Original Article

Spectrophotometric methods for the simultaneous estimation of ofloxacin and tinidazole in bulk and pharmaceutical dosage form

Abstract

Aim: This work deals with the simultaneous estimation of Ofloxacin (OFL) and Tinidazole (TNZ) in in bulk and pharmaceutical dosage form, without prior separation, by three different techniques (Simultaneous equation, Absorbance ratio method and First order derivative method). **Materials and Methods:** The present work was carried out on Shimadzu electron UV1800 double beam UV-Visible spectrophotometer. The absorption spectra of reference and test solutions were carried out in 1 cm matched quartz cell over the range of 200 - 400 nm. Standard gift sample of OFL and TNZ obtain from Torrent pharmaceuticals Ltd., Baddi, Himachal Pradesh. Combined OFL and TNZ tablets were purchased from local market. Methanol from Merck Ltd and distilled water are used as solvent. **Results:** The first method is the application of simultaneous equation. Where the linearity ranges for OFL and TNZ were 5-30 µg/ml and 10-50 µg/ml respectively. The second method is the determination of ratio of absorbance at 278nm, the maximum absorption of TNZ and isobestic wavelength 283 nm, the linearity ranges for OFL and TNZ were 5-30 µg/ml and 10-50µg/ml respectively. The third method is the first order derivative method, where the linearity ranges for OFL and TNZ were 5-30 µg/ml and 10-50 µg/ml respectively. The results of the analysis have been validated statistically and by recovery studies, where the percentage recovery was found to be 100.9±0.49 and 97.30±0.20 using the simultaneous equation method, 98±0.45 and 100.4±0.48 using the graphical absorbance ratio method and 99.10±0.40 and 84.70±0.70 using first derivative method, for OFL and TNZ respectively. **Conclusions:** The proposed procedures are rapid, simple, require no preliminary separation steps and can be used for routine analysis of both drugs in quality control laboratories.

Key words:
Absorbance ratio method, first derivative method, simultaneous equation method, ofloxacin, tinidazole

Introduction

Ofloxacin (OFL) is a synthetic broad-spectrum antibacterial agent. Chemically, OFL,[1] a fluorinated carboxyquinolone, is a racemate, (±)- 9-fluro-2, 3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido [1,2,3-de]-1,4-benzoxazine-6-carboxylic acid. It is official in BP,[2] USP,[3] and EP.[4] It is mainly used as antibacterial for the treatment of urinary tract infection and sexually transmitted diseases.

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Tinidazole (TNZ) [1-(2-ethylsulfonylethyl)-2-methyl-5-nitroimidazole] is a 5-nitroimidazole derivative, an antiparasitic drug used against protozoan infections. It is also used in the treatment of a variety of amebic and parasitic infections.[5]

Literature survey reveals that OFL was determined by several methods including spectrophotometric,[6] HPLC,[7-9] and chemiluminescence method.[10,11] HPLC,[12-18]

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spectrophotometric,[19,20] and capillary electrophoresis[21,22] methods have been reported for the estimation of OFL in combination with the other drugs. TNZ was determined by spectrophotometric,[23,24] HPLC,[25,26] and electrochemical study.[27] Some methods have also been reported for the determination of TNZ in combination with other drugs, including spectrophotometric,[28-31] capillary electrophoresis,[32] and differential pulse polarography.[33] Literature survey revealed that spectrophotometric[34] and HPLC[35,36] methods have been reported for the estimation of OFL and TNZ in pharmaceutical formulations. The aim of this paper was to explore the possibility of using techniques of simultaneous equation, the absorbance ratio (Q-analysis), and first derivative method for quantifying OFL and TNZ simultaneously in their mixture forms. The proposed methods are simple, convenient, precise, accurate, and economical than the reported method.

Materials and Methods

The present work was carried out on Shimadzu electron UV1800 double beam UV-Visible spectrophotometer. The absorption spectra of reference and test solutions were carried out in 1-cm matched quartz cell over the range of 200 to 400 nm. Standard gift samples of OFL and TNZ were from Torrent pharmaceuticals Ltd, Baddi, Himachal Pradesh. Combined OFL and TNZ tablets were purchased from local market. Methanol from Merck Ltd and distilled water are used as solvent.

