated statistically and by recovery studies.

Keywords: nitazoxanide, ofloxacin, ratio spectra derivative spectroscopy, simultaneous determination

Introduction

Nitazoxanide [NTZ] chemically N-(5-nitro-2-thiazolyl) salicylamide acetate [Fig. 1 (a)] is a synthetic nitrothiazole benzamide derivative. It is a broad spectrum antiprotozoal. It is indicated for amebiasis, helminthiasis, giardiasis, etc[1].

Ofloxacin [OFX] is 9-fluoro-2, 3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl) -7-oxo-7H-pyrido [1, 2, 3-de] [1, 4] benzoxazine-6-carboxylic acid [Fig. 1 (b)]. It is a synthetic antibiotic of the fluoroquinolone drug class considered to be a second-generation fluoroquinolone. It is used to treat certain infections including bronchitis, pneumonia and infections of the skin, bladder, urinary tract, reproductive organs, and prostate [2, 3].

Few methods are reported for quantitative determination of NTZ and OFX in single and in combination such as UV Spectrometry [3-5] and RP-HPLC [6-9].

Extensive literature survey revealed that no method available for simultaneous estimation of Nitazoxanide and Ofloxacin in combined dosage form by ratio spectra derivative spectroscopy. Aim of present work was to develop simple, economical, reproducible and rapid method for simultaneous estimation of binary drug formulation.

Theoretical aspects [10-11].

The method is based on dividing the spectrum for a mixture in to the standard spectra for each of the analyte and deriving the quotient to obtain a spectrum that is independent of the analyte concentration used as a divisor. The use of standardized spectra as a divisor minimizes the experimental error and background noise. An accurate choice of standard divisors and working wavelengths is the fundamental for several reasons. Easy measurement on separate peaks, higher values of the analyte signals and no need to work at zero crossing point (some time co-existing compounds have no maximum or minimum at these wavelengths) are advantages for ratio derivative spectrophotometry. Also the presence of a lot of minima and maxima in ratio spectra derivative data is another advantage, since these wavelengths give an opportunity for the determination of these compounds in presence of other active compound and excipients that possibly interfere with the assay. The method basically is dividing the absorption spectrum for a mixture in to the standardized spectra for each of the analytes. Derivatization of the obtained spectra gives derivative spectra that are independent of analyte concentration used as a divisor.

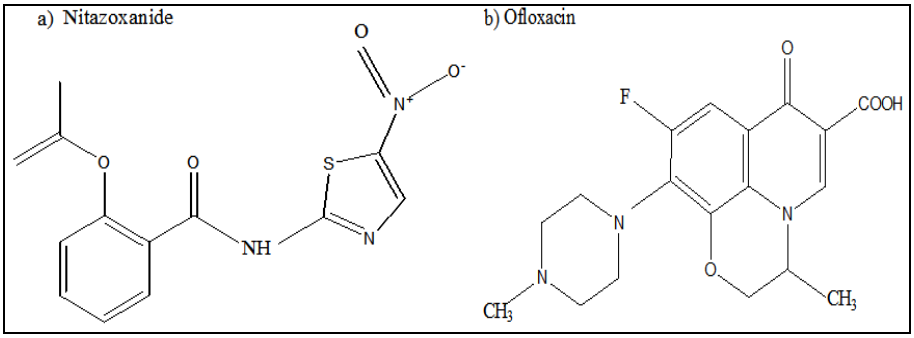

Fig 1: Structure of a) Nitazoxanide (NTZ) and b) Ofloxacin (OFX)

Materials and Methods

1. Instrumentation

Double beam UV- Vis spectrophotometer (Jasco V-730) with matched pair of 1cm quartz cells were used to record spectra of all solutions. The spectra were recorded at spectral band width of 2.0 nm, scanning speed 100 nm/min and data pitch 0.5 nm. Microsoft Excel 2007 were used.

2. Material and Reagents

Reference standard of NTZ and OFX were obtained from Wockhardt Research Centre, Aurangabad as gift samples and methanol (AR grade) purchased from LOBA Chemie, India. NIZONIDE-O tablets containing Nitazoxanide 500 mg and Ofloxacin IP 200 mg were procured from local pharmacy shop.

3. Preparation of standard stock solution

Stock solution of NTZ and OFX were prepared by dissolving accurately weighed 10 mg of standard drug in 10 ml of methanol, separately to get concentration 1000 µg/ml each. Further 5 ml was pipetted and diluted to 50 ml to achieve final concentration of 100 µg/ml of NTZ and OFX, seperately.

