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Spectrophotometric Methods for Simultaneous Determination of Nitazoxanide and Ofloxacin in Combined Bulk and Pharmaceutical Formulations

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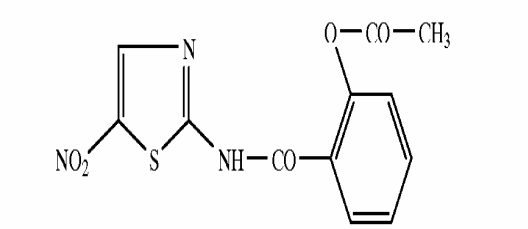
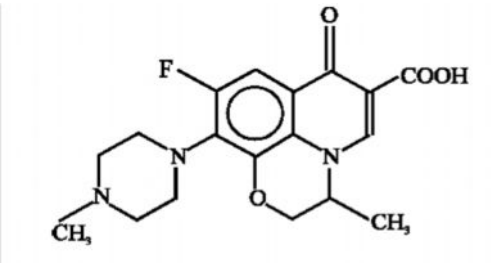
Abstract: The present investigation illustrates the simultaneous determination of nitazoxanide and ofloxacin using three simple, rapid, economical, accurate, precise and reproducible spectrophotometric methods, namely; vierodt's method, Q-analysis method and dual wavelength method. From a solvent effect studies and the spectral behaviours of nitazoxanide and ofloxacin, 1N HCl in methanol was selected as solvent. First method (vierodt's method) is based on the formation and solving of simultaneous equation at 346.361 nm (λ_{max} of nitazoxanide) and 296.496 nm (λ_{max} of ofloxacin). Second method (Q-analysis method) based on absorbance ratio at two selected wavelength, 307.520 (iso-absorption point) and 346.361 nm (λ_{max} of nitazoxanide). In third method (dual wavelength method) two wavelengths were selected for each drug in a way so that the difference in absorbance is zero for another drug. Nitazoxanide show equal absorbance at 333.6 nm and 359.2 nm, where the difference in absorbance were measured for the determination of ofloxacin, similarly difference in absorbance at 302.4 nm and 289.2 nm were measured for determination of nitazoxanide. The results of analysis were validated statistically. Recovery studies give satisfactory results indicating that none of common additives and excipients interfere the assay method. The proposed methods can be used successfully in the quality control of bulk forms, pharmaceutical formulations and routine laboratory analysis.
Keywords: Nitazoxanide, Ofloxacin, Vierodt's method, Q-analysis method, Dual wavelength method.

INTRODUCTION

The scope of developing and validating analytical method is to ensure a suitable method for a particular analyte more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation. Nitazoxanide [NTZ] is a synthetic nitrothiazole benzamide derivative, chemically it is N-(5-nitro-2-thiazolyl)salicylamide acetate[1-3]. Ofloxacin [OFL] is a fluorinated carboxyquinolone derivative is a racemate (±)-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[4]. Both the drugs are formulated in binary solid dosage form [combination 500 mg NTZ and 200 mg OFL in each tablet] as antiparasitic and antiprotozoal drug which is effective against a wide variety of protozoa, helminthes and gram-negative organisms. These combination also used in giardia intestinalis induced diarrhea in patients. In the process of development, fast and reliable analytical method is required for the simultaneous determination of both drugs in combined formulations[5].



(A)



(B)

Fig. 1: Chemical structure of nitazoxanide (A) and ofloxacin (B)

Extensive literature survey revealed that number of methods has been reported for estimation of NTZ individually[6-8] and OFL individually[9, 10] or in combination[11, 12] with other drugs. There are some complicated spectrophotometric[13] and HPLC[14, 15] methods have been reported for the simultaneous estimation of NTZ and OFL. However, there is no simple method for the simultaneous estimation of NTZ and OFL by UV-spectrophotometry using commonly available solvents.

The aim of present work was to develop simple, rapid, economical, accurate, precise and reproducible spectrophotometric methods for determination of drugs in combination. In the proposed methods no separation is required; the method is fast and convenient. The proposed methods were optimized and validated as per International Conference on Harmonization (ICH) guidelines[16].

