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Der Pharma Chemica, 2012, 4 (3):1041-1046
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Application of UV spectrophotometric methods for simultaneous estimation of norfloxacin and tinidazole in bulk and tablet dosage forms

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ABSTRACT

Two simple, accurate and reproducible spectrophotometric methods have been developed for the simultaneous estimation of norfloxacin and Tinidazole in pharmaceutical dosage forms. The first method involves determination using the Vierodt's Method (Simultaneous Equation Method); the sampling wavelengths selected are 273 nm and 319 nm over the concentration ranges of 2.5-20µg/mL and 5-40 µg/mL for Norfloxacin and Tinidazole respectively. The second method involves determination using the Multicomponent Mode Method; the sampling wavelengths selected are 273 nm and 319 nm over the concentration ranges of 2.5-20µg/mL and 5-40 µg/mL for Norfloxacin and Tinidazole respectively. The results of the analysis were validated statistically and recovery studies were carried out as per ICH guidelines.

Key Words-Norfloxacin, Tinidazole, UV Validation.

INTRODUCTION

Norfloxacin (NF), [1-ethyl-6-fluoro-1, 4-dihydro-4-oxo-7-(piperazin-1-yl) quinoline-3-carboxylic acid], is a fluoroquinolone carboxylic acid derivative used as broad-spectrum antibacterial (Fig. 1). The mode of action of NF depends on blocking of bacterial DNA replication through inhibition of the bacterial DNA gyrase enzyme. It is used for treatment of uncomplicated urinary tract infections including cystitis and prostatitis. NF is the subject of a monograph in each of British Pharmacopoeia, (BP) [1] and the United States Pharmacopoeia, USP [2]. The BP and USP recommended non aqueous titration for the raw material and HPLC (High Performance Liquid Chromatography) methods for tablets. Because of the therapeutic importance of NF, numerous analytical methods have been developed for its determination.

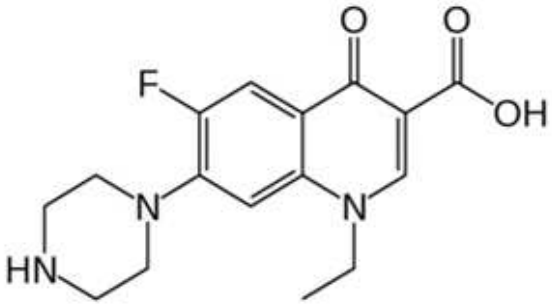
In bulk, pharmaceutical formulations and/or biological fluids. Spectrophotometric technique is the most widely used in pharmaceutical analysis [3-6]. Literature survey revealed that a number of methods have been reported for estimation of Norfloxacin [7-8] and Tinidazole [9-10] individually or in combination with other drugs. Other analytical methods have been used such as, HPLC [11-13], electrochemical analysis [14-15], Difference spectroscopy [16], and capillary electrophoresis [17], Stability studies [18-19].

Tinidazole (TZ), [1-(2-(ethylsulfonyl) ethyl)-2-methyl-5-nitroimidazole], is an effective antiprotozoal and antibacterial agent (Fig. 1). It is used for treatment of amoebiasis, giardiasis and trichomoniasis. TZ is the subject of monograph in each of the BP and the USP. The BP and USP recommended non aqueous titration for determination of TZ. There are several reports on the determination of TZ, both in formulations and biological fluids, *viz*: spectrophotometry [20-21], HPLC [22], and Titrimetric and Spectrophotomertic analysis [23], Potentiometry [24]. The combination of NF and TZ is commercially available in tablet forms to control gastrointestinal infections caused by bacterial or amoebic infection, prostatitis and urinary tract infections due to susceptible uropathogens.