Experiment

Procedure

Simultaneous equation method (Method-I)

Standard stock solutions (1 mg/ml) of OFL and TNZ were prepared by dissolving 25 mg of each in 25 ml 0.1N HCl, which was further diluted with 0.1N HCl to get the working standard solution (100 µg/ml) of OFL and TNZ. From this, suitable aliquots are taken and diluted with 0.1N HCl to get 10 µg/ml of OFL and 30 µg/ml of TNZ. The absorption spectra of all the solutions were recorded between 200 and 400 nm. The absorbances were measured for OFL and TNZ at 227 nm (λ_1) (maximum absorbance of OFL) and 278 nm (λ_2) (maximum absorbance of TNZ), respectively. Wavelengths 227 nm and 278 nm were selected for the formation of simultaneous equation [Figure 1]. The absorbances were measured at the selected wavelengths. The molar absorptivity values were 569.8 at λ_1 and 540 at λ_2 for TNZ. The absorbance and absorptivity values were substituted in the following equation to obtain the concentrations:

Cx=A2ay1 – A1ay2 / ax2ay1 – ax1ay2

Cy=A1ax2 – A2ax1 / ay1ax2 – ay2ax1

Where, A_1 and A_2 are absorbances of the mixture at λ_1 and λ_2, respectively; ax_1 and ax_2 are absorptivity of X at λ_1 and λ_2, respectively; ay_1 and ay_2 denotes absorptivity of Y at λ_1 and λ_2, respectively; and Cx and Cy are concentrations of OFL and TNZ, respectively.

The graphical absorbance ratio method (Q-analysis method) (Method-II)

In the quantitative assay of two components by absorbance ratio method, absorbances were measured at two wavelength, one being the isosbestic wavelength and the other being wavelength of maximum absorption of one of the two components. From overlain spectra of OFL and TNZ, absorbances were measured at the selected wavelength, i.e., 283 nm (isosbestic wavelength) and 278 nm (wavelength of maximum absorption of TNZ) [Figure 2]. The concentration of each component can be calculated by mathematical treatment of the following mentioned equation.

For OFL,

C_1=Qm–Qy/Qx–Qy.A_1/a

For TNZ,

C_2=Qm–Qx/Qy–Qx. A_1/a

Where,

C_1=Concentration of OFL

C_2=Concentration of TNZ

A_1=Absorbance of sample at isosbestic wavelength (283 nm)

a=Absorptivity of OFL and TNZ at isosbestic wavelength (283 nm)

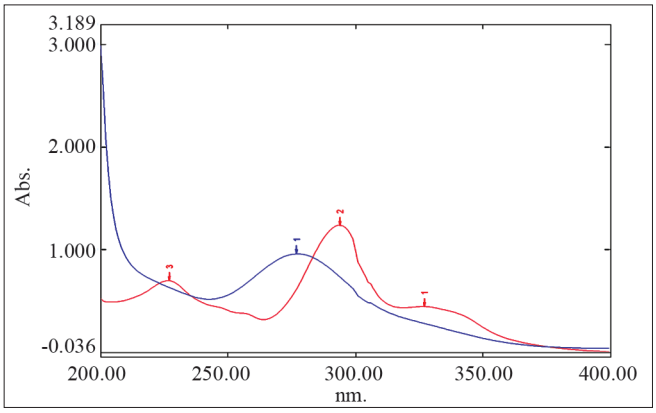

Figure 1: Overlain spectra of OFL and TNZ for simultaneous equation method

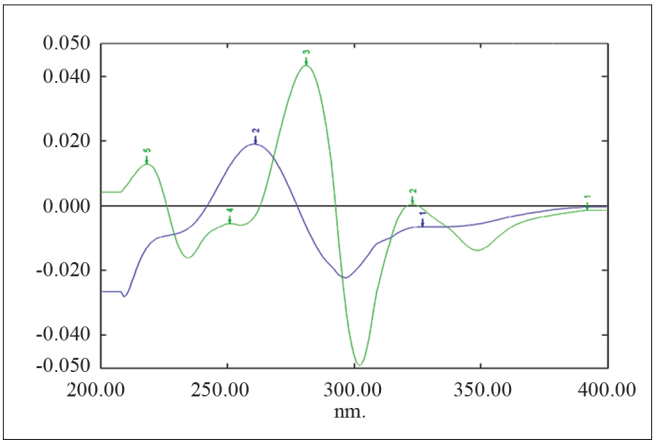

Figure 2: Overlain first derivative spectra of OFL and TNZ

Qx=Absorptivity of OFL at 278 nm/Absorptivity of OFL at 283 nm
Qy=Absorptivity of TNZ at 278 nm/Absorptivity of TNZ at 283 nm
Qm=Absorptivity of sample solution at 278 nm/Absorptivity of sample solution at 283 nm.