EXPERIMENTAL[17-22]

Instrument:

PC based UV-Visible double beam spectrophotometer; model shimadzu 1700 with 1 cm quartz cells was used. All weighing were done on electronic balance (model GL-200, Precisa Limited).

Materials:

All the chemicals and reagents used were of analytical grade. Standard gift sample of nitazoxanide and ofloxacin was provided by Ind-Swift Labs, Chandigarh. Tablet formulations of combined form Nizonide-O and Netazox-OF (500 mg NTZ & 200 mg OFL /tablet) were procured from a local pharmacy.

Methods and Results:

Selection of solvent – In this work, different solvent were investigated to develop a suitable UV-spectrophotometric method for the analysis of NTZ and OFL in combined formulation. For selection of diluents, the criteria employed were the sensitivity of the method, the easiness of the sample preparation and the solubility of the drugs. 1 N HCl in methanol was used as a first diluent because of the total solubilization of the drugs in this diluent.

Preparation of standard solutions and study of spectra- Accurately weighed NTZ and OFL (10.00 mg) was transferred into 100 ml volumetric flask, volume made up to 100 ml by solvent. The final solution contained con of 100 µg/ml of the drug. The stock solutions were further diluted to get standard solutions of NTZ and OFL of the concentration 10µg/ml and 4µg/ml respectively. The resulting solutions were scanned in the range of 400-200 nm. NTZ show λ_{max} at 346.361 nm and OFL show λ_{max} at 296.496 nm. Hence these λ_{max} used for formation of simultaneous equations in vierodt's method. Whereas, 307.520 nm (iso-absorption point) and 346.361 nm (λ_{max} of NTZ) were selected as the wavelengths of determination for NTZ and OFL using Q-analysis method. Dual wavelength method two wavelengths were selected for each drug in a way so that the difference in absorbance is zero for another drug. NTZ show equal absorbance at 333.6 nm and 359.2 nm, where the difference in absorbance were measured for the determination of OFL, similarly difference in absorbance at 302.4 nm and 289.2 nm were measured for determination of NTZ. The both drugs obey beer's law in the concentration range 2-10 µg/ml.

Methods I- Vierodt's method

The absorptivity of NTZ and OFL were determined at two selected wavelengths. The absorptivity values of two drugs are used for framing the simultaneous equation. Set of two simultaneous equations for simultaneous estimation of NTZ and OFL using these absorptivity values are as:

$A_1 = 62.66 \times C_x + 29.21 \times C_y$ ---------- (1)

$A_2 = 156.66 \times C_x + 18.35 \times C_y$ ---------- (2)

Where,

C_x and C_y are concentrations of NTZ and OFL in g/1000 ml in the sample solution.

A_1 and A_2 are the absorbances of the mixture at 346.361 nm and 296.496 nm respectively.

The concentration of C_x and C_y can be calculated from the above-framed equations.