4. Preparation of working stock solution

Working standard solutions were prepared from standard stock solution of 100 µg/ml by appropriate dilution with methanol to obtain final concentration of 5, 10, 15, 20, 25 and 30 µg/ml for NTZ and 2, 4, 6, 8, 10 and 12 µg/ml for OFX.

Experimental

The method involves dividing the spectrum of mixture into the standardized spectra for each of the analyte and deriving the ratio to obtain spectra that is independent of analyte concentration used as a divisor. Using appropriate dilutions of standard stock solution the two solutions were scanned separately. The ratio spectrums of different NTZ standards at increasing concentrations are obtained by dividing each with the stored spectrum of the standard solution of OFX (2 µg/ml, Scaling factor 1) by computer aid are shown in Fig 2 (I) and the first derivative of these spectra traced with the interval of Δλ=21 nm (the influence of for the first derivative of the ratio spectra was tested to obtain the optimum wavelength interval, Δλ=21 nm was considered to be suitable) are illustrated in Fig 2 (II). Wavelength 269 nm was selected for the quantification of NTZ in NTZ + OFX mixture.

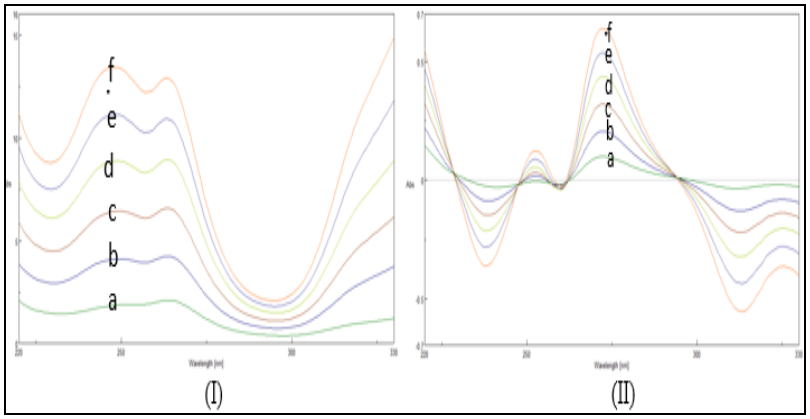

Fig 2: Ratio spectra (I) and first derivative of the ratio spectra (II) of (a) 5.0µg/ml, (b) 10.0µg/ml, (c) 15.0µg/ml, (d) 20.0µg/ml, (e) 25µg/ml and (f) 30.0 µg/ml solution of NTZ when 2µg/ml solution of OFX is used as divisor (Δλ=21nm)

The ratio and ratio derivative spectra of the solutions of OFX at different concentrations traced with the interval of Δλ=21nm by using the standard spectrum of NTZ (20 µg/ml, Scaling factor 2) as divisor by computer aid are demonstrated in Fig 3 (I) and (II), respectively. Wavelength 236.5 nm (maxima) was selected for the quantification of OFX in NTZ + OFX mixture.

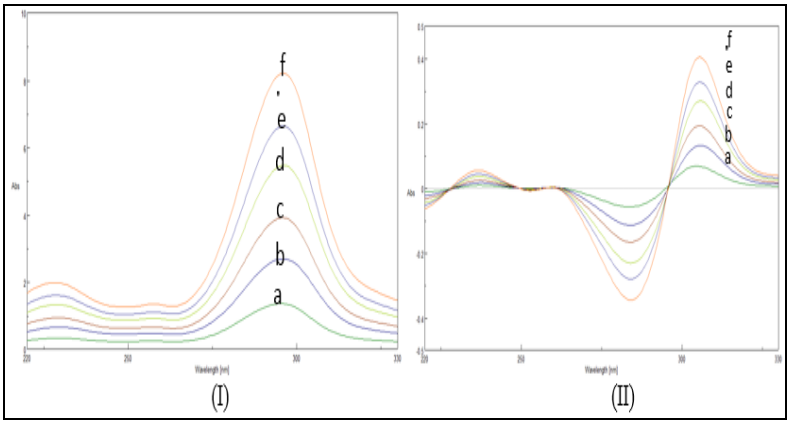

Fig 3: Ratio spectra (I) and first derivative of the ratio spectra (II) of (a) 2.0 µg/ml, (b) 4.0 µg/ml, (c) 6.0 µg/ml, (d) 8.0 µg/ml, (e) 10.0 µg/ml and (f) 12.0 µg/ml solution of OFX when 20 µg/ml solution of NTZ is used as divisor (Δλ=21nm)

Measured analytical signals at these wavelengths are proportional to the concentrations of the drugs. The coincident first derivative ratio spectra of pure and sample solution for estimation of NTZ and OFX are shown in the Fig 4.

