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Simultaneous Spectrophotometric Determination of Ofloxacin and Tinidazole from Combined Dosage Forms from Pharmaceutical Formulations

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A new spectrophotometric method for the simultaneous and separate estimation of ofloxacin and tinidazole in binary tablet formulations has been described. The method is based on the estimation of one drug in presence of another drug by absorbance difference method. The ofloxacin solution was scanned over the range 230–390 nm and tinidazole solution was scanned over the range 220–395 nm. In this method, two wavelengths 270 and 300 nm were selected for ofloxacin. At these wavelengths the absorbance was almost zero and in case of tinidazole there should be a considerable absorbance difference. Similarly, the two wavelengths 280 and 335 nm were selected for tinidazole; at these two wavelengths the absorbance difference was almost zero and there should be considerable absorbance difference in case of ofloxacin. In the mixture of ofloxacin and tinidazole solution the absorbance values of four wavelengths 270, 300, 280 and 335 nm were measured. The amount of tinidazole is directly proportional to the absorbance difference between 270 and 300 nm. Similarly the amount of ofloxacin is directly proportional to the absorbance difference between 280 and 335 nm.

Key Words: Spectrophotometry, Ofloxacin, Tinidazole, Pharmaceutical formulations.

INTRODUCTION

The combination formulations of ofloxacin and tinidazole have been in the market for their use in anaerobic infections. Literature describes various methods for the analysis of ofloxacin[1–4] and tinidazole[5] as individual drug products. Only one spectrophotometric method[6] for simultaneous analysis of ofloxacin and tinidazole has been cited. No method for the simultaneous analysis of ofloxacin and tinidazole in binary tablet formulations has been reported by absorbance difference method. The aim of the present work is to develop a simple, rapid, precise, reproducible and economical method for the simultaneous analysis of the binary drug formulations by using absorbance difference method without any interference from each other.

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EXPERIMENAL

A Spectronics-1001 spectrophotometer with 10 mm quartz cells was used for absorbance values of the drug solution. All the chemicals used were of analytical grade. AR grade methanol was used as solvent.

Preparation of standard ofloxacin solution: Pure ofloxacin (50 mg) was dissolved in 50 mL methanol. Further, 1 mL of the stock solution was further diluted to 100 mL with methanol to get working concentration of 40 μg/mL.

Preparation of standard tinidazole solution: 50 mg of pure tinidazole was dissolved in 50 mL methanol to obtain the working concentration of 1 mg/mL. 1 mL of the above stock solution was further diluted to 50 mL with methanol to get a working concentration of 20 μg/mL.

Preparation of mixed solution: Two solutions, the first containing 40 μg/mL of ofloxacin and the second containing 20 μg/mL of tinidazole were used as mixed solutions. Four mixed standard solutions were made by taking 4, 3, 2 and 1 mL of ofloxacin solution in a series of test tubes and the tinidazole stock solution was also added to a series of test tubes to keep the total volume at 5 mL.

Preparation of ofloxacin curve: Various aliquots (5, 6, 7 and 8 mL) of ofloxacin solution were transferred into a series of 10 mL standard flasks and the volume in each flask was adjusted to 10 mL with distilled water. The absorbance of these solutions was scanned over the range of 230 to 390 nm. Overlain spectrum of ofloxacin was shown in Fig. 1. Again various aliquots (5, 6, 7 and 8 mL) of tinidazole solutions were transferred into a series of 10 mL volumetric flasks and the volume in each flask was adjusted to 10 mL with distilled water.

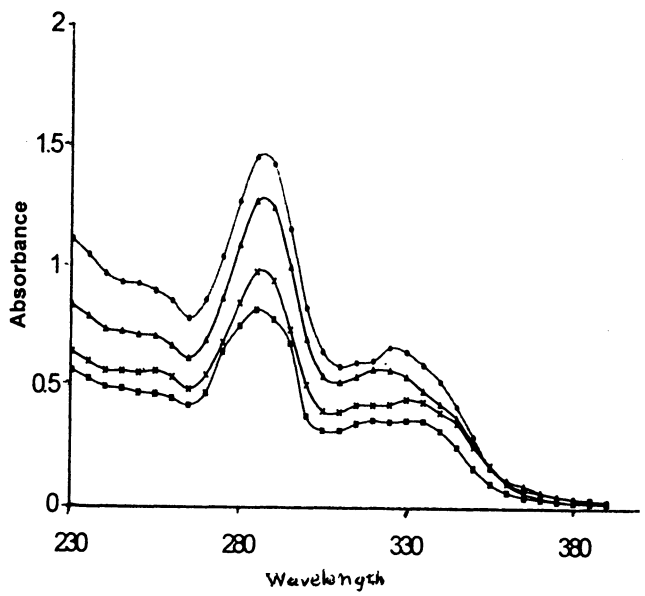

Fig 1. Overlain spectrum of ofloxacin. Absorbance spectra obtained from different concentrations of ofloxacin solution at different wavelengths in the range 206–276 nm. Various concentrations of ofloxacin solution such as 320 (μg/mL) (—•—), 280 (μg/mL) (—▲—), 240 μg/mL (—✖—) and 200 (μg/mL) (—□—).