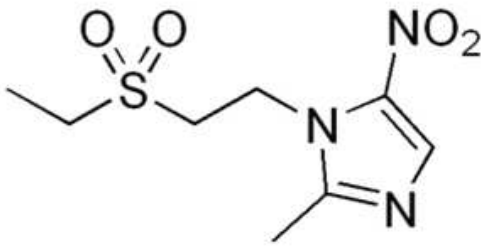
Both drugs were simultaneously determined by spectrophotometry, HPLC, electrochemical analysis, and capillary electrophoresis. Taste masking of Norfloxacin and Tinidazole Tablet by Flavor Coating: A Developmental Approach[25], derivative spectrophotometric procedure[26].

Difference spectrophotometry stability indicating assay method. In the present work, simultaneous equation method and multicomponent analysis spectrophotometric method is described for simultaneous determination of NF and TZ in the presence of each other in pure form and in pharmaceutical dosage forms.

Figure 1. Structural formulae of the norfloxacin (NF) and tinidazole (TZ).



NF



TZ

MATERIALS AND METHDS

Instrumentation:
A Shimadzu UV/Visible spectrophotometer, model 1700 (Japan) was employed with spectral bandwidth of 2 nm and wavelength accuracy of ± 0.5 nm, with automatic wavelength correction was employed. A Shimadzu electronic analytical balance (AX-200) was used for weighing the sample. An ultrasonic cleaner (Art No.400014CL) was used for sonicating the sample solution.

Reagents and Chemicals:
Analytical pure samples of NF and TZ (Hindustan Antibiotic Limited, Pimpri, Pune, India) were used in the study. The pharmaceutical dosage form used in this study was Hindustan Antibiotic Limited, Pimpri, Pune, India labelled to contain 400 mg NF and 600 mg of TZ.

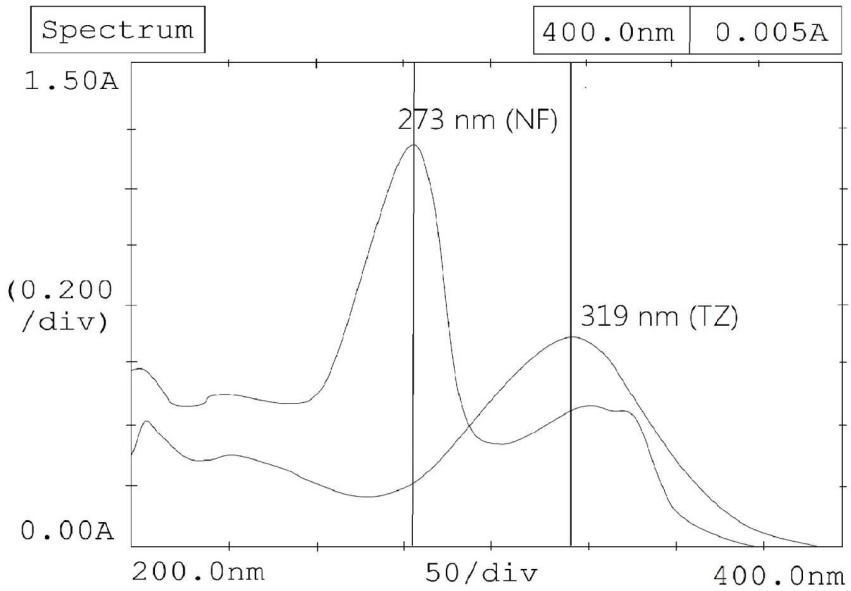
Preparation of Standard Stock Solution:
Standard stock solutions (100µg/mL) of NF and TZ were prepared by dissolving separately 10 mg of drug each in 50 ml methanol and volume is made up with water upto 100 ml. The working standard solutions of these drugs were obtained by dilution of the respective stock solution with water.

Preparation of Sample Stock Solutions:
An accurately weighed powder sample equivalent to 10 mg of NF was transferred to a 100 ml volumetric flask and dissolved in 50ml and sonicated for 15 minutes and volume made to 100ml with doubled distilled HPLC grade water. It was then filtered through Whatmann filter paper No.41. The solution was suitably diluted with double distilled HPLC grade water to obtain sample solutions containing NF and TZ in the concentrations ratio of 2:3 µg/mL respectively as in the formulation. The final concentrations are 10 µg/mL of NF and 15 µg/mL of TZ.

