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UTILIZATION OF UV SPECTROPHOTOMETRIC TECHNIQUES FOR THE CONCURRENT QUANTIFICATION OF NORFLOXACIN AND TINIDAZOLE IN BOTH BULK SUBSTANCES AND TABLET FORMULATIONS

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ABSTRACT

Two simple, accurate simultaneous and reproducible simulations for spectrometric methods for norfloxacin and tinidazole developed pharmaceutical dosage forms..The first method includes determination by the Vierodt method (Simultaneous Equation Method); the selected sample wavelengths are 273 nm and 319 which span the concentration ranges of 2.5-20 µg/mL and 5-40 µg/mL for norfloxacin and tinidazole. The results were analysis were statistically validated and recovery studies were performed according to ICH guidelines. The developed methods are simple, fast, precise and accurate and usable for direct estimation of NF and TZ.

Keywords: Norfloxacin, Tinidazole, UV validation

INTRODUCTION

The spectrophotometric method of this study aims to develop and evaluate the UV validation of norfloxacin and tinidazole Bulkand Tablet Dosage Forms.

1. **Norfloxacin:**

It is a first-generation synthetic floroquinolone as a synthetic chemotherapy antibacterial agent. It is used to treat both common and complicated urinary tract infections (1,2).

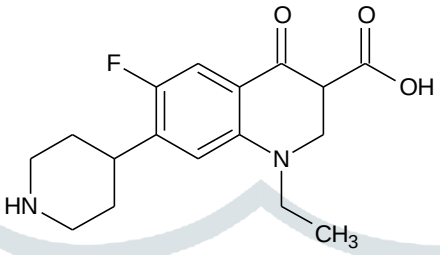

Fig.1. Norfloxacin

- Molecular Formula:C16H18FN3O3
- Chemical Name: 1- Ethyl - 6- fluoro-1,4- dihydro - 4oxo-7-(1-piperazinyl) 3 quinoline carboxylic acid
- Molecular Weight:319.33g/mol
- Description: White to light-yellow crystalline powder
- Solubility: Slightly soluble in ethanol, methanol.Very slightly soluble in water.
- Melting Point: 220-221°C
- Category: Antibacteria

Mechanism of action

It is a broad spectrum antibiotics showing activity against gram negative and gram positive bacteria . It inhibits bacterial cell division by inhibiting DNA gyrase, topoisomerase II and IV which are necessary enzymes for causing separation of bacterial DNA (3). Several methods have been widely used to detect ions in drugs, such as capillary electrophoresis, HPLC, TLC (4), LC, voltammetry, ISE (5), differential pulse polarography, fluorimetry, and potentiometric titration (6).

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 subject of the monograph is the British Pharmacopoeia (BP) (7) and the United State Pharmacopoeia (USP) (8). Due to the therapeutic importance of NF, many

analytical methods have been developed for its deteremination. Bulk goods, dosage forms and biological fluids T. The spectrophotometric technique is that the most generally utilized in pharmaceutical analysis.

A review of the literature indicated that several methods have been reported for the evaluation of norfloxacin[1] and tinidazole[2] (9,10) alone or in combination (11,12) with other drugs. Other analysis methods were also used such as HPLC (13), Electrochemical analysis (14), Difference spectroscopy (15), capillary electrophoresis and stability studies (16).

2. Tinidazole:

It is a derivative of 2methyl imidazole , which belongs to the class of nitroimidazole antibiotics. It is an anti-parasitic drug and used for a treatment of various amoebic and parasitic infections. (17).

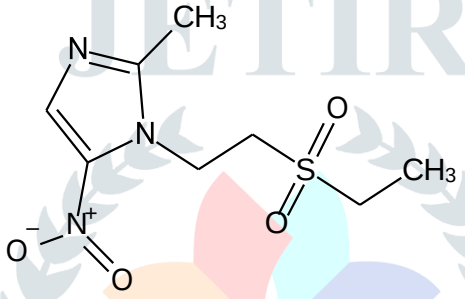

Fig.2. Tinidazole

- Molecular Formula: C8H13N3O4S
- Chemical Name: 1-(2-ethyl sulfonyl ethyl)-2methyl-5-nitro-imidazole
- Molecular Weight: 247.27g/mol
- Description: White or pale yellow crystalline powder
- Solubility: Practically insoluble in water soluble in aetone 6 and in methylene chloride, sparingly soluble in methanol
- Melting Point: 127-128°C
- Category: Antiprotozoal, Antibacterial

Mechanism of action

Tinidazole is a prodrug and antiprotozoal agent. Trichomonas ferredoxin-mediated electron transport system reduces the nitro group of tinidazole. It is believed that the free radicals produced as a result of this reduction are responsible for the anti-protozoal activity. Toxic free radicals have ben hypothesized to covalently bind DNA, causing DNA damage and leading to cell death. The mechanism by which tinidazole affects Giardia and Entamoe species is

unknown, although it is likely to be similar. A review of the literature shows that tinidazole is official in the USP (18) and BP(19). Tinidazole was determined by high performance liquid chromatography (20), spectrophotometry (21), voltammetry (22), capillary electrophoresis (23), flow injection analysis (24) and gas chromatography FID (25) .