These solutions were scanned over the range 220–395 nm. Overlain spectrum of tinidazole is shown in Fig. 2. Two wavelengths 270 and 300 nm are selected for ofloxacin; at these two wavelenghts the absorbance values are almost zero and in case of tinidazole at the same wavelength 270 and 300 nm have maximum absorbance difference. A calibration curve was drawn between the absorbance difference values of tinidazole and the amount of tinidazole in μg/mL. The amount of tinidazole present in the sample was estimated from the calibration curve. Similarly two wavelengths 280 and 335 nm were selected for tinidazole; at these two wavelengths the absorbance difference was almost zero and in case of ofloxacin, maximum absorbance difference values were at the same wavelengths 280 and 335 nm. A calibration curve was drawn between the absorbance difference values of ofloxacin and the amount of ofloxacin in μg/mL. The amount of ofloxacin present in the sample was estimated from the calibration curve.

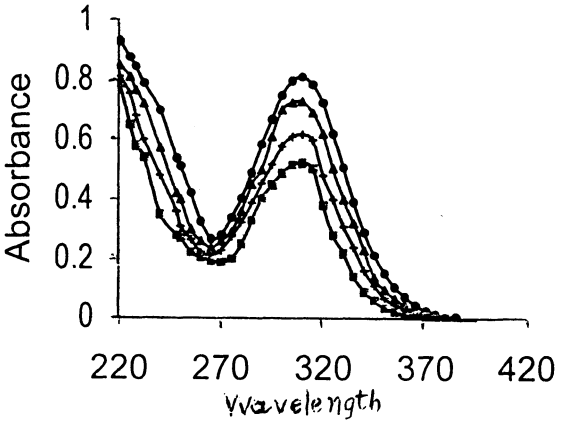

Fig 2. Overlain spectrum of tinidazole. Absorbance spectra obtained from different concentrations of tinidazole solution at different wavelengths in the range 206–276 nm. Various concentrations of tinidazole solution such as 160 (μg/mL) (—•—), 140 (μg/mL) (—▲—), 120 μg/mL (—✚—) and 100 (μg/mL) (—□—).

Various aliquots of mixtures of ofloxacin and tinidazole solutions in different proportions were transferred into a series of test tubes and the volume in each test tube was made 5 mL with distilled water. The abosrbance values were measured at two wavelengths 280 and 335 nm for estimation of ofloxacin and two wavelenghls 270 and 300 nm for estimation of tinidazole. A calibration curve was drawn between the abosrbance difference values of ofloxacin and the amount of ofloxacin present in μg/mL. A calibration graph was drawn between the absorbance difference values of tinidazole and the amount of tinidazole present in μg/mL. A linear curve in each case was obtained. The linearity of the curves obtained indicates that its obeys Beer's law and the suitability of this method for the simultaneous determination of the two drugs in admixture.

Estimation of ofloxacin in pharmaceutical formulations: Tablets were weighed and powdered. An average weight of the tablets containing the two drugs ofloxacin and tinidazole in the ratio of 1 : 3 was taken and an amount of 50 mg was

dissolved in 30 mL methanol by vigorously shaking and the volume was made up to the mark. The solution was then filtered through Whatmann filter paper No. 41 and the solution was diluted to get a final concentration of 20 μg/mL of ofloxacin and 60 μg/mL of tinidazole. The sample solutions were measured at 280 and 335 nm for ofloxacin and 270 and 300 nm for tinidazole in a Spectronics-1001 spectrophotometer. The results are represented in Table-2.

TABLE-1
CONCENTRATION OF THE TWO COMPONENTS IN THE FOUR MIXED STANDARDS

Standard No.	Volume of OX† (mL)	Amountof OX† (μg/mL)	Volume of TZ* (mL)	Amount of TZ* (μg/mL)
1.	4	160	1	20
2.	3	120	2	40
3.	2	80	3	60
4.	1	40	4	80

*Tinidazol. †Ofloxacin.