Method A:
Vierodt's Method (Simultaneous Equation Method)
Construction of calibration curve
For the Vierodt's Method (Simultaneous Equation Method), 273nm, and 319nm were selected as the two sampling wavelengths. Fig.2 represents the overlain UV spectra of NF and TZ.

NF and TZ exhibited linearity with absorbances in the range of 2.5-20 µg/mL and 5-40 µg/mL at their respective selected wavelengths. Co-efficient of correlation was found to be 0.9989 and 0.9987 for NF and TZ respectively. The optical characteristics and regression values for the calibration curves are presented in Table 1. For simultaneous estimation of NF and TZ, mixed standards containing NF and TZ in a concentration ratio of 2:3 µg/mL each were prepared by appropriate dilution of the standard stock solutions with distilled HPLC grade water.

Fig.2: Overlain Spectra of NF and TZ.



The absorbances of the mixed standard solutions were measured at the selected wavelengths. A set of two simultaneous equations were used for obtaining the concentratios of NF and TZ are as follows;

$$C_x = \frac{A_2\, ay_1 - A_1\, ay_2}{ax_2\, ay_1 - ax_1\, ay_2} \quad \text{..... Eq. (i)}$$

$$C_y = \frac{A_1\, ax_2 - A_2\, ax_1}{ax_2\, ay_1 - ax_1\, ay_2} \quad \text{..... Eq. (ii)}$$

Where, A_1 and A_2 are absorbances of mixture at 273.0 nm and 319.0 nm respectively, ax_1 and ax_2 are absorptivities of NF at $\lambda_1$and λ_2 respectively and ay_1 and ay_2 are absorptivities of TZ at λ1 and λ2 respectively. Cx and Cy are concentrations of NF and TZ respectively. The concentration of NF and TZ in mixed standard and tablets formulation can be obtained by solving equation (i) and (ii).

Method B:
Multicomponent Mode Method
For the analysis of NF and TZ by multicomponent method of analysis, the multicomponent mode of the UV visible spectrophotometer was used. For multicomponent method of analysis, 273 nm, and 319 nm were selected as the two sampling wavelengths for NF and TZ respectively. The drugs showed linearity in the concentration ranges of 2.5-20µg/mL, 5-40 µg/mL with regression coefficient (r^2) values of 0.9990, 0.9989 for NF and TZ respectively. Six mixed standards in ratio of 2:3µg/mL showing linearity within the Beer's concentration range of NF and TZ were prepared by appropriate dilution of standard stock solutions (100µg/mL). In multicomponent mode of the

instrument, the mixed standards were scanned over the range of 190-400 nm at the selected sampling wavelengths. The overlain spectra of the six mixed standards were then employed to determine the concentration of the drugs in sample solutions by analysis of the spectral data of sample solution with reference to that of mixed standards.

Assay of Tablet Formulation:

Powder equivalent to 10 mg of NF and 15 mg of TZ was weighed and transferred100 ml and dissolved in 50 mL methanol and volume is made up with water upto100ml with the aid of ultrasonication for 15 min. The solution was then filtered through Whatmann filter paper No.41 and diluted further to obtain final concentration of 10 µg/mL of NF and 15 µg/mL of TZ. The sample solutions were analyzed as per the procedure for mixed standards. The concentrations of each drug in sample solutions were calculated using equations (I) and (II) for the Vierodt's Method (Simultaneous Equation Method) and using the multicomponent mode of the instrument for the Multicomponent method of analysis. The proposed methods were validated as per ICH guidelines[12]. The accuracy of the proposed methods was determined by performing recovery studies at 80%, 100% and 120% of the test concentration. The results of the analysis and statistical validation data of the Tablet formulation are given in table 1. The statistical validation data of recovery study are given in table 2.