First-order derivative method (Method III)
Solutions of 10 µg/ml of OFL and TNZ were prepared separately. Both the solutions were scanned in the spectrum mode from 200 to 400 nm. The absorption spectra thus obtained were derivatized from first to fourth order. First-order derivative (n=1) was selected for analysis of both the drugs. The zero crossing wavelengths, 277.6 nm and 261.7 nm were selected for OFL and TNZ, respectively [Figure 2].

Preparation of calibration curve
Six mixed standards having concentration 5, 10, 15, 20, 25, and 30 µg/ml of OFL and 10, 20, 30, 40, 50, and 60 µg/ml of TNZ, respectively, were prepared and scanned in the spectrum mode from 200 to 400 nm. The absorption spectra so obtained were derivatized to obtain first derivative order spectra. The absorbances of OFL and TNZ were measured at 277.6 nm and 261.7 nm, respectively, and calibration curve of both the drugs were plotted separately. The concentration of individual drug present in the mixture was determined against calibration curve in quantitation mode.

Application of the proposed procedure for the estimation of OFL and TNZ in tablet
Twenty tablets were weighed and average weight was calculated. The tablets were crushed to fine powder. The powder equivalent to 10 mg of OFL and 30 mg of TNZ was transferred to 100 ml volumetric flask. The powder was dissolved in 70 ml of 0.1N HCl by intermittent shaking followed by sonication for 30 minutes and then the volume was made up to 100 ml with 0.1N HCl. The solution was then filtered through a Whatman filter paper (No. 41). The solution was diluted further with 0.1N HCl to obtain 15 µg/ml of OFL and 30 µg/ml of TNZ. The concentrations of both OFL and TNZ were determined by measuring the absorbance of the samples at 227 nm (λ_{max} for OFL), 278 nm (λ_{max} for TNZ), and 283 nm (isosbestic point). The recorded data were then substituted in the equation and results obtained are summarized in Table 1. The analysis procedure was repeated three times. The selectivity of the proposed procedure was examined by determining the recovery of the two drugs by standard addition method [Table 2].

Results and Discussion

The proposed methods were found to be simple, accurate, economic, and rapid for routine simultaneous estimation of two drugs. The values of relative standard deviation (RSD) are satisfactorily low and recovery was closed to 100%, indicating reproducibility and accuracy of all methods. These methods also gave excellent result and can be employed for routine analysis of these two drugs in combined dosage form.

In simultaneous equation method, the overlay spectra of OFL and TNZ show overlap that prevents the use of direct absorbance measurement for determination of both the

Table 1: Determination of OFL and TNZ in tablet using the proposed methods

	Simultaneous equation method recovery (%)±SD (n=3)		Graphical absorbance ratio method recovery (%)±SD (n=3)		First derivative method recovery (%)±SD (n=3)	
	OFL	TNZ	OFL	TNZ	OFL	TNZ
OFLER-TZ Tablet	100.9±0.49	97.3±0.20	98.0±0.45	100.4±0.48	99.1±0.42	84.7±0.71
RSD %	0.491	0.207	0.455	0.483	0.426	0.814

SD – Standard deviation; RSD – Relative standard deviation; OFL - Ofloxacin; TNZ - Tinidazole

Table 2: Results of the application of the standard addition technique to the simultaneous determination of OFL and TNZ in tablet by the proposed method (n=3)

