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New Validated UV Spectrophotometric Method for Estimation of Norfloxacin and Tinidazole in Bulk and Tablet Dosage Forms

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ABSTRACT

The simple, specific, accurate, and precise validated UV Spectrophotometric method has been developed for the simultaneous determination of Norfloxacin and Tinidazole in pharmaceutical dosage forms. The method is often used for the estimation of a combination of those drugs in tablets. The method involves determination using the Vierodt's Method (Simultaneous Equation Method); the sampling wavelengths selected are 276 nm and 316 nm over the concentration ranges of 01-11µg/mL and 01-25µg/mL for Norfloxacin and Tinidazole respectively. The results of the analysis were validated statistically and recovery studies were administered as per ICH guidelines.

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INTRODUCTION

The aim of the present study is the Development and Validation of a new UV Spectrophotometric Method for Estimation of Norfloxacin and Tinidazole in Bulk and Tablet Dosage Forms.

Norfloxacin (NF), [1-ethyl-6-fluoro-1, 4-dihydro-4-oxo-7-(piperazine-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 the treatment of uncomplicated urinary tract infections including cystitis and prostatitis. NF is the subject of a monograph in each of British Pharmacopoeia, (BP) [3] and the United States Pharmacopoeia (USP) [4]. The BP and USP recommended non-aqueous titration for the staple and HPLC (High-Performance Liquid Chromatography) methods for tablets. Because of the therapeutic importance of NF, numerous analytical methods are developed for its determination. In bulk, pharmaceutical formulations, and/or biological fluids. The spectrophotometric technique is that the most generally utilized in pharmaceutical analysis. A literature survey revealed that several methods [13-20] have been reported for estimation of Norfloxacin [1] and Tinidazole [2] individually [1-2,5-9] or in combination [10-12] with other drugs Other analytical methods have been used such as HPLC [13-15], electrochemical analysis [19]; Difference spectroscopy[16-17], and capillary electrophoresis[20], Stability studies[21,22].

Figure No. 1: Norfloxacin

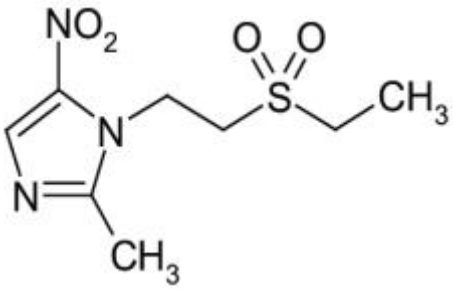

Figure No. 2: Tinidazole

Tinidazole (TZ), [1-(2-(ethylsulfonyl) ethyl)-2-methyl-5-nitroimidazole], is an efficient antiprotozoal and antibacterial agent (Fig. 1). It is used for the treatment of amoebiasis, giardiasis, and trichomoniasis. TZ is the subject of a monograph in each of the BP [3], IP [2], and the USP [4]. BP and USP followed the non-aqueous titration for the determination of TZ. There are several reports applicable to the determination of TZ [5-9], both in formulations and

biological fluids, viz: spectrophotometry, HPLC [13-15], and Titrimetric and Differential Spectrophotometric analysis [16-17], Potentiometry [18]. The combination of NF and TZ is commercially available in tablet forms to regulate gastrointestinal infections caused by bacterial and/or amoebic infection, prostatitis, and tract infections due to susceptible uropathogens. Both drugs were simultaneously determined by spectrophotometry, HPLC, electrochemical analysis [19], and capillary electrophoresis [20]; Difference spectrophotometry stability-indicating assay method [21,22]. In the present work, the simultaneous equation method is described for the determination of NF and TZ within the presence of every other in pure form as well as in pharmaceutical dosage forms.

MATERIALS AND METHODS [30]

Instrumentation:

A Shimadzu UV/Visible spectrophotometer, model 1800 (Japan) was employed with a 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 was used for sonicating the sample solution.