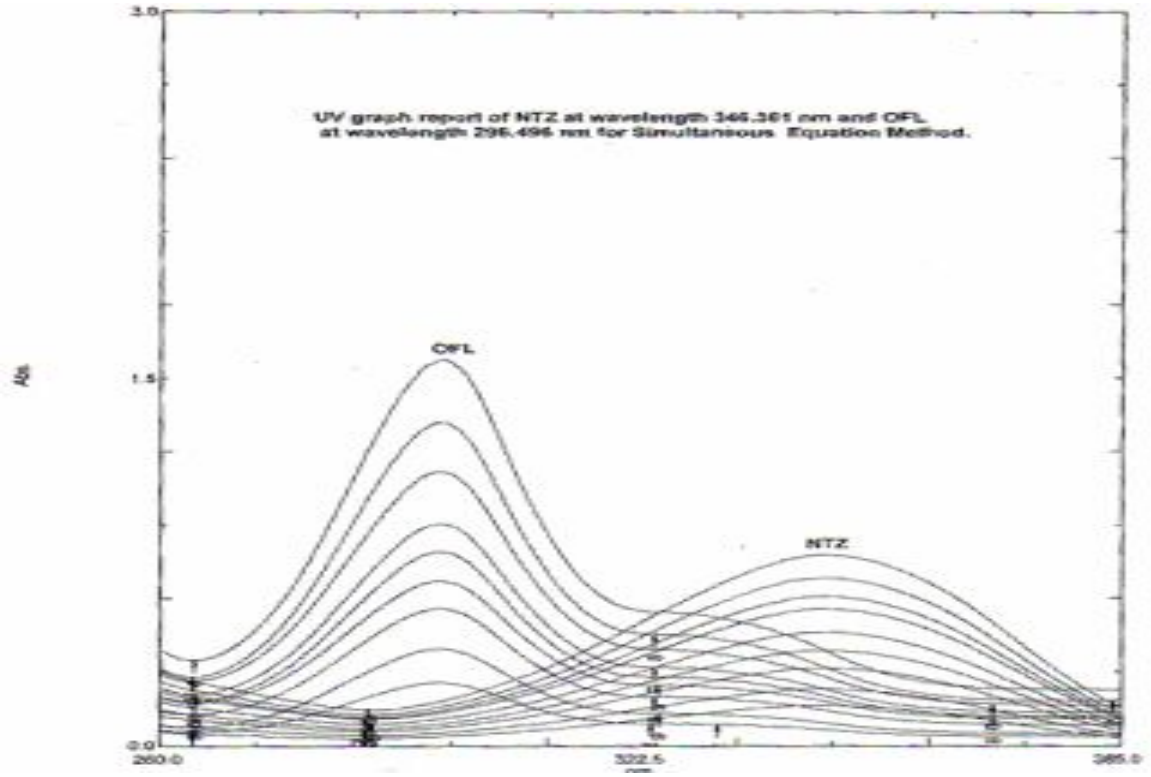


Fig. 2: Overlain spectra of NTZ and OFL for vierodt's method

Standardization of the vierodt's method by analysis of laboratory prepared samples:

To check the validity of above framed equations four mixed standards were prepared using pure sample of two drugs. The results of validation studies are reported in table 1.

Methods II- Q-analysis method

The absorptivity of NTZ and OFL were determined at two selected wavelengths. The absorptivity values of two drugs are used for framing the equation. Set of two equations for simultaneous estimation of NTZ and OFL using these absorptivity values are as:

$C_x = (Q_M - Q_Y) A_1 / (Q_X - Q_Y) a_{x1}$ ---------- (1)

$C_y = (A_1 – A_{x1}.C_x) / A_{y1}$---------- (2)

Where,

$Q_X = a_{x2} / a_{x1,}$

$Q_Y = a_{y2} / a_{y1,}$

$Q_M = A_2 / A_1$

a_{x1} and a_{x2} are absorptivity of NTZ at 307.520 nm and 346.361 nm, respectively.

a_{y1} and a_{y2} are absorptivity of OFL at 296.496 nm and 346.361 nm, respectively.

C_x and C_y are concentrations of nitazoxanide and ofloxacin in g/1000 ml in the sample solution.

Table 1: Results of validation studies of simultaneous equation method using mixed standards

S.No.	Concentration (µg/ml)		% concentration found	
	NTZ	OFL	NTZ	OFL
1	2	8	98.90	99.78
2	4	6	99.42	99.77
3	6	4	99.47	99.42
4	8	2	99.65	98.70

