ORIGINAL RESEARCH

DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHODS FOR SIMULTANEOUS ESTIMATION OF OFLOXACIN AND TINIDAZOLE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT: A sensitive and validated UV method have been developed for the simultaneous estimation of Ofloxacin (OFL) and Tinidazole (TNZ) in bulk and pharmaceutical dosage form, without prior separation, by three different techniques (Simultaneous equation, Absorbance ratio method and Dual wavelength method). The 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. The first method is the application of simultaneous equation, where the linearity ranges for Ofloxacin and Tinidazole were 2-10 µg/ml and 5-15 µg/ml respectively. The second method is the dual wavelength method, where the linearity ranges for Ofloxacin and Tinidazole were 2-10 µg/ml and 5-15 µg/ml respectively. The third method is the determination of ratio of absorbance at 294.6 nm, the maximum absorption of Tinidazole and isobestic wavelength 285.6 nm, the linearity ranges for Ofloxacin and Tinidazole were 2-10 µg/ml and 5-15 µg/ml respectively. The results of the analysis have been validated statistically and by recovery studies. 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.

KEYWORDS: Ofloxacin, Tinidazole, UV spectroscopy and Validation.

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INTRODUCTION[1-7]

Chemically, Ofloxacin (Figure 1) is a racemate, (±)-9-fluro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido [1, 2, 3-de]-1,4-benzoxazine-6-carboxylic acid.[1] It is a synthetic fluoroquinolone antibacterial agent that inhibits the supercoiling activity of bacterial DNA gyrase, halting DNA replication. It is mainly used as antibacterial for the treatment of urinary tract infection and sexually transmitted diseases.

Chemically, Tinidazole (Figure 1) is 1-[2-(ethanesulfonyl)ethyl]-2-methyl-5-nitro-1*H*-imidazole.[2] It is a nitroimidazole antitrichomonal agent effective against Trichomonas vaginalis, Entamoeba histolytica, and Giardia lamblia infections. It is also used in the treatment of a variety of amebic and parasitic infections. Thus, the two drugs can be used in combination to improvise the activity by killing the infection causing bacteria and reducing the infection.[3]

Literature survey reveals that some Spectrophotometric[4] and HPLC[5,6] methods have been reported for the estimation of Ofloxacin and Tinidazole in pharmaceutical formulations.

The aim of this paper was to explore the possibility of using techniques of simultaneous equation, the dual wavelength and the absorbance ratio (Q-analysis) method for quantifying Ofloxacin and Tinidazole simultaneously in their mixture forms. The proposed methods are simple, convenient, precise, accurate, and economical than the reported method and validated as per ICH guidelines.[7]

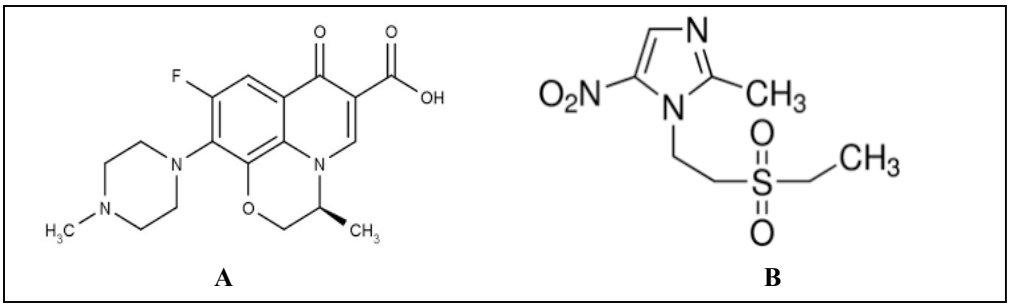

Figure 1. Chemical structures of analytes Chemical structure of A. Ofloxacin and B. Tinidazole

MATERIALS AND METHOD

Instrumentation: To develop a UV spectroscopic method for simultaneous estimation of Ofloxacin and Tinidazole, Shimadzu electron UV1800 double beam UV-Visible spectrophotometer was used. The instrument is equipped with Silicon photodiode detector.

Chemicals and solvents: The working standards of Ofloxacin and Tinidazole were provided as gift samples from Spectrum Pharma Research Solutions, Hyderabad, India. HCl was purchased from E.Merck (India) Ltd., Mumbai, India. Combined Ofloxacin and Tinidazole tablets were purchased from local market.