Reagents and Chemicals:

Analytical pure samples of NF and TZ (Hindustan Antibiotic Limited, Pimpri, Pune, India) were utilized in the study. The pharmaceutical dosage form used in this study was taken from the local market labeled 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 10mg of the drug each in 50 ml acetonitrile and volume is made up of water up to 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 drug sample equivalent weight to 10 mg of NF and 15 mg of TZ was transferred to a 100 ml volumetric flask and dissolved in 50ml acetonitrile and sonicated for 10-15 minutes and volume made to 100ml with doubled distilled HPLC grade

water. It was then filtered through Whatman 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 ratio of the concentration of 2:3 µg/mL respectively as in the formulation. The final concentrations are obtained as 10 µg/mL of NF and 15 µg/mL of TZ.

Calibration curve

Calibration curves were prepared by making appropriate dilutions of standard norfloxacin and tinidazole stock solutions in different 10 ml volumetric flask and diluted up to the mark with mobile phase to obtain final concentrations of 01-11 µg/ml of norfloxacin and 1-25 µg/ml tinidazole. Standard solutions were analyzed at 276 nm and 316 nm wavelength for norfloxacin and tinidazole respectively. The calibration curve was constructed by plotting the absorbance against concentration and the regression equation was computed.

Method:

Vierodt's Method (Simultaneous Equation Method) [30]

Construction of calibration curve

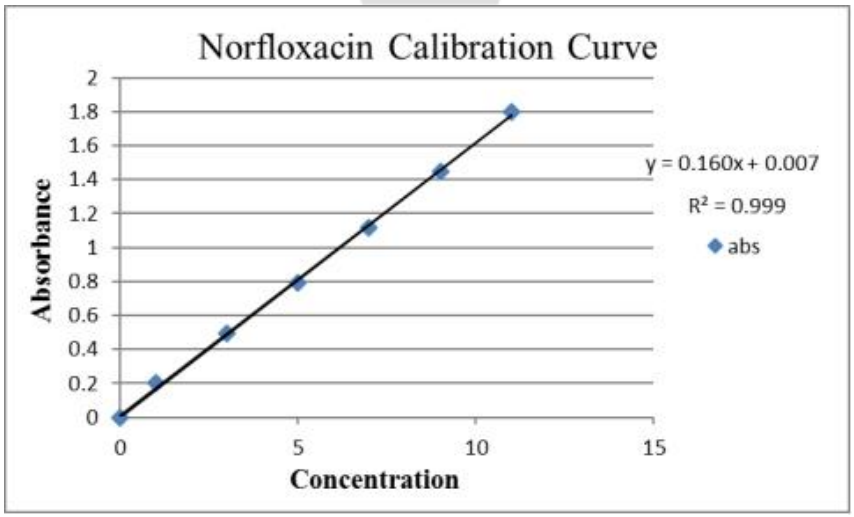

Figure No.3: Calibration curve of Norfloxacin.