TABLE-2
ESTIMATION OF OFLOXACIN AND TINIDAZOLE IN PHARMACEUTICAL PREPARATIONS

Sample	Label Claim (mg/tab)		Amount found by proposed method (mg)**		% Recovery by proposed method*	
	OX†	TZ‡	OX†	TZ‡	OX†	TZ‡
Tablet 1[a]	200	600	199.96	599.76	99.98	99.95
Tablet 2[b]	200	600	199.92	599.94	99.96	99.80
Tablet 3[c]	200	600	199.90	599.98	99.90	99.85
Tablet 4[d]	200	600	200.02	600.04	99.70	99.90

* Average of five determinations. † Ofloxacin. ‡ Tinidazole
**Determination of ofloxacin and tinidazole in combined dosage form pharmaceutical preparations by proposed method.
[a]Moflox-TZ marketed by Marc India. [b]Obactin-TZ, marketed by Blue Cross.
[c]Ofax-TZ, marketed by Alde. [d]Ocent-TZ, marketed by Indus Drugs.

Validation of the method: The method was validated in terms of linearity, accuracy, precision, specificity and reproducibility of the sample applications. The linearity of the method was investigated by serially diluting the stock solutions of ofloxacin (40 μg/mL) and tinidazole (20 μg/mL) and measured the absorbance values at 280 and 335 nm for ofloxacin and at 270 and 300 nm for tinidazole in a Spectronics-1001 spectrophotometer. Calibration curves were constructed by plotting the absorbance difference values against the amount of drug in μg/mL.

Statistical analysis: A statistical analysis was performed on the statistically significant variables using the statistical software. The following parameters were determined: coefficient of variation, standard deviation and student t-test.

Recovery experiment: To ensure the accuracy and reproducibility of the results obtained, recovery experiments were performed by adding a known amount of standard drug to previously analysed pharmaceutical preparations. The results are recorded in Table-2.

RESULTS AND DISCUSSION

The present study was carried out to develop a simple, rapid, sensitive, precise, reproducible and accurate spectrophotometric method for the estimation of simultaneous determination of ofloxacin and tinidazole in pharmaceutical dosage forms. The proposed absorbance difference method was simple, less time consuming, low cost and found to be one of the best versatile analytical techniques employed for routine analysis purposes like assay and pharmaceutical formulation. No method for the simultaneous analysis of ofloxacin and tinidazole in binary tablet formulations has been reported in literature by absorbance difference method. The content of ofloxacin and tinidazole in four different tablet dosage forms is shown in Table-2. The absorbance of various aliquots of the mixture of ofloxacin and tinidazole solutions was measured at two wavelengths 280 and 335 nm for ofloxacin and 270 and 300 nm for tinidazole A calibration curve was drawn between the absorbance difference of tinidazole and the amount of ofloxacin in μg/mL. The amount of ofloxacin present in the sample was computed from the calibration curve. Similarly, for the estimation of tinidazole, a calibration curve was plotted between the absorbance difference values of tinidazole against the amount of tinidazole in μg/mL. The amount of tinidazole in the sample was estimated from the calibration curve.

The results obtained by the proposed method are in good agreement with the label claim of the tablets. The additive and excipients usually present in tablets do not interfere. As a check on accuracy of the method, recovery experiment was performed and per cent recovery values also tabulated (Table-2). The statistical analysis was studied by the proposed method. The values of standard deviation and coefficient of variation were satisfactorily low, indicating the accuracy and the reproducibility of the method. Student t-test shows that the calculated 't' values are less than the theoretical value 2.78 with 4 degrees of freedom at 5% level of significance, indicating that there is no significant difference between the proposed and official values.

TABLE-3
STATISTICAL ANALYSIS OF ESTIMATION OF OFLOXACIN AND TINIDAZOLE

Sample	S.D.*		C.V.**		t_{cal} [a]	
	OX[b]	TZ[c]	OX[b]	TZ[c]	OX[b]	TZ[c]
Tablet 1	0.3049	0.4560	0.1524	0.1001	0.2934	1.1770
Tablet 2	0.4764	0.2701	0.2382	0.0450	0.3755	0.4971
Tablet 3	0.2449	0.3193	0.1224	0.0532	0.9132	0.1401
Tablet 4	0.1788	0.2073	0.0894	0.0345	0.2503	0.4314

* Standard deviation. ** Coefficient of variation

[a] Calculated 't' value by proposed method. Theoretical values at 95% confidence limit, 't' 2.78.
[b] Ofloxacin. [c] Tinidazole.

In conclusion, the results indicate that the proposed absorbance difference method was found to be simple, rapid, precise and accurate, and it can be used for the routine analysis of simultaneous determination of ofloxacin and tinidazole in pharmaceutical formulations.

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