Preparation of standard stock and working standard solutions of Ofloxacin: 100 mg of Ofloxacin was weighed in to 100 ml volumetric flask and dissolved in 0.1N HCl and then dilute up to the mark with 0.1N HCl to get a concentration of 1000 µg/ml. The solution was diluted accordingly to get a concentration of 100 µg/ml and was kept as the stock solution. The prepared stock solution was diluted with 0.1N HCl solution to get working standard solutions of concentrations 2-10 µg/ml.

Preparation of standard stock and working standard solutions of Tinidazole: 100 mg of Tinidazole was weighed and transferred in to 100 ml volumetric flask and dissolved in 0.1N HCl and then make up to the mark with 0.1N HCl to get a concentration of 1000 µg/ml. The solution was diluted accordingly to get a concentration of 100 µg/ml and was kept as the stock solution. The prepared stock solution was diluted with 0.1N HCl solution to get working standard solutions of concentrations 5-15 µg/ml.

Simultaneous equation method (Method-I): Standard stock solutions (1 mg/ml) of Ofloxacin and Tinidazole 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 Ofloxacin and Tinidazole. From this, suitable aliquots are taken and diluted with 0.1N HCl to get 10 µg/ml of Ofloxacin and Tinidazole. The absorption spectra of all the solutions were recorded between 200 and 400 nm. The absorbances were measured for Ofloxacin and Tinidazole at 294 nm (λ_1) (maximum absorbance of Ofloxacin) and 276 nm (λ_2) (maximum absorbance of Tinidazole), respectively. Wavelengths 294 nm and 276 nm were selected for the formation of simultaneous equation (Figure 2). The absorbances were measured at the selected wavelengths. The absorbance and absorptivity values were substituted in the following equation to obtain the concentrations:

$$C\ x = (A1\ aY2 - A2\ Ay1) / (aX1\ aY2 - aX2\ aY1)$$

$$C\ y = (aX1\ A2 - aX2\ A1) / (aX1\ aY2 - aX2\ ay1)$$

Where, A_1, A_2 are abs. of components, aX_1, aX_2 are absorbitivity of first drug at λ_1 and λ_2 respectively; aY_1, aY_2 are absorbitivity of second drug at λ_1 and λ_2 respectively.

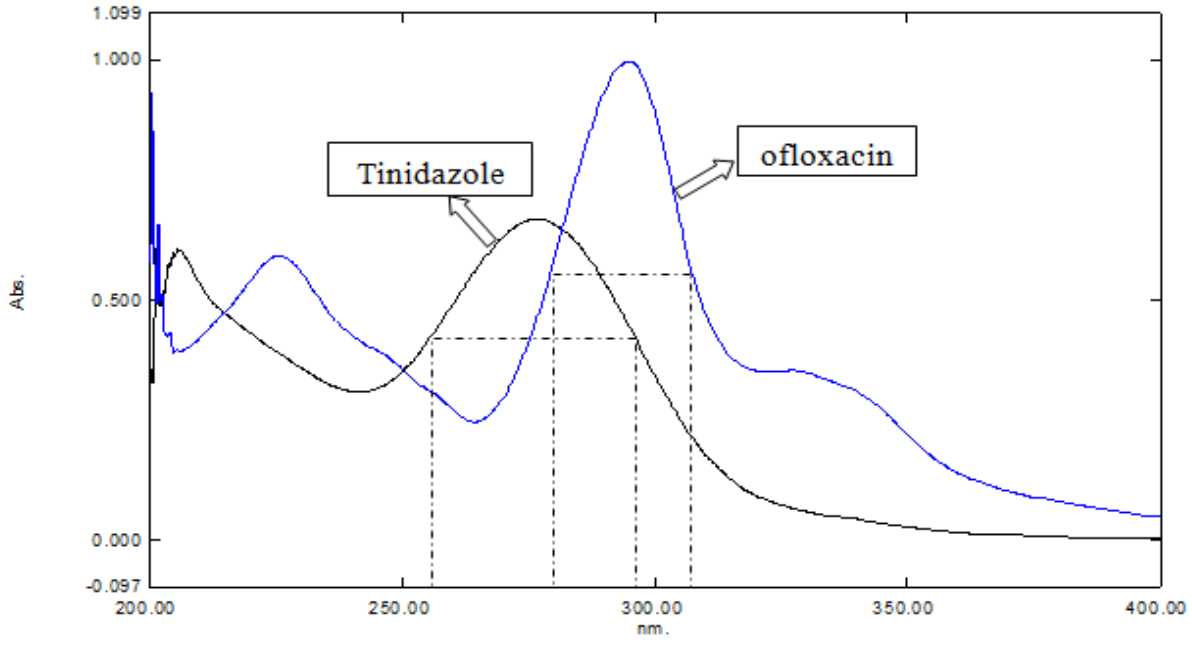

Figure 2. Overlain spectra of Ofloxacin and Tinidazole for simultaneous equation method