Table 1: Optical Characteristics and Validation Data of NF and TZ.

Parameters	NF		TZ	
	Method-A	Method- B	Method-A	Method-B
Working wavelengths	273nm	273nm	319nm	319nm
Beer-Lamberts Law range (µg/mL)	2.5-20	2.5-20	5-40	5-40
Precision*				
Interday (%RSD)	0.412	0.5396	0.143	0.309
Intraday (%RSD)	0.167	0.143	0.410	0.1141
LOD (µg/mL)*	2.223	0.3779	0.342	1.5120
LOQ (µg/mL)*	6.736	1.1453	1.129	4.5820
Regression Values:				
I. Slope*	0.1245	0.1239	0.0380	0.0378
II.Correlation Coefficient (r^2)*	0.9989	0.9990	0.9987	0.9989

**Denotes average of five estimations.*
Method A – Vierodt's Method (Simultaneous Equation Method)
Method B – Multicomponent Mode Method.

Table 2: Statistical Validation Data of Tablet Formulation
Results of Commercial Sample Analysis
The % drug obtained and % recovery value are mean of five determinations.

Component	Methods	Labeled Drug (Mg/tablet)	Amount obtained (mg)	% Amount Found	S.D.*	% R.S.D.*
NF	A	400	406.54	100.63	0.04289	0.412
	B	400	394.18	98.55	0.0141	0.143
TZ	A	600	600.04	100.01	0.0389	0.2537
	B	600	606.24	101.39	0.01732	0.1141

S.D. = Standard deviation, n= 5, RSD= Relative standard deviation.*
Tablet Formulation, NOR TZ, manufactured by Omega Biotech Ltd. Dehradun, Uttarakhand

Table 3: Statistical Validation of Recovery Studies

Level of % Recovery	Methods	% Recovery*		% R.S.D.*	
		NF	TZ	NF	TZ
80	A	99.39	100.247	0.3653	0.6779
	B	99.78	100.02	0.4019	0.1379
100	A	99.76	99.79	0.3815	0.3010
	B	99.73	100.46	0.4738	0.3461
120	A	99.83	99.97	0.2598	0.2018
	B	100.11	99.96	0.3409	0.1792

***Denotes average of three estimations at each level of recovery.*

RESULTS AND DISCUSSION

Under the experimental conditions described, calibration curves, assay of Tablet and recovery studies were performed. The developed methods were validated as per ICH guidelines for linearity, repeatability, intermediate precision (inter-day and intra-day precision studies), LOD, LOQ as shown in Table 1. The mean % content of 99.73% and 99.91% formulation by the developed methods were 100.32% and 100.56% respectively (Table 2). The mean % recoveries of NF and TZ were found to be 99.87% and 100.29 % respectively (Table 3). The ruggedness of

the developed methods was determined by evaluating the effect of change in instruments and analysts on the % mean content of drugs.

CONCLUSION

The combination of NF and TZ is commercially available in tablet forms to control gastrointestinal infections caused by bacterial or amoebic infection, prostatitis and urinary tract infections due to susceptible uropathogens. Here, two simple UV spectrophotometric methods (Vierodt's Method (Simultaneous Equation Method), Multicomponent Mode Method) were developed for their simultaneous analysis. The standard deviation, RSD and standard error calculated for the methods are low, indicating high degree of precision of the methods. The RSD is also less than 2% as required by ICH guidelines. The % recovery was between 98- 102% indicating high degree of accuracy of the proposed methods. The developed methods are simple, rapid, precise, accurate and can be employed for the routine estimation of NF and TZ in both bulk and injection dosage form.

Acknowledgements
The authors express their gratitude to Mission Vivacare Limited, Pithampur, Indore for providing samples of pure NF and TZ and for providing necessary facilities and Jodhpur National University, Department of Chemistry, Faculty of Engineering & Technology, Jodhpur-Rajasthan, INDIA.

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