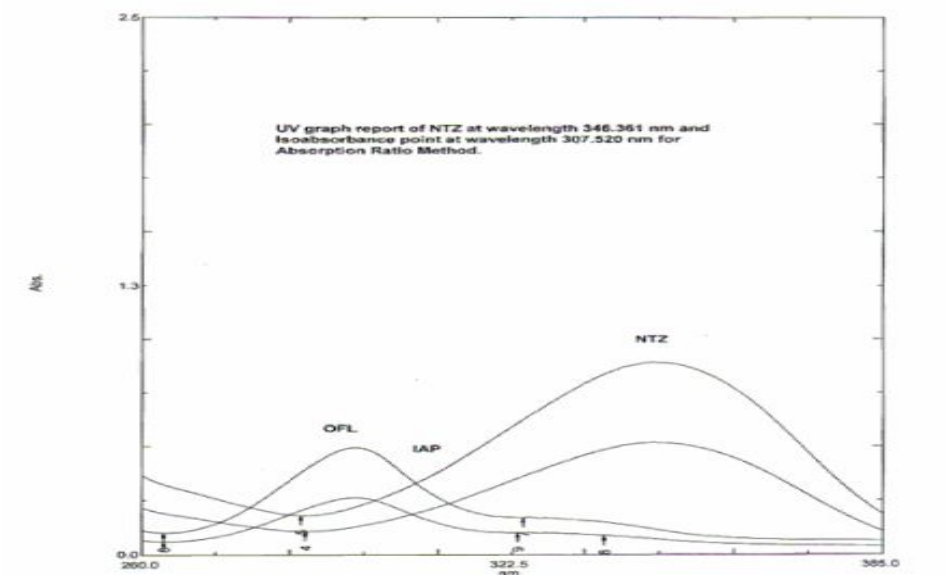


Fig. 3: Overlain spectra of NTZ and OFL for Q-analysis method

Standardization of the Q-analysis method by analysis of laboratory prepared samples:

To check the validity of above framed equations three mixed standards were prepared using pure sample of two drugs. The results of validation studies are reported in table 2.

Methods III- Dual wavelength method

It was observed that NTZ shows same absorbance at wavelength of 333.6 nm and 359.2 nm and OFL shows marked difference of absorbance at these two wavelengths, while OFL shows same absorbance at wavelength of 302.4 nm and 289.2 nm and NTZ shows marked difference of absorbance at these two wavelengths. Absorbance difference values were recorded at respective set of two wavelengths and calibration curve was plotted between concentration and absorbance difference values for both the drugs.

Table 2: Results of validation studies of Q-absorbance method using mixed standards

S.No.	Concentration (µg/ml)		% concentration found	
	NTZ	OFL	NTZ	OFL
1	3	7	99.65	98.70
2	5	5	98.29	100.00
3	7	3	99.88	100.00

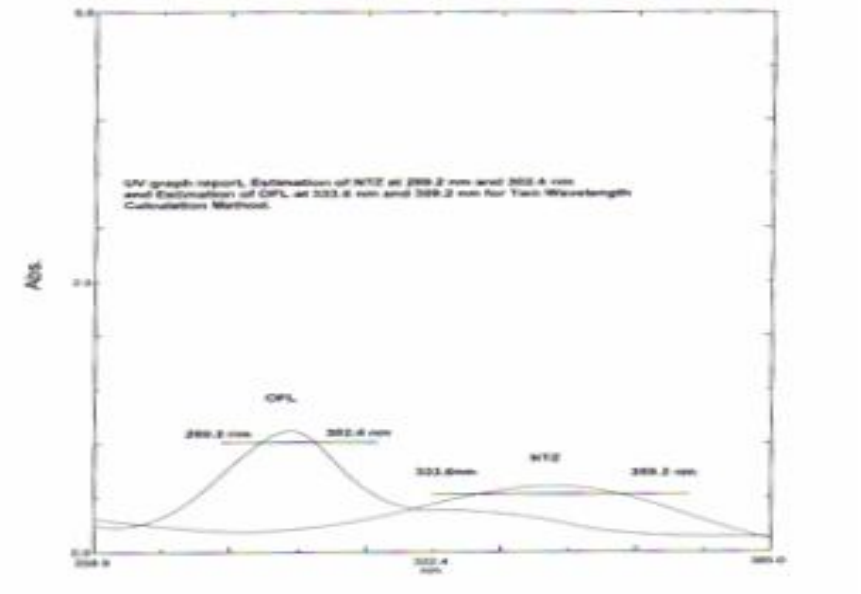


Fig. 4: Overlain spectra of NTZ and OFL for dual wavelength method

Standardization of the dual wavelength method by analysis of laboratory prepared samples:
To check the validity of above selected wavelengths three mixed standards were prepared using pure sample of two drugs. The concentrations of two components of mixed standards were calculated using the equations of calibration curves.