Dual Wavelength Method: The utility of dual wavelength data processing programme is to calculate the unknown concentration of a component of interest present in a mixture containing both the component of interest and an unwanted interfering component by the principle of difference in the absorbance between two points on the mixture spectrum. This is directly proportional to the concentration of the component of interest, independent of the interfering components. The prerequisite for dual wavelength method is the selection of two such wavelengths where the interfering component shows same absorbance whereas the component of interest shows significant difference in absorbance with concentration. Based on this criterion, two wavelengths 297.8 nm and 252.2 nm were selected as λ_1 and λ_2 for the estimation of Ofloxacin. Tinidazole shows the same absorbance at these wavelengths. Similarly, wavelengths 277.4 nm and 309.6 nm were selected as λ_3 and λ_4 for the estimation of Tinidazole as Ofloxacin shows the same absorbance at these wavelengths. For calibration curve, the standard stock solutions of these drugs were diluted in the concentration range of 2-10 µg/ml (2, 4, 6, 8 and 10 µg/ml) for Ofloxacin and 5-15 µg/ml (5, 7.5, 10, 12.5 and 15 µg/ml) for Tinidazole. Absorbances were recorded at selected wavelengths.

Absorbance ratio method (Q-analysis method): The absorbance ratio method is a modification of the simultaneous equation procedure. In the USP, this ratio is referred to as Q value. In the quantitative assay of two components in mixture by the absorbance ratio method, absorbance is measured at two wavelengths, one being the λmax of one of the components (λ2) and the other being a wavelength of equal absorptivity of the two components (λ1), i.e., an iso-absorptive point. A series of standard solutions of Ofloxacin and Tinidazole in the concentration range of 2-10 µg/ml and 5-15 µg/ml respectively were prepared in 0.1N HCl and the absorbance of these solutions were measured at 285.6 nm (iso-absorptive point) and 275.8 nm (λmax of Tinidazole). Calibration curves were plotted to verify the Beer's law and the absorptivity values calculated at the respective wavelengths for both the drugs. The concentration of two drugs in mixture was calculated by using the following equations:

$$C_x = (Q_m - Q_y / Q_x - Q_y) \times (A_1 / ax_1)$$

$$C_y = (Q_m - Q_x / Q_y - Q_x) \times A_1 / ay_1)$$

Where, ax_1, ax_2 are A (1%, 1cm) of Ofloxacin at 285.6 nm and 275.8 nm respectively; ay_1, ay_2 are A (1%, 1cm) of Tinidazole at 285.6 nm and 275.8 nm respectively; A_1 and A_2 are the absorbances of mixture at 285.6 nm and 275.8 nm; C_x and C_y are the concentrations of Ofloxacin and Tinidazole in gm/100 ml respectively in sample solution; Q_m is A_2 / A_1, Q_x is ax_2 / ax_1 and Q_y is ay_2 /ay_1.

VALIDATION OF DEVELOPED METHODS

Linearity: A stock solutions were prepared by dissolving 50 mg of the drugs in 50 ml of mobile phase. Then from these stock solutions dilutions of various concentration from 2 to 10 µg/ml and 5-15 µg/ml were prepared for Ofloxacin and Tinidazole respectively. Each dilution was analysed in series to construct the calibration curves. Absorbance of each

dilution was noted and plotted against the concentration of each dilution.

Accuracy: Accuracy was determined by calculating %recovery of Ofloxacin and Tinidazole by standard addition method. The pre-analyzed sample solutions (4 and 5 µg/ml of Ofloxacin and Tinidazole respectively) were spiked with standard drug solutions at three different levels- 50, 100 and 150 %. The resulting mixtures were reanalyzed using the proposed method. The experiment was conducted in triplicates accuracy was reported as % recovery.

Precision: Precision of the proposed method was calculated by conducting intermediate precision.