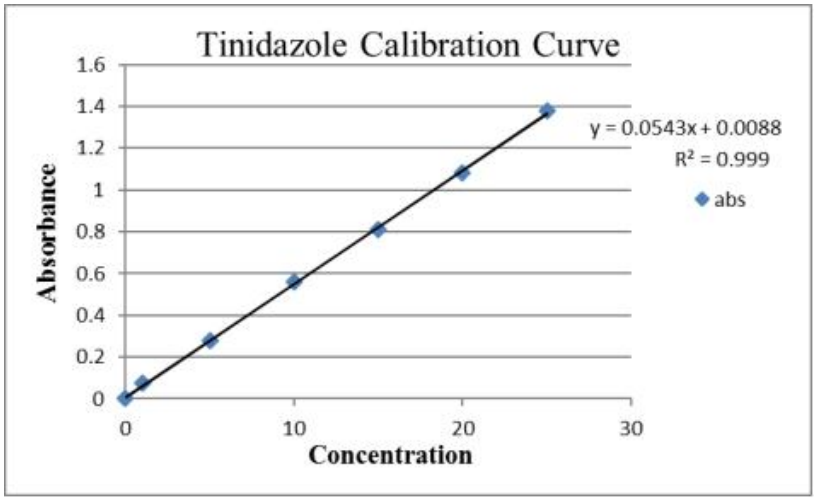

Figure No. 4: Calibration curve of Tinidazole

For the Vierodt's Method (Simultaneous Equation Method), 276nm, and 316nm were selected as the two sampling wavelengths. Fig.5 represents the overlain UV spectra of NF and TZ. NF and TZ exhibited linearity with absorbances in the range of 01-11µg/mL and 01-25µg/mL at their respective selected wavelengths. The coefficient of correlation was found to be 0.9999 and 0.9998 for NF and TZ respectively. The optical characteristics and regression values for the calibration curves are presented in Table 1. For the simultaneous determination of NF and TZ, mixed standards containing NF and TZ in such a concentration ratio of 2:3 µg/mL each were prepared by appropriate dilution of the standard stock solutions with distilled HPLC grade water.

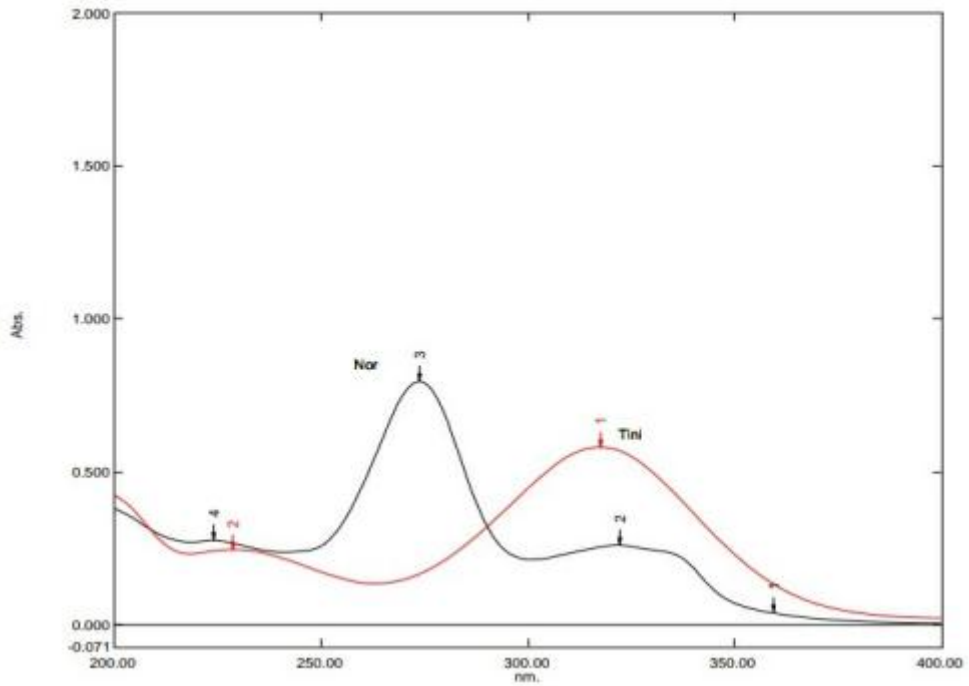

Figure No. 5: Overlain Spectra of NF and TZ.

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

$$Cx = \frac{A1\,ay2 - A2\,a\,y1}{ax1ay2 - ax2ay1} \quad Cx = \frac{A1\,ay2 - A2\,a\,y1}{ax1ay2 - ax2ay1} \quad \text{.....Eq. (i)}$$

$$Cy = \frac{A1ax2 - A2ax1}{ay1ax2 - ay2ax1} \quad Cy = \frac{A1ax2 - A2ax1}{ay1ax2 - ay2ax1} \quad \text{.....Eq. (ii)}$$

Where, A1 and A2 are absorbances of the mixture at 276.0 nm and 316.0 nm respectively, ax1 and ax2 are absorptivities of NF at λ1and λ2 respectively and ay1 and ay2 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).

Table No. 01: Optical Characteristics and Validation Data of NF and TZ

Parameter	**Norfloxacin**	**Tinidazole**
Beer-Lamberts Law range (µg/mL)	01-11	01-25
Selected Wavelengths	276nm	316nm
Regression equation	Y=0.160x + 0.007	Y= 0.054x + 0.008
Slope*	0.160	0.054
Y intercept	0.007	0.008
Correlation Coefficient (r2)*	0.9999	0.9998
LOD (µg/mL)*	0.0825	0.1222
LOQ (µg/mL)*	0.25	0.3703

S.D. = Standard Deviation, R.S.D. = Relative Standard Deviation

* = Average of 3 Estimates.