Tinidazole (TZ) [1-(2-(ethyl sulphonyl ethyl)-2-methyl-5-nitro imiddazole] is effective against protozoa and bacteria (Fig.2). It is used to treat amoebiasis, giardiasis and trichomoniasis. TZ is the subject of a monograph in each BP (7) IP, there are several reports for the determination of TZ (26,27) and preparation and biological fluids, namely: spectrophotometry, HPLC (13), titrimetric differential spectrophotometric analysis (28) and potentiometry (29). The combination of NF and TZ is commercially available as tablets for the control of gastrointestinal infections caused by bacterial and / or amoebic infections, prostatitis and tract infections caused by susceptible uropathogens. Both drugs were simultaneously determined by spectrophotometry, HPLC, electrochemical analysis (14) and capillary electrophoresis (10). Differential spectrophotometric (15) stability-indicating method of analysis (16).

MATERIALS AND METHODS (30)

Instrumentation

A Shimadzu UV/Visible spectrophotometer, Model 1700 (Japan) with a spectral band width of 2 nm and a wavelength accuracy of ±0.5 nm was used, and automatic wavelength correction was used. 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.

Chemicals and Reagents

Analytical pure NF and TZ samples (Hindustan Antibiotic Limited, Pimpri, Pune, India) were used in the study. Hindustan Antibiotic Limited, Pimpri, Pune, India. contains 400 mg NF and 600 mg TZ.

Preparation of Standard Stock Solution:

NF and TZ standard stock solutions (100 µg/ml) were prepared by dissolving 10 mg of each drug separately in 50 ml of methanol and adding water to make up to 100 ml.

Working standard solutions of these drugs were obtained by diluting the corresponding stock solution with water.

Preparation of Sample Stock Solution

An accurately weighed powder sample corresponding to 10 mg of NF was transferred to a 100 mL volumetric flask and dissolved in 50 mL and sonicated with 1500 mL double distilled HPLC grade water. It was then filtered through Whatmann No. 41 filter paper. 1 The solution was appropriately diluted with double-distilled HPLC-grade water to obtain sample solutions containing NF and TZ concentrations of 2:3 µg/ml, respectively, as in the formulation. The final concentrations are 10 µg/ml NF and 15 µg/ml TZ.

Calibration curve

Calibration curves were prepared by making appropriate dilutions of stock solutions of norfloxacin and tinidazole in different 10 ml volumetric flasks and diluted to the mark with the mobile phase to give final norfloxacin concentrations of 01-11 µg/ml and 1-25 µg/ml tinidazole. Standard solutions were analyzed at wavelengths of 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) (31)

Construction of calibration curve

For the simultaneous equation method, two sample wavelengths and TZ showed linearity with absorbances in the range of 01-11 µl /mL and 01-25 µg/mL at the respective selected wavelengths. The correlation coefficient of NZ and TZ was 0.9998, and 0.9998, respectively.