1. Intra-Day Precision: The intra-day precision was determined by estimating the corresponding absorbance of the drug solution (in triplicates) three times on the same day.

2. Inter-Day Precision: The inter-day precision was established by analysing the drug solution (in triplicates) on three different days.

3. Analyst-Analyst: The analyst to analyst precision was established by analysing the drug solution by different analyst.

The standard deviation, %relative standard deviation and estimated concentrations based on standard curve were reported for each set of data.

Robustness: Robustness of the developed method was determined by injecting the drug solution in triplicates by varying the wavelength (±2). Robustness is reported in %RSD.

LOD and LOQ: Detection limit and Quantitation limit of the drug is calculated by using the calibration curve standards. Detection limit and Quantitation limit were calculated from the equation $3.3\sigma/S$ and $10\sigma/S$ respectively, where σ is the standard devition of y-intercept and S is the slope of the calibration curve.

Specificity: The specificity of the developed method was seen by analyzing solutions containing excipients and pure drug and demonstrating that the result is unaffected by the presence of the excipients present in it.

Assay: 20 tablets were taken and weighed accurately. Then the tablets are crushed to powder. The weight of tablet contents equivalent to 100 mg of Tinidazole and Ofloxacin was calculated and were taken into 100 ml volumetric flask. 50 ml of HCl was added to it and soicated for 10 mins and diluted upto the mark to prepare 1 mg/ml solutions. The resultant solutions were filtered through 0.22 µm syringe driven filter unit. From these solutions, 10 µg/ml dilutions of the drugs were prepared and their absorbances were taken. From the absorbance of the drug solutions, the amounts of each drug in the sample solutions were computed. The results were compared with the label claim of Ofloxacin and Tinidazole in tablet dosage forms. From the results the average %Assay was calculated.

RESULTS AND DISCUSSION

Linearity: The calibration curves (Figure 3) drawn by using the proposed method were found to be linear in the range (2-10 and 5-15 µg/ml for Ofloxacin and Tinidazole respectively). Table 1 shows the calibration data with regression coefficient and %RSD was found to be less than 2.

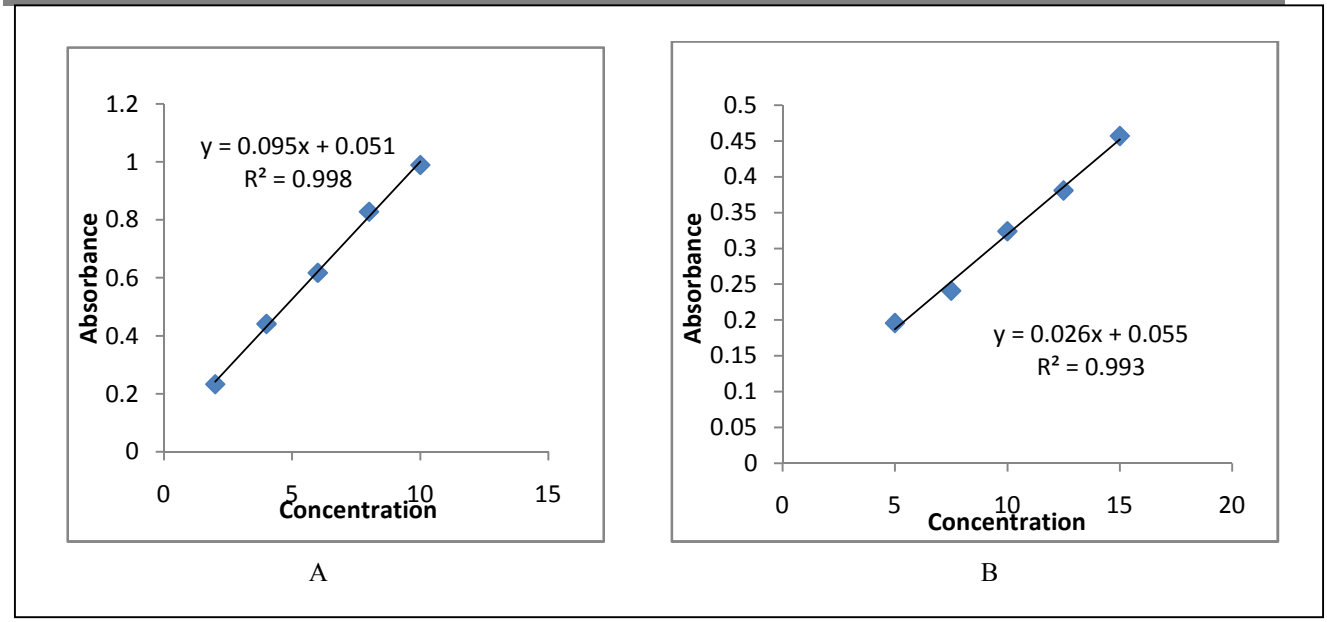

Figure 3. Calibration curves of analytes Calibration curves of A. Ofloxacin and B. Tinidazole