Assay of Tablet Formulation: [21-22]

Powder equivalent to 10 mg of NF and 15 mg of TZ was weighed and transferred100 ml and dissolved in 50 mL acetonitrile and volume are made up with water upto100ml with the aid of sonication for 10-15 min. The solution was then filtered through Whatman filter paper No.41 and diluted further solution to obtain final concentrations of 10 µg/mL of NF and 15 µg/mL of TZ. The sample solutions were analyzed as per the procedure for the mixed standards method. The concentrations of each drug in sample solutions were calculated using equations (I) and (II) for the Vierodt's Method (Simultaneous Equation Method). The proposed methods were validated as per ICH guidelines [23,24]. 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 2.

Table No. 02: Result of Assay of analysis for marketed formulations

Tablet Brands	**Nor TZ**		**Norflox TZ**	
Analyte	Norfloxacin	Tinidazole	Norfloxacin	Tinidazole
%Estimated	99.66	99.35	98.27	101.12
Amount found in mg	398.64	596.10	393.08	606.72
SD*	0.11	0.2637	0.4114	0.1014
%R.S.D.*	0.1102	0.2647	0.4170	0.100

* = Average of 3 Estimates.

Method Validation [23]

The proposed UV Spectrophotometric method was validated as per ICH guidelines.

Linearity

Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for norfloxacin was found to be 01- 11 µg/ml and tinidazole was found to be 5-25 µg/ml. The regression equation for norfloxacin was found to be y = 0.160x + 0.007 and for tinidazole was found to be y = 0.054x + 0.008 with coefficient of correlation 0.9999 and 0.9998 respectively.

Table No. 03: Result of Linearity studies

Contents	Conc.	Abs. Mean	SD	%RSD
NF	1	0.191	0.0013	0.7172
	3	0.516	0.0010	0.1930
	5	0.791	0.0017	0.2183
	7	1.124	0.0015	0.1333
	9	1.484	0.0005	0.038
	11	1.776	0.0026	0.1463
TZ	5	0.0517	0.0001	0.2938
	10	0.557	0.0020	0.3590
	15	0.797	0.0020	0.2509
	20	1.083	0.0021	0.1957
	25	1.391	0.0030	0.2191

Precision

The precision of the methods was studied as intraday, interday, and repeatability. An intraday study was performed by analyzing three times on the same day (morning, afternoon, and evening). Inter day precision was performed by analyzing three different concentrations of the drug for two days. Repeatability was performed by analyzing the same concentration of drugs. The precision of the method was expressed as a relative standard deviation (%RSD) and standard deviation (SD). Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise.

Table No. 04: Result of Precision studies

Precision

Contents	Conc.	Amount Recover	% Recovery	SD	%RSD
Intraday					
NF	5	4.95	99	0.3559	0.3589
	7	6.98	99.71		
	10	9.94	99.40		
TZ	2	2.004	100.2	0.7251	0.7288
	4	3.95	98.75		
	6	5.97	99.50		
Interday					
NF	5	4.97	99.40	0.06428	0.6469
	7	6.96	99.42		
	10	9.93	99.30		
TZ	2	1.99	99.50	0.7329	0.7382
	4	3.96	99		
	6	5.96	99.33		

Repeatability

Table No. 05: Result of Repeatability studies

Repeatability		NF	TZ
	SD*	0.0052	0.0011
	%RSD*	0.666	0.4135

* = Average of 6 Estimates.

Accuracy (Recovery studies)

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80%, 100%, and 120%. Known amounts of a standard mixture of norfloxacin and tinidazole were added to pre-analyzed samples and were subjected to the proposed UV Spectrophotometric method. The results of recovery studies are shown in Table 6.

