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Spectrophotometric assays of diloxanide furoate and tinidazole in combined dosage forms

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Abstract: Two spectrophotometric methods have been developed for the simultaneous determination of diloxanide furoate and tinidazole in combined dosage formulations without prior separation. The first method is based on the measurement of the absorbance of a methanolic solution of the sample at 259 and 311 nm and application of a simplified Vierordt equation for the determination of diloxanide furoate, whereas tinidazole is determined by direct spectrophotometry. The second method is a difference spectrophotometric procedure based on measurement of the absorbance of the sample solution in water relative to that of an acidic or alkaline solution of identical concentration at the appropriate wavelength of maximum difference absorption. The results are calculated by reference to standard absorptivity values.

Keywords: *Difference spectrophotometry; two-wavelength simultaneous spectrophotometry; diloxanide furoate-tinidazole determination; pH-induced spectral changes.*

Introduction

Diloxanide furoate [DLF; 4-(*N*-methyl-2,2-dichloroacetamido)phenyl 2-furoate] is used extensively for the treatment of chronic amoebiasis. Tinidazole {TDZ; 1-[2-(ethylsulphonyl)ethyl]-2-methyl-5-nitroimidazole} is a new broad-spectrum anti-protozoal agent. Combinations of DLF and TDZ are widely used for the treatment of chronic intestinal amoebiasis. DLF, official in the *British Pharmacopoeia* [1] and the *Indian Pharmacopoeia* [2], is assayed by non-aqueous titrimetry using tetrabutylammonium hydroxide as titrant whereas TDZ, included in the *Extra Pharmacopoeia* [3], can be determined by perchloric acid titration by a method similar to that described for metronidazole [4]. A number of visible spectrophotometric methods are reported for DLF [5–9]. Sanghavi *et al.* [10] reported a difference spectrophotometric method for its assay in the presence of degradation products. Methods reported for TDZ include ultraviolet [11–14] and visible [15, 16] spectrophotometry, chromatography [16, 17] and polarography [18]. A review of the literature has shown that no methods for the simultaneous spectrophotometric determination of both the components have been published. In the present investigation, a two-wavelength spectrophotometric method and a difference spectrophotometric method have been applied to the simultaneous determination of both the drugs without prior separation.

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Experimental

Measurements were carried out on a Beckman 24 ultraviolet-visible recording double beam spectrophotometer using 1-cm matched silica cells. Spectra were recorded in the range 360–220 nm with a scan speed of 20 nm min^{-1}.

Materials

DLF and TDZ reference standards were obtained from the Central Drug Laboratory, Calcutta, India. Hydrochloric acid, sodium hydroxide and methanol were of analytical grade. Glass distilled water (approximately pH 7). Stock solution contained 0.25 mg ml^{-1} of DLF and TDZ in methanol.

Two-wavelength spectrophotometric method

Standard solutions. A 2 ml aliquot of each of the stock solutions was diluted to 50 ml with methanol. The absorbance of the DLF solution was measured at 259 nm and that of TDZ solution at 311 and 259 nm using methanol in the reference cell. Standard absorptivity values of DLF and TDZ at the respective wavelengths were calculated using the mean of five determinations.

Sample solutions. A quantity of powdered tablets equivalent to about 25 mg of DLF was accurately weighed and extracted with about 60 ml of methanol. The solution was filtered through a sintered-glass funnel under suction, the residue was washed well with methanol and the filtrate was diluted to 100 ml with methanol. A 2-ml aliquot of the resultant solution was diluted to 50 ml with methanol. The absorbance of this solution was measured at 259 and 311 nm using methanol in the reference cell. The concentration of TDZ in the sample was calculated by using the standard absorptivity value at 311 nm. The concentration of DLF in the sample was calculated by applying the following equation:

$$C = \frac{(A_1 \times a_2) - (A_2 \times a_1)}{a_2 - a_3}$$

where C is the concentration (g l^{-1}) of DLF in the final sample solution, A_1 and A_2 are the absorbances of a 1-cm layer of sample solution at 259 and 311 nm respectively, a_1 and a_2 are the absorptivity (l/gm.cm) values of TDZ at 259 nm and 311 nm respectively and a_3 is the absorptivity value of DLF at 259 nm. The values for a_1, a_2 and a_3 determined with the standard solutions were; $a_1 = 7.2$, $a_2 = 35.5$ and $a_3 = 69.91\ g^{-1}\ cm^{-1}$. Thus, the equation for calculating the content of DLF simplifies to:

$$C\ (mg\ l^{-1}) = (14.3062 \times A_1) - (2.9015 \times A_2)$$

Difference spectrophotometric method

Standard solutions. A 2-ml aliquot of each of the stock solutions was separately diluted to 50 ml with water, 0.02 M sodium hydroxide and 0.1 M hydrochloric acid.

The absorbance of the DLF solution in water was measured at 267 nm (ΔA_{267}^{std}) relative to that of the alkaline solution and the absorbance of the TDZ solution in water was measured at 320 nm (ΔA_{320}^{std}) relative to that of the acidic solution. The appropriate solvent corrections were carried out at the respective wavelengths and the net

absorbance-difference (ΔA) values of each substance were calculated as the average of five determinations.

Sample solutions. A quantity of the powdered tablets equivalent to 25 mg of TDZ was accurately weighed and extracted with about 60 ml of methanol. The solution was filtered through a sintered-glass funnel under suction, the residue was washed well with methanol and the filtrate was diluted to 100 ml with methanol. Three 2-ml aliquots of this solution were separately diluted to 50 ml with water, 0.02 M sodium hydroxide and 0.1 M hydrochloric acid. The absorbance of the solution in water was measured at 267 nm ($\Delta A_{267}^{\text{sample}}$) relative to that of the alkaline solution and at 320 nm ($\Delta A_{320}^{\text{sample}}$) relative to that of the acidic solution. The appropriate solvent corrections were carried out. The content (g per tablet) of DLF (C_D) and TDZ (C_T) in a tablet of average weight were calculated using:

$$C_D = \frac{\Delta A_{267}^{\text{sample}} \times C_D^{\text{std}} \times \text{average weight} \times 25}{\Delta A_{267}^{\text{std}} \times \text{weight of sample taken}}$$

$$C_T = \frac{\Delta A_{320}^{\text{sample}} \times C_T^{\text{std}} \times \text{average weight} \times 25}{\Delta A_{320}^{\text{std}} \times \text{weight of sample taken}}$$

where C_D^{std} and C_T^{std} are the concentrations (g 100 ml^{-1}) of the final standard solutions of DLF and TDZ respectively and weights are in gram.

Results and Discussion

For the two-wavelength simultaneous spectrophotometric determination of both components, the UV spectra of the compounds were studied in several solvents. It was observed that the methanolic solution of DLF shows maximum absorbance at 259 nm and zero absorbance at 311 nm, the absorption maximum for TDZ (Fig. 1). Thus TDZ could be directly assayed by measuring the absorbance of the sample solution at 311 nm, without the need to apply the Vierordt equation [19]. For the assay of DLF, the absorbance of the sample solution was measured at 259 nm and the concentrations were calculated by using a simplified Vierordt equation. The absorbance is linear over a concentration range of 4–14 $\mu g\ ml^{-1}$.