Accuracy (recovery): Accuracy of the methods was determined by using standard addition method and %recovery was found in the range 98-102% and %RSD was within the range for both drugs. Table 1 shows the results of accuracy studies.

Limit of Detection and Limit of Quantification: LOD and LOQ were calculated from the calibration curves of the drugs itself and the results are shows in Table 1.

% Assay: %Assay of the formulation was calculated by three different techniques and %recovery was found in the range 98-102% and %RSD was within the range for both drugs. Table 1 shows the results of %assay studies.

Precision: Intra-day precision was assessed by analyzing the drug at 3 different times in the same day. The method passed the test as the %RSD was found to be less than 2. Inter-day precision was assessed by analyzing the drug for three different days. The method passed the test as the %RSD was found to be less than 2. Analyst to analyst Precision was conducted by two different analysts at the same experimental conditions. Results are shown in Table 1.

Table 1. Summarized results of linearity, accuracy, LOD, LOQ, %assay and Precision

PARAMETERS	Method - I		Method - II		Method - III	
	OFL	TNZ	OFL	TNZ	OFL	TNZ
Detection Wavelength	294 nm	276 nm	252.2 nm 297.8 nm	277.4 nm 309.6 nm	285.6 nm 275.8 nm	285.6 nm 275.8 nm
Linearity and range	2-10 µg/ml	5-15 µg/ml	2-10 µg/ml	5-15 µg/ml	2-10 µg/ml	5-15 µg/ml

Correlation coefficient	0.9983	0.9932	0.9982	0.9973	0.3922	0.5696
Slope	0.095	0.0265	0.0541	0.0205	0.03855	0.2907
Intercept	0.0519	0.055	0.0199	0.0112	0.28517	4.375
Accuracy	100.21	100.77	99.19	100.04	99.98	100.12
Limit of detection	1.218	0.665	1.218	0.665	1.218	0.665
Limit of quantitation	3.692	2.016	3.692	2.016	3.692	2.016
% Assay	100.9±0.4	97.3±0.2	98.0±0.4	100.4±0.4	99.1±0.4	84.7±0.7
Precision						
Intra-day (n=3)	97.63±0.80	97.10±1.0	96.00±0.88	97.13±0.8	97.13±0.8	96.43±5.3
Inter-day (n=3)	87.66±10.4	92.63±7.4	95.36±5.15	101.40±1.7	81.53±17.3	87.36±14.3
Analyst-Analyst	101.40±0.1	99.33±0.6	100.16±0.7	100.86±2.7	101.0 ±0.8	99.23 ±0.8

Where, Method I - Simultaneous equation method, Method II - Dual wavelength method and Method III- Q-ratio method.

Robustness: Robustness studies were performed by varying the detection wavelength (±2). The method was found to be robust. Results are shown in Table 2.

Table 2. Results of robustness studies

λ_{max} (±2 nm)	Concentration (µg/ml)	Observed absorbance			Mean	SD	%RSD
Ofloxacin							
294+2	10	0.91	0.9	0.88	0.896	0.0152	1.7
294-2		0.9	0.91	0.9	0.903	0.0070	0.78
Tinidazole							
276+2	10	0.324	0.333	0.331	0.329	0.0047	0.47
276-2		0.330	0.329	0.337	0.332	0.0043	0.43

Result of specificity: Specificity studies were performed by spiking the formulation and standard drug using two tailed unpaired t-test.

CONCLUSION

The proposed method based on simultaneous equation, dual wavelength and absorption ratio methods can be used for the simultaneous estimation of Ofloxacin and Tinidazole in their bulk and pharmaceutical dosage form. The proposed methods are accurate, reproducible, repeatable, linear, precise, selective, reliable and simple to perform. Also, no separation step is required. These results indicate that the proposed method may find practical applications as a quality-control tool in the simultaneous analysis

of the two drugs in combined dosage forms in quality-control laboratories.

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