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Spectrophotometric Analysis of Nitazoxanide in Single and Combined Dosage Form

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Two simple sensitive UV spectrophotometric methods for the analysis of nitazoxanide in single and in combined dosage form with ofloxacin were developed and statistically validated. Estimation of nitazoxanide in tablet dosage form was carried out by calibration curve method while graphical absorbance ratio method was used for nitazoxanide and ofloxacin combined tablets. The absorption maxima (λ_{max}) for nitazoxanide and ofloxacin are 268 and 302 nm, respectively. The most striking feature of this method is its simplicity, sensitivity and reproducibility. The method is economical and suitable for routine analysis of nitazoxanide tablets and nitazoxanide and ofloxacin in combined tablet dosage form.

Key Words: Spectrophotometric Analysis, Nitazoxanide.

INTRODUCTION

Nitazoxanide is a synthetic antiprotozoal drug available in single and combined dosage form with ofloxacin. The drug samples were scanned between 400-200 nm to determine the λ_{max}, the sampling wavelengths selected were 268 nm for nitazoxanide and 302 nm for ofloxacin. For the quantitative analysis of nitazoxanide tablets, by simple and direct measurement of absorbance at its λ_{max} 'calibration curve method' and for nitazoxanide and ofloxacin combined tablets having overlapping spectra Q-analysis (graphical absorbance ratio method) is based on the property that for a substance which obeys beers law at all the wavelengths, the ratio of absorbance at any two wavelength is a constant value independent of concentration or path length. Spectroscopic methods for the estimation of nitazoxanide are not available but for ofloxacin in combination with ornidazole[1] and norfloxacin[2] spectroscopic methods are available in literature. In the present studies, single estimation of nitazoxanide and the simultaneous estimation of nitazoxanide in combination with ofloxacin were reported.

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EXPERIMENTAL

For the spectrophotometric analysis of this drug, Shimadzu UV/Vis double beam recording spectrophotometer (model UV-160A) with 1.0 cm path length quartz cells was used. The solvent selected for present study is dimethyl formamide (AR, Qualigens).

Calibration curve method[3,4]: Stock solutions containing 1000 µg/mL of nitazoxanide was prepared by accurately weighed 50 mg of nitazoxanide in 50 mL volumetric flask, dissolved in 25 mL of DMF and then the final volume was made up to the mark with DMF. Aliquot of solution containing 10.0 to 50.0 µg/mL of nitazoxanide, by measuring the absorbance at 268 nm calibration curve was prepared. The concentration of nitazoxanide was calculated from calibration graphs.

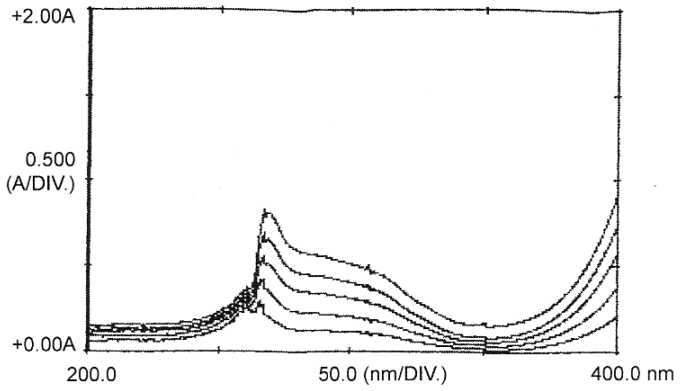

Fig. 1. Overlain spectra of nitazoxanide in (DMF)

TABLE-1
LINEARITY OF NITAZOXANIDE AND OFLOXACIN

Observation	Method A	Method B	
Drugs	Nitazoxanide	Nitazoxanide	Ofloxacin
Absorption maxima (λ_{max}) nm	268	268	302
Beer s law limit (µg/mL)	10.0-125.0	10. 0-125.0	2.0-25.0
Correlation coefficient	0.9996	0.9994	0.9980
Regression equation (y=mx+c)			
Slope (m)	0.0169	0.0170	0.1270
Intercept (c)	+0.0083	+ 0.0069	+0.021
%Relative Standard Deviation (n = 5)	0.4680	0.4520	0.5010

Analysis of nitazoxanide tablet: Twenty tablets of three different batches were taken and their average weight was determined. They were crushed to a fine powder. Powder containing amount equivalent to 500 mg of nitazoxanide was taken in 100 mL

volumetric flask and dissolved in DMF by intermittent shaking for *ca.* 0.5 h. The volume was made up to the mark with DMF and then the solution was filtered through Whatmann filter paper (No. 41). The filtrate was farther diluted to obtain the final concentration of 100 µg/mL and working concentration was obtained (30 µg/mL). The diluted samples were scanned over the range of 400-200 nm and the absorbance at wavelength 268 nm was measured. From calibration curve the final drug concentration in tablet was calculated. The results are shown in Table-2 and the statistical validation is recorded in Table-3.

