***Original article***

# Facile derivative UV spectroscopy method: simultaneous estimation of tinidazole and fluconazole in combined tablet dosage form

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## Abstract:

Two derivative spectrophotometric methods have been developed for simultaneous determination of tinidazole and fluconazole in pharmaceutical formulations. The first method depends on utilization of first derivative UV spectrophotometry, with zero-crossing and peak-to-base measurement at 260.57 & 264.23 nm for fluconazole and 263.86 & 318.85 nm for tinidazole. The second method, compensation technique depends on first derivative of the ratio-spectra by measurements of the amplitudes for tinidazole and fluconazole. Calibration graphs were established for tinidazole and fluconazole in the range of 10-100 μg $ml^{-1}$ and 2-20 μg $ml^{-1}$, respectively. All proposed methods have been extensively validated. The results were found to be precise and free from interferences. The described methods can be readily utilized for analysis of pharmaceutical formulations. There was no significant difference between the performances of all of the proposed methods regarding the statistical values.

**Keywords:** Tinidazole; Fluconazole; Derivative spectroscopy; Simultaneous determination

## Introduction

Derivative spectrophotometry has been found to be a useful technique, in the identification and quantification of drugs in combination formulations even when the drugs have overlapping spectra, and in eliminating interference from formulation matrices by using the zero crossing technique [1]. However, in the absence of zero crossing point (ZCP) or when the zero crossing wavelengths are very close, the estimation of two drug combinations cannot be achieved easily. In such cases, the compensation technique (CT) is found to be valuable in the estimation of two-component drug mixtures [2].

Fluconazole (FZ, α-(2, 4-diflurophenyl)-α-(1*H*-1,2, 4-triazol-1-yl-methyl)-1*H*-1,2,4-trizol-1-ethanol) (Figure 1A) is a broad spectrum antifungal agent and recommended for the treatment and prophylaxis of disseminated and deep organ candidiasis and used against trichomoniasis, giardiasis, and amoebiasis. Tinidazole (TZ, 1-[2-(ethylsulfonyl)-ethyl]-2-methyl-5-nitroimidazole) (Figure 1B) belongs to the group of 5-nitroimidazoles, which are used in the chemotherapy of infectious diseases such as amoebiasis, giardiasis, and trichomonasis and against anaerobic bacteria. Both the drugs are now used in treatment of systemic fungal infection either as two different tablets in a form of a kit or as combined dosage form tablet.

The official monographs describe the procedure for the individual assay of FZ and TZ [3]. Literature survey has revealed various methods for estimation of FZ in biological fluids and in pharmaceutical formulations, such as IR spectroscopy [4], UV spectrophotometry [5-9], microbiology [10-11], HPLC in human plasma [11-16], TLC [17] and HPTLC [18]. Couple of individual methods reported for assay of TZ by using potentiometry [19-20]. Similarly, various literature revealed spectrophotometric methods for the estimation of TZ [21-25], an electrochemical method based on single-wall carbon nanotubes [26], HPLC [27], TLC [28] and HPTLC [29] methods reported in literature.

Though literature reports various methods for simultaneous estimation of TZ with other drugs by using spectrophotometer [30-32], but currently there is no such spectrophotometric method reported for simultaneous estimation of TZ and FZ from tablet dosage forms. The present paper describes simultaneous estimation of TZ and FZ from tablet dosage form by UV spectrophotometry. The developed method is validated and found to be rapid and sensitive.

## Experimental

### *Chemicals and reagent*

FZ was obtained as a gift sample from Sunrise International Labs Ltd., Hyderabad, India, and TZ was kindly supplied by Alkem Laboratories, Mumbai, India. Dipotassium Hydrogen Phosphate, Disodium hydrogen phosphate, Orthophosphoric acid from Qualigens, Mumbai, India. All chemicals and solvents were of analytical-reagent grade. The combination formulations are procured from a local pharmacy.