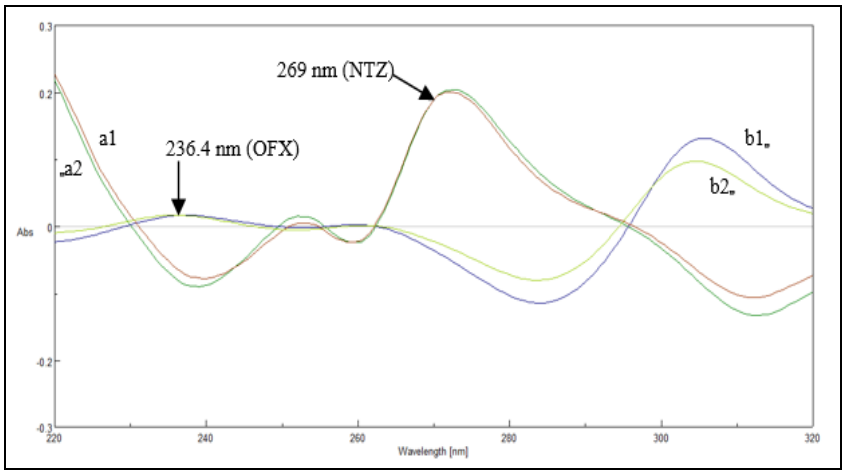

Fig 4: The coincident first derivative ratio spectra of (a1) 10 µg/ml of pure NTZ and (a2) sample solution (10 µg/ml of NTZ and 4 µg/ml of OFX); 2 µg/ml OFX as a divisor and (b1) 4 µg/ml of pure OFX and (b2) sample solution (10 µg/ml of NTZ and 4 µg/ml of OFX); 20 µg/ml of NTZ as a divisor

Results and Discussion

Under experimental conditions described, calibration curve, assay of tablets and recovery studies were performed. A critical evaluation of proposed method was performed by statistical analysis of data where slopes, intercept, correlation coefficient are shown in Table 1.

Table 1: Optical characteristics of the proposed method

Parameters	Nitazoxanide	Ofloxacin
λ_{max}	269 nm	236.5
Beer's law limit (µg/ml)	5-30 µg/ml	2-12 µg/ml
Molar Absorbtivity	1.733×10^{-1}	2.91×10^{-2}
Regression Equation (y = mx + c)	y=0.018632x-0.01131	y=0.004629x-0.00221
Slope (m)	0.018632	0.004629
Intercept (c)	-0.01131	-0.00221
Correlation Coefficient	0.999	0.996

Table 2: Results of analysis of commercial formulation

Drug	Label Claim (mg/tablet)	% of Label claim estimated	Standard Deviation	% RSD
NTZ	10	99.126	1.659	1.674
OFX	4	100.696	1.270	1.261

Results of analysis of commercial formulation are reported in Table 2. Low standard deviation values of determination indicate reproducibility of the method. Recovery studies were carried out by the addition of standard drug solution to preanalyzed tablet sample solution at three different concentration levels within the range of linearity for both the drugs. Results of recovery studies are shown in Table 3.

Table 3: Recovery studies of Nitazoxanide and Ofloxacin

Level of % Recovery	% Mean Recovery		Standard Deviation		% RSD	
	NTZ	OFX	NTZ	OFX	NTZ	OFX
50	100.576	100.057	0.992	1.569	0.986	1.568
100	99.279	99.697	0.811	1.848	0.817	1.854
150	101.142	99.215	0.279	0.769	0.276	0.776

Conclusion

The developed method was found to be simple, sensitive, accurate, precise and repeatable and can be used for routine analysis of Nitazoxanide and Ofloxacin in bulk and pharmaceutical dosage form without any interference from the excipients. The method was validated as per ICH guidelines. Statistical analysis proved that the method is repeatable for analysis of NTZ and OFX.

Acknowledgement

Authors are thankful to the Principal and Management, AISSMS College of Pharmacy, Pune for providing required facilities for research work.

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