TABLE-2
ANALYSIS OF NITAZOXANIDE TABLET

S. No.	Amount present (µg/mL)	Amount found	% Amount found
1	30.0	29.45	98.16
2	30.0	29.95	99.83
3	30.0	29.98	99.93
4	30.0	30.20	100.66
5	30.0	30.05	100.16

TABLE-3
STATISTICAL VALIDATION OF NITAZOXANIDE TABLET

Batch	Mean Percentage	(±) Standard deviation	% Coefficient of Variance
1	29.926	0.2531	0.8460
2	28.512	1.3564	1.6732
3	29.354	0.8952	1.2510

Q-Analysis (graphical absorbance ratio method) for combined dosage form: Stock solutions each containing 1000 µg/mL of nitazoxanide and ofloxacin was prepared by accurately weighed 50 mg of nitazoxanide and 50 µg/mL of ofloxacin in two separate 50 mL volumetric flasks. This was dissolved in 25 mL of DMF and the final volume was made up to the mark with DMF. Aliquot of solution containing 10 to 40 µg/mL of nitazoxanide and 4 to 12 µg/mL of ofloxacin were prepared by further dilutions, by measuring the absorbance at 268 and 302 nm for nitazoxanide and ofloxacin, respectively, calibration curves were prepared.

Preparation of mixed standards: From the standard stock solutions of (1000 µg/mL) each of nitazoxanide and ofloxacin, different mixed standards were prepared by mixing in appropriate proportions as shown in Table-4. The absorbance at isoabsorptive point 279 nm ($\lambda 1$) and λ_{max} of ofloxacin 302 nm ($\lambda 2$) were noted and absorbance ratio was calculated from the two values. A graph of relative concentration *vs.* absorbance ratio was plotted for nitazoxanide and ofloxacin, respectively. These plots are shown in Figs. 3 and 4.

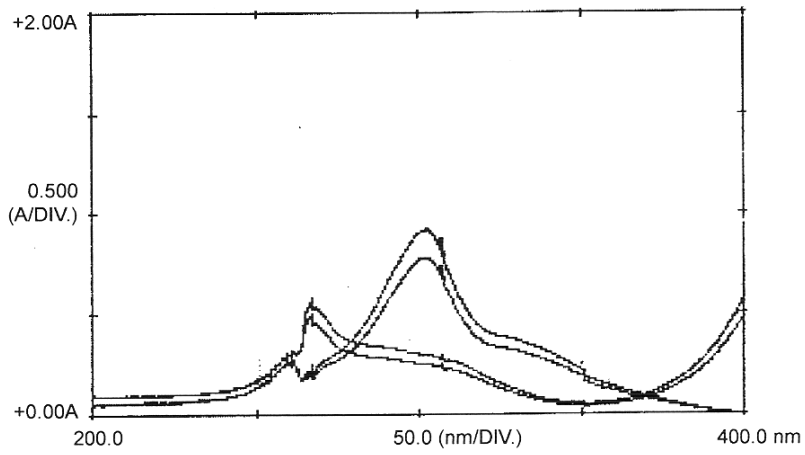

Fig. 2. Overlain spectra of nitazoxanide and ofloxacin in DMF

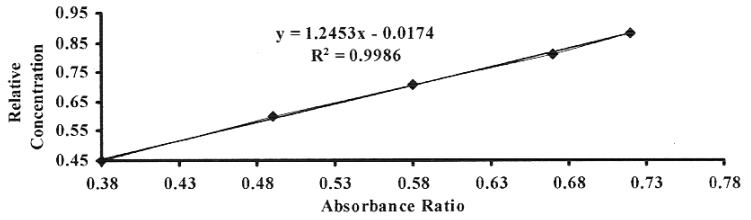

Fig. 3. Relative concentration (nitazoxanide / ofloxacin) and absorbance ratio

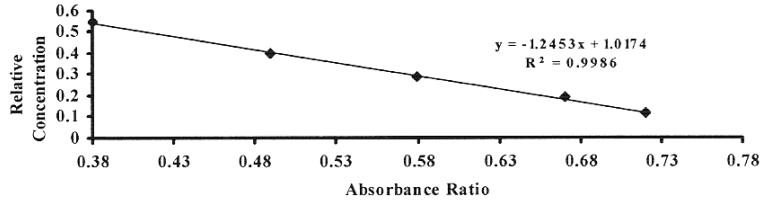

Fig. 4 Relative concentration (ofloxacin/nitazoxanide) and absorbance ratio

TABLE-3
STATISTICAL VALIDATION OF NITAZOXANIDE TABLET

Batch	Mean Percentage	(±) Standard deviation	% Coefficient of Variance
1	29.926	0.2531	0.8460
2	28.512	1.3564	1.6732
3	29.354	0.8952	1.2510