### *Instrument*

A Shimadzu UV 1700 double beam spectrophotometer equipped with 1.0 cm quartz cells with a fixed slit width (2 nm) was used, coupled a Dell-PC computer running spectrophotometric software UV Probe Version 2.33 (Schimadzu). A scan speed of 480 nm $min^{-1}$ and a chart speed of 10 nm $min^{-1}$ were maintained. Ordinate maximum and minimum were adjusted to the magnitude of derivative values. Analytical balance (XS 205 from

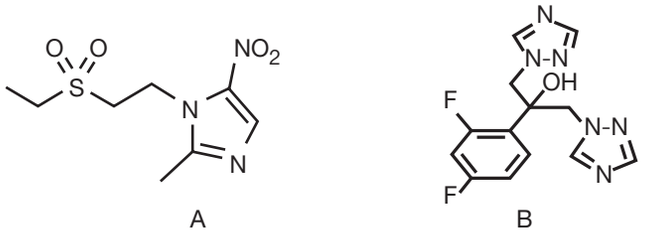


**Figure 1** Chemical structures of A) tinidazole and B) fluconazole

Mettler Toledo), autopippette (100-1000 µL from Eppendorf), Oven (VA1, VA3 from SHEL LAB) were used.

***Standard solution***

The stock solutions of pure TZ (1000 µg $ml^{-1}$) and FZ (75 µg $ml^{-1}$) were prepared by dissolving appropriate amount of the pure drugs in phosphate buffer (PB) of pH 6.8. A series of dilutions for TZ in the range of 10-100 µg $ml^{-1}$ and FZ in the range of 2-20 µg $ml^{-1}$ were prepared separately. Similarly, two series of 10 ml of each mixture solution were also prepared in buffer from the stock solutions. The first series contained a constant concentration of TZ (60 µg $ml^{-1}$) and a varying concentration of FZ (3.75, 4.5 and 5.25 µg $ml^{-1}$). The second series contained a constant concentration of FZ (4.5 µg $ml^{-1}$), and a varying concentration of TZ (50, 60 and 70 µg $ml^{-1}$).

***Sample preparation***

Ten tablets labeled to claim 1000 mg of TZ and 75 mg FZ and excipients were accurately weighed and powdered. The powder weight equivalent to 10 mg of TZ (corresponds to 0.75 mg of FZ) was dissolved in PB by mixing and made up to volume in a 50 ml volumetric flask. The sample was filtered through Whatman filter paper No. 1. After filtration, appropriate solutions were prepared by taking suitable aliquots of clear filtrate and diluting with PB in order to obtain a final solution of 60 µg $ml^{-1}$ TZ (corresponding amount of FZ 4.5 µg $ml^{-1}$).

## Method

In the ZCP technique, the absorbance of the sample and standard solutions of TZ and FZ were recorded from 200-400 nm against the blank solution. The first derivative absorption spectra for each set of solutions were subsequently recorded and were measured at the zero crossing wavelengths of the other drug. The values of the $\lambda^1$ amplitudes were measured at 318.85 nm & 263.86 nm (zero-crossing of TZ) and 260.57 & 264.23 nm (zero-crossing of FZ) for the determination of both drugs, respectively.

For the CT, the sample cell contained the TZ (60 µg $ml^{-1}$) and FZ (4.5 µg $ml^{-1}$) mixture and the reference cell contained a series of standard solutions (TZ and FZ) with different concentrations. By using an appropriate wavelength range (296.26 & 341.06 nm for TZ, 268.13 & 265.09 nm for FZ) and set parameters mentioned in ZCP technique, the derivative spectra were recorded. The derivative spectra of standard solutions were recorded against a blank in each instance. Different ratios of wavelengths i.e. maxima to minima or vice versa for the pure drugs and mixture were calculated. At the exact balance point (isobestic point) the concentration of one of the analyte components of a mixture becomes equal to that of the reference solution and ratios should be equal. The procedure was repeated to obtain concentration of the analytes in the mixture at the balance point.

Both proposed methods were validated as to linearity (evaluated by regression equations), ruggedness and precision (reported as the relative standard deviation i.e. % RSD in same and different days), detection limit (LOD) and determination limits (LOQ) were calculated according to the 3 s/m and 10 s/m criterions, respectively, where s is the standard deviation of the absorbance ($n$ = 5) of the sample and $m$ is the slope of the corresponding calibration curve, accuracy (evaluated by recovery studies). The validation data for both methods are shown in Table 1.