Claimed amount taken (µg/ml)		Standard added (µg/ml)		Recovery of added standard (%)±SD					
				OFL			TNZ		
OFL	TNZ	OFL	TNZ	SEM	Q– analysis	First derivative	SEM	Q–analysis	First derivative
20	60	8	24	95.5±1.7	98.5±0.222	88.2±0.305	93.12±0.7	93.4±0.177	100.3±0.24
20	60	10	30	90.0±1.1	86.23±0.244	93.23±0.3	96.3±1.3	102.4±0.444	102.4±0.39
20	60	12	36	99.4±0.8	92.2±0.444	98.12±0.299	84.3±0.9	98.0±0.466	107.2±0.6
Mean				94.9	92.3	93.18	91.24	99.93	103.3
SD				1.2	0.303	0.298	0.96	0.362	0.40

SD – Standard deviation; SEM – Simultaneous equation method; Q analysis – Graphical absorbance ratio method; OFL - Ofloxacin; TNZ - Tinidazole

Table 3: Precision and accuracy of spectrophotometric method developed for analysis of tablet

	Simultaneous equation method	Graphical absorbance ratio method	First derivative method
OFL			
Amount found (Mean % ± SD)	94.9 ± 1.2	92.3 ± 0.303	93.18 ± 0.298
Accuracy, Bias (%)	-0.05	-0.083	-0.073
Precision, RSD (%)	1.56	0.99	0.188
TNZ			
Amount found (Mean % ± SD)	91.24 ± 0.96	99.93 ± 0.362	103.3 ± 0.40
Accuracy, Bias (%)	-0.096	-0.021	0.031
Precision, RSD (%)	0.7	0.819	0.65

SD – Standard deviation; % Bias – [100(found-added)/added]; RSD – Relative standard deviation

drugs in their mixture. Figure 1 shows the λ_{max} for OFL at 227 nm and for TNZ at 278 nm. The absorbance curves at the selected wavelengths were found to be proportional to the corresponding concentration of the two drugs in the range of 5 to 30 μg/ml for OFL and 10 to 50 μg/ml for TNZ. The absorptivity values of the drugs were determined at selected wavelengths. The absorptivity is the ratio of mean absorbance of the drug at selected wavelength with the concentration of component in mg/ml. These absorptivity values were the mean of six independent determinations. A set of two simultaneous equations obtained by using mean absorptivity values are given below:

A_1=569.8 C_{OFL}+195.4 C_{TNZ} ---------- (at λ_{227})

A_2=540 C_{OFL}+291.4 C_{TNZ} ---------- (at λ_{278})

Here, A_1 and A_2 are absorbance of the sample at 227 nm and 278 nm, respectively. 569.8 and 540 are the absorptivity values of OFL at 227 nm and 278 nm, respectively. 195.4 and 291.4 are the absorptivity values of TNZ at 227 nm and 278 nm, respectively. C_{OFL} is the concentration of the OFL and C_{TNZ} is the concentration of TNZ in mg/ml.

The proposed absorbance ratio method is also a simple method. In this method, the absorbances of the sample solution at the two selected wavelengths, 278 nm and 283 nm, were measured and few calculations were done.

The first derivative spectrophotometry method requires spectral data processing and hence can be applied only on recording spectrophotometers with such facilities. This method was employed to totally eliminate the spectral interference from one of two drugs while eliminating the other drug. This was achieved by selecting the zero crossing point on the derivative spectra of one drug as the wavelength for the estimation of other drug. First derivative method is simple, less time consuming, no manual calculation, and gives marginally better result than absorbance ratio method.

Validation of methods

The methods were validated with respects to linearity, limit of detection, limit of quantification, precision, accuracy, and selectivity/sensitivity.

Accuracy was investigated by analyzing three different concentration of binary mixture of OFL and TNZ in linear range in six independent replicates. The data evaluated using equations are summarized in Table 3. Accuracy was expressed as bias (%). The bias values were close to zero [Table 3]. The RSD values and also the low RSD values obtained from the analysis of pharmaceutical formulations indicated that the intermediate precision of the method was good.

Conclusion

The proposed method based on simultaneous equation, graphical absorbance ratio, and first-order derivative methods can be used for the simultaneous estimation of OFL and TNZ in their bulk and pharmaceutical dosage form. The proposed methods are precise, accurate, and simple to perform. Also, no separation step is required. Hence, the proposed methods can be used for the routine analysis of OFL and TNZ.

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