Table No. 06: Result of Accuracy (Recovery studies)

Content	**% Level of Addition**	**Conc. µg/ml**		**Total Conc. µg/ml**	**Drug Recover µg/ml**	**% Recovery**	**Mean**	**SD**	**%RSD**
		Std	**Addition**						
NF	80%	5	4	9	8.98	99.77	99.73	0.0630	0.0631
		5	4	9	8.98	99.77			
		5	4	9	8.97	99.66			
	100%	5	5	10	9.96	99.60	99.63	0.0570	0.0580
		5	5	10	9.97	99.70			
		5	5	10	9.96	99.60			
	120%	5	6	11	10.97	99.72	99.75	0.4110	0.4120
		5	6	11	10.93	99.36			
		5	6	11	11.02	100.18			
TZ	80%	5	4	9	8.97	99.66	99.73	0.2347	0.2353
		5	4	9	8.96	99.55			
		5	4	9	8.90	100			
	100%	5	5	10	9.97	99.70	99.93	0.3223	0.3225
		5	5	10	10.03	100.30			
		5	5	10	9.98	99.80			
	120%	5	6	11	10.98	99.81	99.78	0.0519	0.0520
		5	6	11	10.97	99.72			
		5	6	11	10.98	99.81			

Ruggedness of method

To evaluate the robustness of the developed UV Spectrophotometric method, small deliberate variations in the optimized method parameters were done. The ruggedness of the proposed method was determined by analysis of dilutions from standard solutions by different analysts using similar operational and environmental conditions.

Table No. 07: Result of Ruggedness studies

Contents	Analyst	Conc. µg/ml	Mean Absorbance	SD	%RSD
NF	Analyst I	5	0.793	0.0035	0.4418
		5			
		5			
	Analyst II	5	0.794	0.0020	0.2621
		5			
		5			
TZ	Analyst I	5	0.282	0.0036	1.275
		5			
		5			
	Analyst II	5	0.279	0.0036	1.29
		5			
		5			

Robustness of method

Robustness of the proposed method was determined by analysis of dilutions from standard solution by measuring the absorbance 278nm, 276nm, 272nm, and 312nm, 316nm, 318nm for Norfloxacin and Tinidazole respectively.

Table No. 08: Result of Robustness studies

Contents	Conc. µg/ml	λmax (nm)	Absorbance			Mean	S. D.	(%) RSD
			I	II	III			
NF	5	278	0.768	0.767	0.768	0.767	0.001	0.1303
	5	276	0.798	0.799	0.800	0.799	0.001	0.1251
	5	272	0.756	0.767	0.767	0.756	0.001	0.1322
TZ	5	312	0.256	0.257	0.257	0.256	0.001	0.3906
	5	316	0.279	0.280	0.281	0.280	0.001	0.3571
	5	318	0.265	0.266	0.264	0.265	0.001	0.3773

RESULTS AND DISCUSSION

To develop and validate a precise, accurate and suitable UV visible spectrophotometric method for the estimation of norfloxacin and tinidazole, in acetonitrile and water at 50:50 ratio were tried and validated as per ICH guidelines for linearity [Table 3], repeatability [Table 5], intermediate precision (inter-day and intra-day precision studies) [Table 4], LOD [Table 1], LOQ [Table 1] shows within a range. The proposed spectrophotometric method were found to be appropriate for the quantitative determination. Under the experimental conditions described, calibration curves, an assay of Tablet, and recovery studies were performed. The mean % content of Norfloxacin 99.66% & 98.27 and Tinidazole 99.35% & 101.12 for marketed formulation Nor-TZ and Norflox-TZ respectively [Table 2]. The mean % recoveries of NF and TZ from bulk were found to be 99.70% and 99.81 % respectively [Table 6]. The ruggedness and robustness of the developed methods were determined by evaluating the effect of change in instrument wavelength 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, the straightforward UV spectrophotometric method (Vierodt's Method (Simultaneous Equation Method) was developed for his or her simultaneous analysis. The standard deviation, % RSD calculated for the methods are low, indicating a high degree of precision of the methods. The %RSD is additionally but 2% as needed by ICH guidelines. The % recovery was between 98- 102% indicating a high degree of accuracy of the proposed UV spectrophotometric method. The developed method is simple, rapid, precise, accurate, and can be employed for the routine estimation of NF and TZ in both bulk and injection dosage form.

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