TABLE-4
RELATIVE CONCENTRATION *vs.* ABSORBANCE RATIO

Concentration in mixed standard		Relative concentration		Absorbance ratio A_1/A_2
Nitazoxanide	Ofloxacin	Nitazoxanide	Ofloxacin	
10.0	12.0	0.45	0.55	0.38
15.0	10.0	0.60	0.40	0.49
20.0	08.0	0.71	0.29	0.58
25.0	06.0	0.81	0.19	0.67
30.0	04.0	0.88	0.12	0.72

Analysis of nitazoxanide and ofloxacin combined tablet: Twenty tablets of three different batches were taken and their average weight was determined. They were crushed to a fine powder and powder equivalent of 500 mg of nitazoxanide and 200 mg of ofloxacin was taken in 100 mL volumetric flask. It was then dissolved in DMF by intermittent shaking for *ca.* 0.5 h and then the volume is make up to the mark. The solution was filtered through Whatmann filter paper (No. 41). The filtrate was further diluted to obtain working concentration of 25 µg/mL of nitazoxanide and 10 µg/mL of ofloxacin.

The final tablet dilution was scanned in multi component mode over the wavelength ranging from 400 to 200 nm and the absorbance at 279 and 302 nm was noted and from the relative concentration *vs.* absorbance ratio graph the concentration of each drug in tablet was obtained. The procedure was repeated six times. The result of analysis and statistical validation are shown in Tables 5 and 6, respectively.

TABLE-5
ANALYSIS OF COMBINED NITAZOXANIDE/OFLOXACIN TABLETS

Amount present (µg/mL)		Amount found (µg/mL)		% Amount found	
Nitazoxanide	Ofloxacin	Nitazoxanide	Ofloxacin	Nitazoxanide	Ofloxacin
25.0	10.0	25.29	9.95	101.18	99.5
25.0	10.0	25.44	10.12	101.76	101.2
25.0	10.0	25.01	10.09	100.06	100.9
25.0	10.0	24.98	10.15	99.96	101.5
25.0	10.0	25.06	9.96	100.26	99.6
25.0	10.0	25.35	9.85	101.42	98.5

TABLE-6
STATISTICAL VALIDATION OF COMBINED
NITAZOXANIDE/OFLOXACIN TABLETS

Batch	Mean percentage		(±) Standard deviation		% Coefficient of variation	
	Nitazoxanide	Ofloxacin	Nitazoxanide	Ofloxacin	Nitazoxanide	Ofloxacin
1	100.77	100.2	1.768	1.073	0.702	1.071
2	098.60	98.96	0.672	0.862	1.846	2.150
3	097.35	98.61	0.674	1.860	2.043	1.490

RESULTS AND DISCUSSION

Nitazoxanide tablet: For nitazoxanide tablets, standard calibration curve method was developed and validated[5]. The procedure was adopted as per designed protocol, based on ICH guidelines[6,7]. The mean percentage estimation of nitazoxanide from standard solution was found to be 99.92 % with standard deviation of 0.1130. The accuracy of the method was determined by recovery studies, here the mean percentage estimation of nitazoxanide was found to be 99.74 % with standard deviation of 0.2531. The linearity was determined by its response ratio as 0.0173 and precision has been calculated by repeatability and intermediate precision, for the validation of the method.

Nitazoxanide and ofloxacin tablet: For simultaneous estimation of nitazoxanide with ofloxacin in tablet dosage form, Q analysis technique (graphical absorbance ratio) was developed and the procedure was adopted as per designed protocol, based on ICH guidelines. The method was validated[5,8] by its linearity, accuracy, precision and recovery studies. The linearity was measured by response ratio 0.0174 for nitazoxanide and 0.126 for ofloxacin and regression coefficient for nitazoxanide 0.999 and for ofloxacin 0.997. For accuracy the recovery studies were performed for nitazoxanide is 99.50 % with standard deviation of 0.482 and for ofloxacin is 98.70 % with standard deviation of 0.363 in tablet dosage form. Analysis of laboratory samples for accuracy the mean percentage amount found for nitazoxanide was 100.05 with standard deviations of 0.706 and for ofloxacin was 99.84 with standard deviation of 1.073 and the precision has been calculated by repeatability and intermediate precision.

Both the methods are simple, accurate and precise and gave satisfactory results. They are recommended for quality control and routine analysis where time, cost effectiveness and high specificity of analytical techniques are of great importance.

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