## Result and Discussion

***ZCP method***

Since the zero-order spectra of pure drug combinations of both the drug were found to overlap (Figure 2), derivative spectroscopy was used for their simultaneous determination. The first derivative curve of both drug shows zero crossing point. The ZCP is found to be 318.85 nm and 263.86 nm for TZ, and 260.57 and 264.23 nm for FZ (Figure 3). The derivative curves plotted for 6 pairs of solutions ($n$ = 6) in both cases, showed linear response to selected analyte concentration at the definite wavelengths (Figures 3, 4). The presence of distinct isosbestic points suggested no interferences in the estimation of TZ and FZ. The statistical analysis of the data obtained is shown in Table 1. The ZCP technique was compared with the CT and validated against each other.

**Table 1** Statistical data for the validation of tinidazole and fluconazole by zero crossing point technique and compensation technique (N=5)

| Drug | λ (nm) | Linearity (μg ml$^{-1}$) | Slope | Correlation coefficient | LOD (μg ml$^{-1}$) | LOQ (μg ml$^{-1}$) | Repeatability | Reproducibility |
|---|---|---|---|---|---|---|---|---|
| TZ | 318.85 | 10-100 | 0.00048 | 0.9988 | 0.231 | 0.386 | 0.31 | 0.50 |
| (ZCP) | 263.86 | 10-100 | 0.00023 | 0.9990 | 0.341 | 0.484 | 0.21 | 0.42 |
| FZ | 264.23 | 2-20 | 0.00082 | 0.9997 | 0.645 | 1.010 | 0.58 | 0.81 |
| (ZCP) | 260.57 | 2-20 | 0.00076 | 0.9989 | 0.882 | 1.210 | 0.69 | 0.91 |
| TZ | 341.06 | 10-100 | 0.009 | 0.9991 | 0.208 | 0.518 | 0.18 | 0.23 |
| (CT) | 296.26 | 10-100 | 0.011 | 0.9994 | 0.223 | 0.591 | 0.24 | 0.30 |
| FZ | 268.13 | 2-20 | 0.005 | 0.9994 | 0.608 | 0.932 | 0.39 | 0.46 |
| (CT) | 265.09 | 2-20 | 0.006 | 0.9993 | 0.701 | 0.991 | 0.40 | 0.52 |

LOD = limit of detection; LOQ = limit of quantification; ZCP = zero crossing point technique; CT = compensation technique; TZ = tinidazole; FZ = fluconazole; N = repetition of experiment