Table 3: Results of validation studies of two wavelength method using mixed standards

S.No.	Concentration (µg/ml)		% concentration found	
	NTZ	OFL	NTZ	OFL
1	2.5	7.5	99.56	99.88
2	5	5	99.58	99.64
3	7.5	2.5	99.85	99.64

Assay procedure
Twenty tablets [500 mg NTZ & 200 mg OFL /tablet] were weighed accurately. The tablets were finely powdered and powder equivalent to 10 mg of NTZ and 4 mg OFL was weighed and extracted with 40 ml of solvent, sonicated for 10 min. The resultant was filtered through Whatman filter paper no. 41 into 100 ml volumetric flask. The filter paper was washed several times with solvent. The washings were added to the filtrate and final volume was made up to the mark with the solvent. Filtrate (0.5 ml) of the sample solution was diluted to 10 ml with solvent.
For all three methods, different sample solutions prepared and the absorbances of these final dilutions were measured at respective wavelengths. Concentration of two drugs in sample were calculated.
The procedure of analysis for tablet formulation was repeated five times with two different formulations and results are reported in table 4.

Recovery studies
Recovery studies were carried out by adding a known quantity of pure NTZ and OFL at three levels to the pre-analyzed formulations solutions and than percentage recovery was calculated.

Table 4: Results of analysis of commercial formulations

Method	Brand name	% labelled claim estimated*		± standard deviation		% relative standard deviation	
		NTZ	OFL	NTZ	OFL	NTZ	OFL
Method I	Nizonide-O	98.67	98.93	0.2525	0.2307	0.2559	0.2331
	Netazox-OF	98.78	99.01	0.4154	0.4006	0.4205	0.4046
Method II	Nizonide-O	99.47	99.68	0.4103	0.1890	0.4124	0.1896
	Netazox-OF	99.20	99.01	0.5468	0.6436	0.5512	0.6500
Method III	Nizonide-O	98.93	99.41	0.5497	0.2945	0.5556	0.2962
	Netazox-OF	99.01	99.72	0.4063	0.1857	0.4103	0.1862

*Each value is an average of five determinations.

Table 5: Results of recovery studies

Brand name	% amount added	% recovery					
		Method I		Method II		Method III	
		NTZ	OFL	NTZ	OFL	NTZ	OFL
Nizonide-O	80	99.65	98.45	99.75	99.35	98.35	98.75
	100	99.42	99.72	98.25	98.20	99.56	98.55
	120	99.85	99.56	99.61	99.30	99.32	99.21
Netazox-OF	80	98.36	98.65	99.45	99.66	99.21	99.78
	100	99.02	98.35	98.31	99.51	99.45	99.35
	120	99.15	99.25	98.65	98.56	98.56	99.25

DISCUSSION

Under experimental condition described, calibration curve, assay of tablets and recovery studies were performed. The both drugs obey beer's law in the concentration range 2-10 µg/ml for all the methods with good correlation coefficient >0.999.

The tablet assay results obtained by proposed method were very closed to labelled claim and low value of standard deviation, suggesting that the developed methods has high precision.

In order to check the accuracy of the developed methods, known quantities of standard drugs of NTZ and OFL in three different levels were added to its preanalyzed tablet samples and analyzed by the developed methods. The mean percentage recoveries were found in the range of 98.20-99.85 and it indicated the non interference of the excipients in the tablet formulations.

CONCLUSION

The developed and validated spectrophotometric methods are simple, rapid, economical, accurate, precise and reproducible as highly desirable. Analysis of authentic samples containing NTZ and OFL showed no interference from the common additives and excipients. Thus these can be used as IPQC test and for routine simultaneous determination of NTZ and OFL in combined formulations.

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