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SIMULTANEOUS DETERMINATION OF NITAZOXANIDE AND OFLOXACIN IN TABLET BY ULTRAVIOLET SPECTROPHOTOMETRY (DUAL WAVELENGTH METHOD)

Shailendra Bindaiya, Shravan Bankey* and Deepti Jain

School of Pharmaceutical Sciences, R.G.P.V., Bhopal, M.P- 462036, India.

*Corres.author: bankey_shravan@yahoo.co.in
Tel. No- +91755-2678883, +91-9425004366*

ABSTRACT:A simple, accurate, and precise dual wavelength spectrophotometric method was developed for simultaneous determination of nitazoxanide and ofloxacin in combined pharmaceutical dosage forms. The principle for dual wavelength method is "the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest". The wavelengths selected for determination of nitazoxanide were 271.5nm & 359.5nm, whereas, the wavelengths selected for determination of ofloxacin were 300.5nm and 365.5nm. A mixture of dichloromethane and n-Hexane (60:40) was taken as a solvent. Regression analysis of beers plots showed good correlation in concentration range of 5-25µg/ml for nitazoxanide and 2-10 µg/ml for ofloxacin. Accuracy of method was found between 98.5-101.5%. The precision (intra-day, inter-day and analyst to analyst) of method was found within limits (%CV≤2). The proposed method was successfully applied to determination of these drugs in laboratory-prepared mixtures and commercial tablets.

Keywords: - Ofloxacin, Nitazoxanide, Dual wavelength method, UV spectrophotometric method.

1. INTRODUCTION

Nitazoxanide, 2-acetyloxy-N-(5-nitro-2-thiazolyl) benzamide, has an antiparasitic activity, it's a prodrug (Figure 1). The active metabolite of nitazoxanide is tizoxanide. Tizoxanide interfers with pyruvate ferredoxin oxidoredutase enzyme (PFOR) dependent electron transfer reaction that is important for anaerobic glucose energy metabolism - this results in impaired parasitic function. Studies have shown that the PFOR enzyme from Giardia lamblia directly reduces nitazoxanide by transfer of electrons in the absence of ferredoxin. Ofloxacin, (+/-)-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 [1], is an anti-bacterial agent (Figure 2). It acts on DNA gyrase, an enzyme which, like human topo-isomerase, prevents the excessive super coiling of DNA during replication or transcription. There have been several reports on the determination of ofloxacin individually or in its combination with other drugs, including HPLC [2, 3, 4, 5], spectrofluorimetry [6], and UV-Spectrophotometric [7] methods are reported but they are applicable for the determination of either for nitazoxanide or ofloxacin individually from pharmaceutical dosage forms or biological fluids. No single method was reported for the determination in combined dosage form. This study attempts to describe a simple, accurate and precise analytical spectrometric method, which can quantify these drugs simultaneously from a combined dosage form.

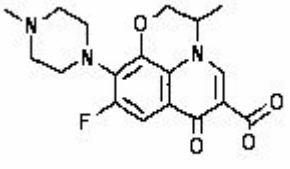

Figure 1 Structure of nitazoxanide

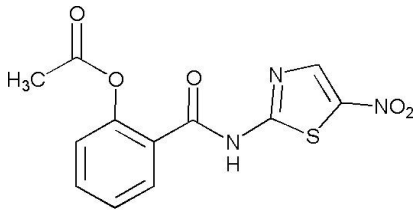

Figure 2 Chemical structure of ofloxacin

2. EXPERIMENTAL

2.1 *Apparatus:*

Spectrometric method was carried out using Shimadzu 1700 series a double beam UV/Visible spectrophotometer.

2.2 *Reagents and chemicals:*

Nitazoxanide API sample (purity 99.88%) was kindly provided by Glane Mark India Ltd. Mumbai and ofloxacin API sample (purity 99.79%) was kindly provided by FDC Ltd. Mumbai, purity of the samples was checked by TLC and HPLC. Dichloromethane and n-hexane were purchased from Merck. All reagents and chemicals used were of analytical grade.

2.3 *Marketed formulation:-*

The marketed formulation studied was Zenflox-NT tablets manufactured by Mankind Pharma. Each tablet contains 500mg nitazoxanide and 200mg ofloxacin.

2.4 *Standard solutions:*

Standard stock solutions of nitazoxanide and ofloxacin, each of 1mg/ml concentration in solvent (a mixture of dichloromethane and n-hexane in the ratio of 60:40) were prepared. From these stock solutions appropriate dilutions in the range of 5-25 µg/ml and 2-10 µg/ml for nitazoxanide and ofloxacin respectively were prepared and analyzed. Mixed standards were prepared in the ratio of 5:2, as the formulation contains nitazoxanide and ofloxacin 500mg and 200mg, respectively.

2.5 *Method development:*

Study of overlain spectra and selection of wavelengths:

Standard stock solutions of 100µg/ml were prepared for both the drugs using a mixture of dichloromethane and n-hexane in the ratio of 60:40 as solvent. From these stock solutions appropriate dilutions of nitazoxanide (10µg/ml) and ofloxacin (4µg/ml) were prepared and scanned over the range of 200-400 nm and the overlain spectra was observed for development of suitable method for analysis. The overlain spectra of nitazoxanide and ofloxacin were shown in figure 3.