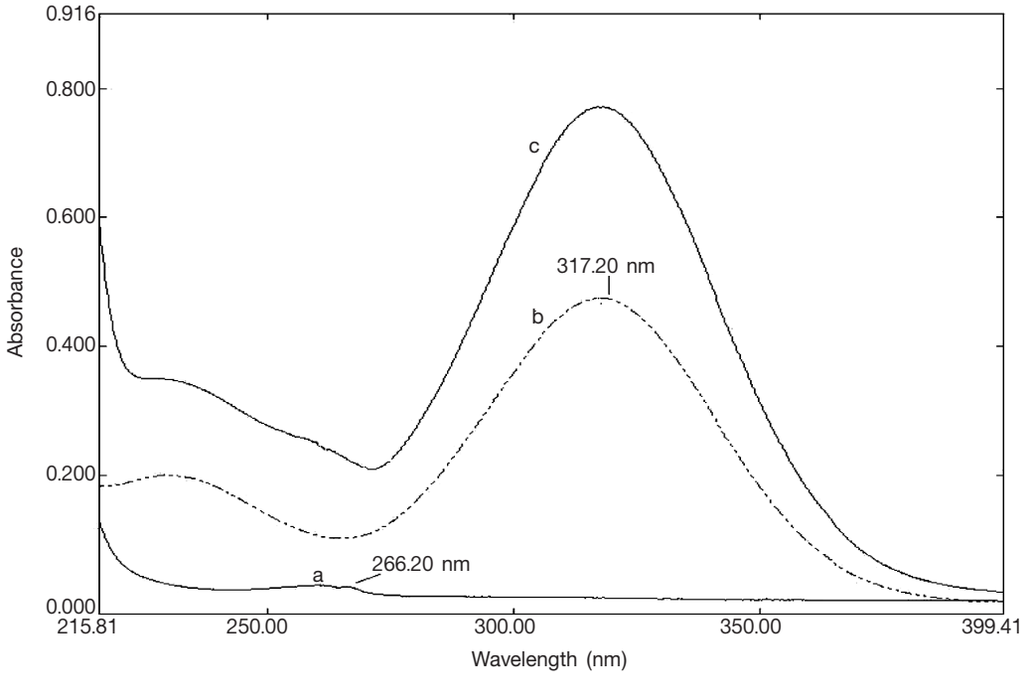


**Figure 2** Zero order absorption spectra of a) fluconazole (4.5 μg ml$^{-1}$), b) tinidazole (60 μg ml$^{-1}$), and c) a mixture of both drugs in phosphate buffer

***Compensation technique***

To optimize the CT, for simultaneous estimation of TZ and FZ, it is significant to test the influence of the variables such as divisor standard concentration, wavelength and smoothing function. The influence of $\Delta\lambda$ for obtaining the first derivative of the ratio-spectra was tested and $\Delta\lambda = 2$ nm was selected as an optimum value. An accurate choice of divisor standard concentration is fundamental for couple of reasons [33,34] hence; the method was trialed with various divisor concentrations. The results of all the tests do not add to the scientific value of the work and not shown. A standard spectrum of 60 μg ml$^{-1}$ of TZ was considered as suitable for the FZ determination and a standard spectrum of 4.5 μg ml$^{-1}$ of FZ as divisor was considered as suitable for the TZ determination.

Similarly, the $\lambda 1$ values at zero crossing wavelengths for TZ (60 μg ml$^{-1}$) and FZ (4.5 μg ml$^{-1}$) and, the ratios of standard solutions of TZ (60 μg ml$^{-1}$) and FZ (4.5 μg ml$^{-1}$) given in Table 1, were used in the determination of TZ and FZ in their combined tablet formulation. Results obtained from proposed methods of the analysis of both drugs in

**Table 2** Recovery analysis of tinidazole (TZ) and fluconazole (FZ) in commercial tablet dosage forms using first derivative ZCP ($\lambda^1$); first derivative of the ratio-spectra, CT ($\lambda\lambda^1$)

| Tablet Brand | Drug (Method) | Labelled claim ($\mu g\ ml^{-1}$) | Pure drug added ($\mu g\ ml^{-1}$) | $\lambda$ (nm) | % Mean labelled amount recovered±SD (n=3) | % RSD |
|---|---|---|---|---|---|---|
| Fluier TZ® | TZ (ZCP) | 1000 | 50 | 318.85 | 99.81 ± 0.62 | 0.13 |
| | | | 60 | 263.86 | 99.21 ± 0.54 | 0.19 |
| | | | 70 | | | |
| | FZ (ZCP) | 75 | 3.75 | 264.23 | 98.64 ± 0.23 | 0.21 |
| | | | 4.50 | 260.57 | 96.43 ± 0.81 | 0.29 |
| | | | 5.25 | | | |
| Flucoti® | TZ (CT) | 1000 | 50 | 341.06 | 99.91 ± 0.19 | 0.05 |
| | | | 60 | 296.26 | 99.51 ± 0.31 | 0.21 |
| | | | 70 | | | |
| | FZ (CT) | 75 | 3.75 | 268.13 | 99.89 ± 0.28 | 0.09 |
| | | | 4.50 | 265.09 | 97.49 ± 0.32 | 0.17 |
| | | | 5.25 | | | |

ZCP = zero crossing point technique; CT = compensation technique; RSD = relative standard deviation; n = repetition of experiment