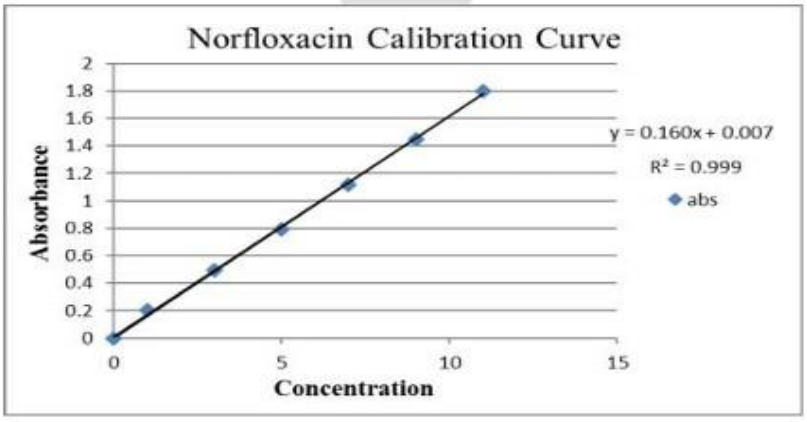

Fig.3. Calibration curve of Norfloxacin

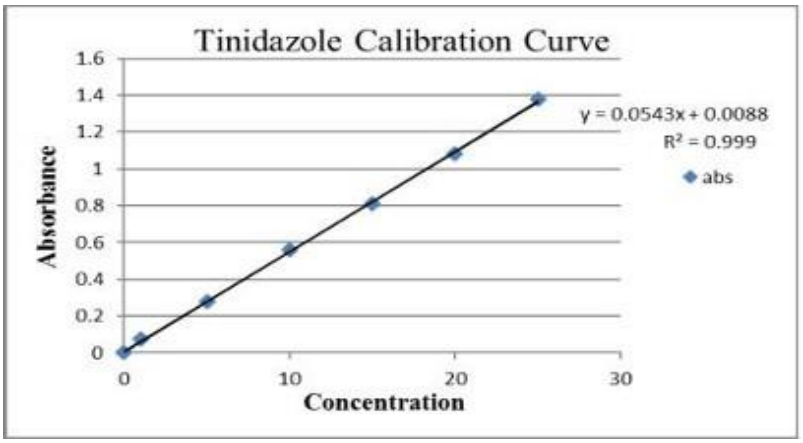

Fig.4. Calibration curve of Tinidazole

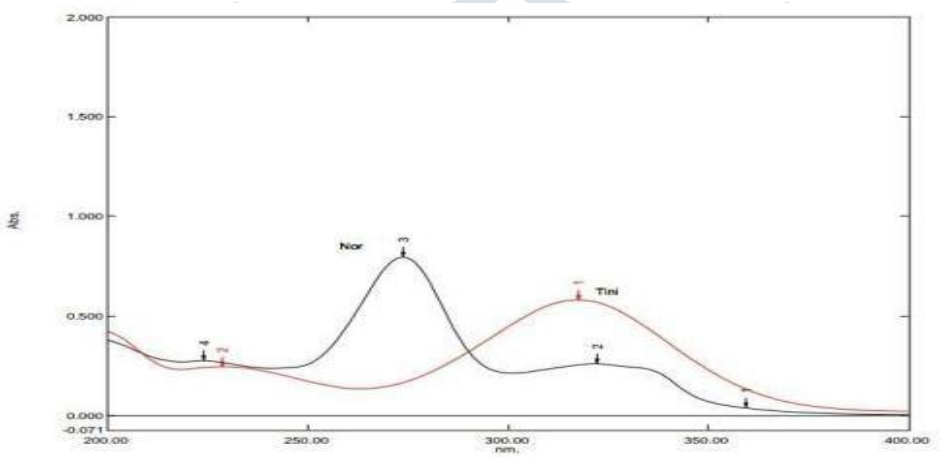

Fig.5. Overlain spectra of NF and TZ

Assay of Tablet Formulation

The powder corresponding to 10 mg of NF and 15 mg of TZ was weighed and transferred to 100 mL and dissolved in 50 mL of methanol, and water was added to 100 mL by sonication for 15 min. The solution was then filtered through Whatmann filter paper No.41 and further diluted to give a final NF concentration of 10 µg/ml and a TZ concentration of 15 µg / ml. Sample solutions were analyzed according to the mixed standard procedure. The concentrations of each drug in the sample solutions were calculated using equations (I) and (II) of the simultaneous equation method of Vierodt and, for multicomponent analysis, using the multicomponent mode of the instrument for the Multicomponent method of analysis. (32)

Method Validation (33)

Linearity

Linearity was investigated by preparing standard solutions at different concentration levels. The linearity range was found to be 01-11 µg/ml for norfloxacin and 5-25 µg/ml for

tinidazole. The regression equation was found to be y = 0.160x + 0.007 for norfloxacin and y = 0.054 x + 0.008 for tinidazole with correlation coefficients of 0.9999 and 0.9998.

Table No.01: 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 accuracy of the methods was investigated in terms of intraday , interday and repeatability. The intraday study was performed analysing three times in the same day (morning, afternoon and evening). Interday precision was performed by analyzing 17 three different drug concentrations over two days. Reproducibility was performed when analyzing the same drug concentration. Method precision was expressed as relative standard deviation (% RSD) and standard deviation (SD). The percent relative standard deviation (%RSD) was found to be less than 2% for intraday and day variation, indicating that the method is accurate.

Table No.2: Result of Precision Studies

Contents	Conc.	Amount Recover	% Recovery	SD	% RSD
Intraday					
NF	5	4.97	99	0.3559	0.3589
	7	6.96	99.71		
	10	9.93	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		

Accuracy

Accuracy (Recovery studies) To check the degree of 17 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 21 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 3.

Table No.3: 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 the method

To assess the robustness of the developed UV spectrometric method, small deliberate variations were made in the parameters of the optimized method. The robustness of the proposed method was determined by analyzing dilutions of standard solutions with different analysts using similar operational and environmental conditions.

Robustness of the method

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

LOD (Limit of Detection)

The detection limit of an analytical procedures the smallest amount of analyte in a sample that can, but not necessarily, be detected.quantified as an exact value. For assay purpose limit of detection was not considered as the validation parameter as per ICH guideline.

LOQ (Limit of Quantification)

The limit of quantification of a single analytical procedure is the smallest amount of analyte in a sample that can be quantified with reasonable accuracy. The limit of quantification is a parameter for the low-level quantification of compounds in sample matrices and is used specifically for the determination of impurities and/or degradation products. Therefore, this analysis is not considered a valid parameter.

RESULTS AND DISCUSSION

An accurate, precise and suitable UV-visible spectrophotometric method for the determination of norfloxacin and tinidazole in acetonitrile and water in a ratio of 50:50 has been tested and validated and validated according to ICH guidelines for linearity [Table 1], intermediate precision (inter-day and intra-day precision studies). The proposed spectrophotometric method were found to be appropriate for the quantitative determination. Calibration curves, array determination and recovery studies were performed under the described experimental conditions..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 3]. 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

A combination of gastrointestinal NF infections and infections caused by TZ is a commercially available bacterial infection or amoebic tablet infection to control prostatitis and 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.

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