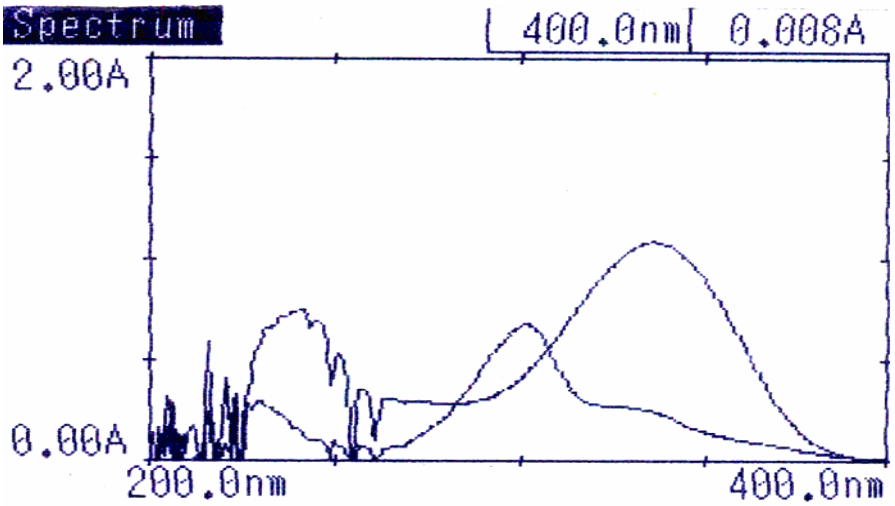

Figure 3 Overlain spectra of the nitazoxanide and ofloxacin

2.6 *Validation of the Method:*

2.6.1 *Linearity:*

Linearity of the proposed method was verified by analyzing five different concentrations in the range of 5-25µg/ml for nitazoxanide and 2-10µg/ml for and ofloxacin. Each concentration was made in triplicate.

2.6.2 *Accuracy:*

The accuracy of the method was performed by conducting the recovery studies (80, 100 and 120%) of pure drugs from marketed formulation, by standard addition method. The actual and measured concentrations were then compared.

2.6.3 *Precision:*

The intra day precision of the developed method was evaluated by analyzing samples of three different concentrations of nitazoxanide (5, 15, and 25µg/ml) and ofloxacin (2, 6, and 10µg/ml) in triplicates on the same day. The inter day precision was evaluated from the same concentration on three consecutive days, and analyst to analyst precision was evaluated from the same concentration by three different analysts.

2.6.4 *Stability:*

Stability was observed by scanning the drug solutions in selected solvent system in time scan mode of UV-spectrophotometer for 12 hours.

2.6.5 *Analysis of Marketed formulation:*

To determine the content of nitazoxanide and ofloxacin in commercial tablets (each tablet containing 500mg nitazoxanide and 200mg ofloxacin), 20 tablets were weighed and finely powdered. A quantity of powder equivalent to 50mg of nitazoxanide and 20mg of ofloxacin was weighed accurately and transferred to a 50ml volumetric flask and the volume was made up with the solvent. It was sonicated for 30 minutes and then filtered through 0.5µm whatman paper. From the above prepared solution, further dilutions were prepared in the linearity range using solvent. The absorbance was taken at selected wavelengths and concentrations were found out. The analysis was done in triplicates.

3. RESULTS AND DISCUSSION

3.1 Method development and Validation:

The overlain spectrum of the drugs suggested that a dual wavelength spectrophotometric method was the most suitable method for simultaneous determination of nitazoxanide and ofloxacin. A mixture of dichloromethane and n-hexane in the ratio of 60:40 was taken as solvent system, as both the drugs were soluble in this system. In Dual wavelength method absorption difference at 359.5nm and 271.5nm was selected for determination of nitazoxanide, whereas 300.5nm and 365.5nm were selected for determination of ofloxacin.

3.1.1 *Linearity:*

The calibration curves of nitazoxanide and ofloxacin were linear in the range of 5-25µg/ml and 2-10µg/ml respectively. The regression equations of calibration curves were $Y_{NTZ} = 0.0605x + 0.012$, $r^2 = 0.9997$ and $Y_{OFX} = 0.0915x - 0.0013$, $r^2 = 0.9998$ for nitazoxanide and ofloxacin, respectively.

The linear regression analysis data are summarized in table 1 and 2.

3.1.2 *Accuracy:*

The percentage recovery was calculated of pure drugs from marketed formulation by standard addition of pure drugs at three known concentrations (80, 100 and 120%) and excellent recoveries were obtained at each level. The respective % recovery and %RSDs for nitazoxanide at three levels (80, 100 an120%) were found 100 $\pm$ 1.2, 99.8 $\pm$ 2.1 and 98.9 $\pm$ 1.7 respectively. The respective % recovery and %RSDs for ofloxacin at three levels (80, 100 an120%) were found 101 $\pm$ 1.8, 99.7 $\pm$ 1.1 and 99.9 $\pm$ 1.6 respectively. The results of accuracy studies are shown in table 3.