**Table 3** Comparison of proposed method (N=3) to reported HPLC method for drug analysis

| | ZCP Wavelength (nm) | | CT Wavelength (nm) | | HPLC* | |
|---|---|---|---|---|---|---|
| | 318.85 | 264.23 | 341.06 | 268.13 | | |
| Drug | TZ | FZ | TZ | FZ | TZ | FZ |
| Linearity ($\mu g\ ml^{-1}$) | 10-100 | 2-20 | 10-100 | 2-20 | 79.98-186.62 | 6-14 |
| LOD ($\mu g\ ml^{-1}$) | 0.231 | 0.645 | 0.208 | 0.608 | 4.39 | 0.30 |
| LOQ ($\mu g\ ml^{-1}$) | 0.386 | 1.01 | 0.518 | 0.932 | 14.64 | 0.99 |
| Recovery % | 99.81 | 98.64 | 99.91 | 99.89 | 99.04 | 100.06 |

*From ref [35]

TZ = tinidazole; FZ = fluconazole; ZCP = zero crossing point technique; CT = compensation technique; LOD = limit of detection; N = repetition of experiment; LOQ = limit of quantification

tablets indicate that the proposed techniques can be used for simultaneous quantitation and routine quality control analysis of this binary mixture in pharmaceuticals (Table 2). The method is found to be precise and reproducible. The ZCP method is more rapid and simple than the CT method, while the proposed CT method has greater sensitivity and accuracy. No such method was reported for simultaneous estimation of both drugs in official pharmacopoeias and literature so far, so comparison of proposed methods has not been possible. A reported HPLC results [35] were then compared with the proposed spectrophoto-metric methods (Table 3). The quantification of TZ and FZ by both proposed methods shows lower LOD and LOQ values than HPLC method. The recovery values found were similar for all methods.

## Conclusion

The two proposed methods for simultaneous estimation of TZ and FZ are suitable for quality control laboratories, where economy and time are essential. All validation parameters were found to be highly satisfactory, indicating linearity, precision and adequate detection and quantification limit. High percentage recovery shows that the methods are free from the interferences of the commonly used excipients and additives in the formulations of drugs.

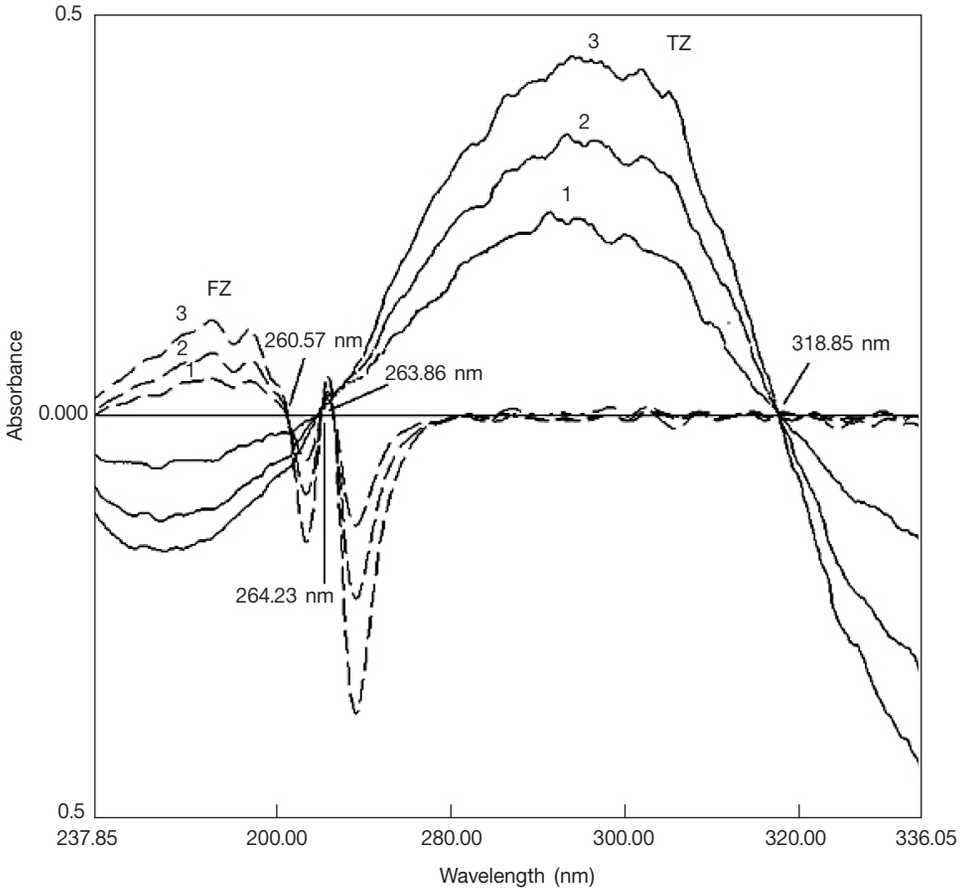


**Figure 3** First derivative absorbance spectra of fluconazole (dotted line) (1) 3.75 μg ml$^{-1}$, (2) 4.5 μg ml$^{-1}$ and (3) 5.25 μg ml$^{-1}$; tinidazole (countinous line) (1) 50 μg ml$^{-1}$, (2) 60 μg ml$^{-1}$ and (3) 70 μg ml$^{-1}$

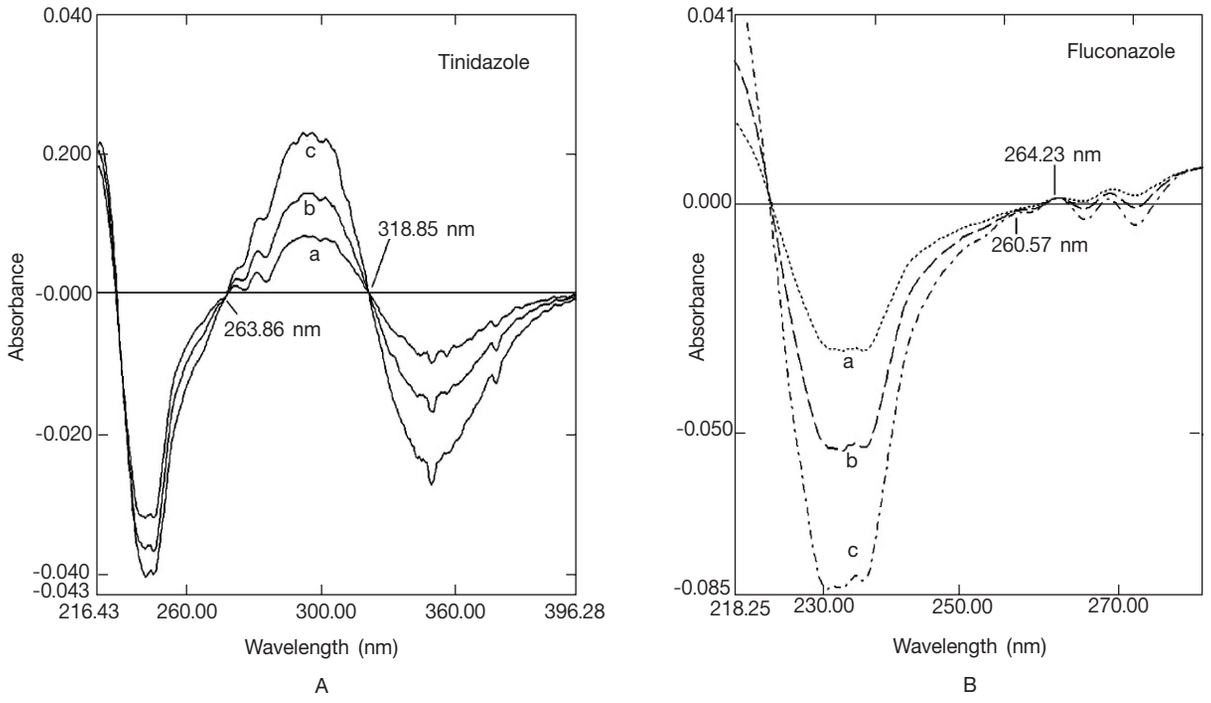


**Figure 4** First derivative of the ratio-spectra for different concentrations of A) tinidazole i.e. 50 (a), 60 (b), 70 (c) μg/ml (divisor fluconazole of 4.75 μg ml$^{-1}$, and B) fluconazole i.e. 3.75 (a), 4.50 (b), 5.25 (c) μg ml$^{-1}$ (divisor tinidazole of 60 μg ml$^{-1}$). The working wavelengths are indicated

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