3.1.3 *Precision:*

The intraday precision showed a relative standard deviation (R.S.D. %) of 1.2-2.6% for nitazoxanide and 1.5-2.4% for ofloxacin. The inter day precision showed a R.S.D. % were 2.1-2.8% and 1.6-2.5% for nitazoxanide and ofloxacin, respectively. The analyst to analyst precision R.S.D. % was found 1.3-2.6% and 1.6-2.7% for nitazoxanide and ofloxacin, respectively. Intra day, inter day and analyst to analyst precision of method is illustrated in Table 4, 5 and 6.

3.2 Application of the Method in Tablets

The proposed UV method was applied for the determination of nitazoxanide and ofloxacin in their combined pharmaceutical formulations and the results are shown in table 7. The high percentage recoveries (99.38-101.2) and low %CV (1.01-2.01) values confirm the suitability of the proposed method for the routine determination of these components in combined formulation.

4. CONCLUSION

The proposed dual wavelength method gives accurate and precise results for determination of nitazoxanide and ofloxacin in marketed formulation (tablet) without prior separation and is easily applied for routine analysis. The most striking feature of the dual wavelength method is its simplicity and rapidity. Method validation has been demonstrated by variety of tests for linearity, accuracy, precision and stability. The developed method has several advantages, as it is simple, accurate, precise and economical. The proposed method was successfully applied to determination of these drugs in commercial tablets.

Table 1 Linearity of nitazoxanide at 337nm

Sr. No.	Concentration in µg/ml (n=3)	Absorbance found ± S.D.
1.	5	0.319 ± 0.015
2.	10	0.622 ± 0.022
3.	15	0.932 ± 0.019
4.	20	1.223 ± 0.026
5.	25	1.513 ± 0.043

Table 2 Linearity of ofloxacin at 302nm

Sr. No.	Concentration in µg/ml (n=3)	Absorbance found ± S.D.
1.	2	0.179 ± 0.021
2.	4	0.371 ± 0.034
3.	6	0.553 ± 0.029
4.	8	0.741 ± 0.037
5.	10	0.913 ± 0.043

Table 3 Recovery study data of nitazoxanide for accuracy (n=3)

Nitazoxanide				
Pre-analyzed conc. (µg/ml)	**Added conc. (µg/ml)**	**Measured ± S.D.**	**R.S.D. %**	**% Accuracy**
10.05	8	7.95 ± 0.08	1.01	99.38
10.05	10	10.02 ± 0.19	1.89	100.2
10.05	12	12.07 ± 0.21	1.74	100.6

Table 4 Recovery study data of ofloxacin for accuracy (n=3)

Ofloxacin				
Pre-analyzed conc. (µg/ml)	**Added conc. (µg/ml)**	**Measured ± S.D.**	**R.S.D. %**	**% Accuracy**
6.03	4	3.98 ± 0.05	1.26	99.50
6.03	6	6.07 ± 0.09	1.48	101.2
6.03	8	7.97 ± 0.16	2.01	99.63

Table 5 Intra-day precision for determination of nitazoxanide and ofloxacin (n=3)

Nitazoxanide			Ofloxacin		
Added conc. (µg/ml)	Measured ± S.D.	R.S.D.%	Added conc. (µg/ml)	Measured ± S.D.	R.S.D.%
5	4.92 ± 0.08	1.63	2	1.98 ± 0.05	2.52
15	15.02 ± 0.19	1.26	6	6.07 ± 0.09	1.48
25	24.67 ± 0.35	1.42	10	9.92 ± 0.16	1.61

Table 6 Inter-day precision for determination of nitazoxanide and ofloxacin (n=3)

Nitazoxanide			Ofloxacin		
Added conc. (µg/ml)	Measured ± S.D.	R.S.D.%	Added conc. (µg/ml)	Measured ± S.D.	R.S.D.%
5	5.05 ± 0.11	2.17	2	2.06 ± 0.04	1.94
15	15.19 ± 0.18	1.18	6	6.12 ± 0.12	1.96
25	25.34 ± 0.26	1.03	10	9.98 ± 0.21	2.10

Table 7 Analyst to analyst precision for determination of nitazoxanide and ofloxacin (n=3)

Nitazoxanide	Ofloxacin				
Added conc. (µg/ml)	Measured ± S.D.	R.S.D.%	Added conc. (µg/ml)	Measured ± S.D.	R.S.D.%
5	5.12 ± 0.14	2.73	2	1.92 ± 0.06	3.12
15	14.93 ± 0.23	1.54	6	5.96 ± 0.12	2.01
25	25.14 ± 0.17	0.68	10	9.99 ± 0.13	1.30

Table 8 Analysis of nitazoxanide and ofloxacin in tablets by proposed method (n=3)

Drug	Amount (mg/tablet)		% Label claim	% Found
	Labeled claim	Found (mean ± S.D.)		
Nitazoxanide	500	498.4 ± 1.06	99.88	99.68
Ofloxacin	200	198.8 ± 1.11	98.79	99.40

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