The difference spectrophotometric assays of DLF and TDZ are based on pH-induced spectral changes [20–21]; TDZ solution displays a marked bathochromic shift between

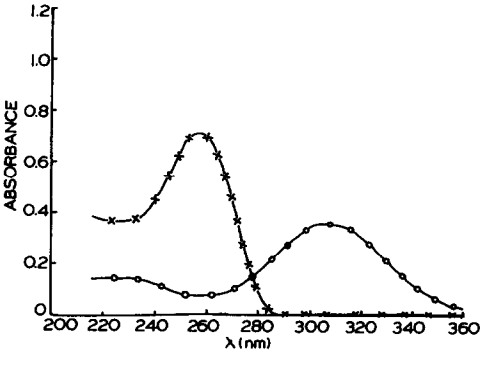

Figure 1
U.V. absorption spectra of (0.01 $g\ l^{-1}$) diloxanide furoate (——×——×——) and tinidazole (——○——○——) in methanol.

acidic and alkaline media whereas DLF solution shows a slight hyposochromic shift. When the absorbance of the solution of DLF in water was measured against that of the alkaline solution, the maximum absorbance-difference (ΔA) was observed at 267 nm, whereas the solutions of TDZ when measured similarily show zero absorbance at 267 nm (Fig. 2). Similarly when the absorption of the solution of TDZ in water was measured against that of the acidic solution, the maximum absorbance-difference was observed at 320 nm, whereas the solutions of DLF when measured similarly show zero ΔA at 320 nm (Fig. 2).

DLF and TDZ solutions exhibit a linear relationship between ΔA and concentration within the concentration range 2–18 μg ml^{-1} and 4–24 μg ml^{-1} respectively at their

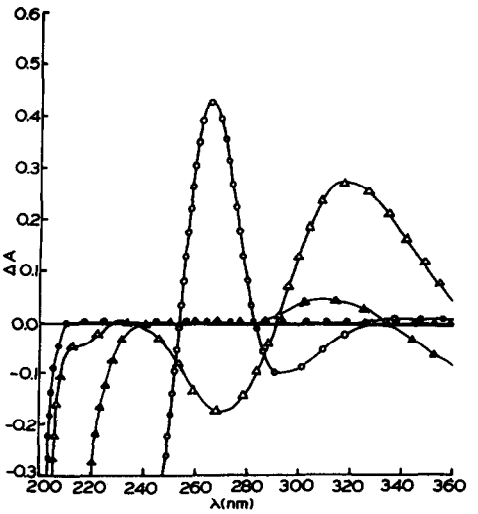

Figure 2
Difference absorption spectra of diloxanide furoate solution (0.01 g l^{-1}) in water versus equimolar solutions in alkali (——○——○——) and acid (——●——●——) and tinidazole solution (0.01 g l^{-1}) in water versus equimolar solutions in acid (——△——△——) and alkali (——▲——▲——).

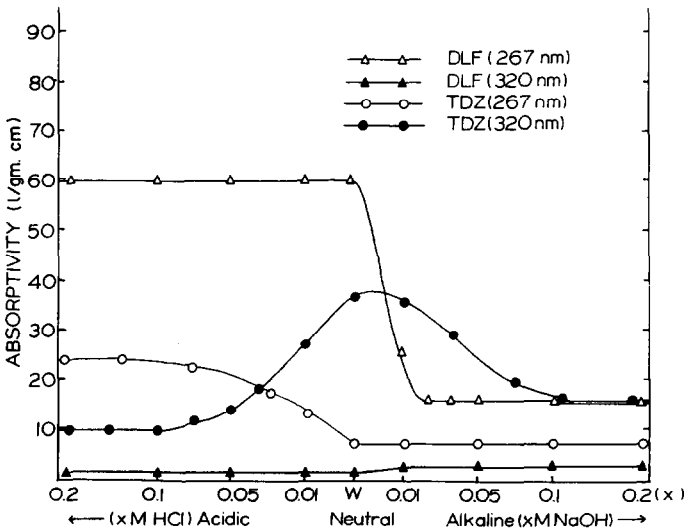

Figure 3
Variation of absorptivity of diloxanide furoate and tinidazole at 267 and 320 nm in acidic, neutral and alkaline media.

Table 1
Assay results of diloxanide furoate and tinidazole formulations by two-wavelength simultaneous spectrophotometric method

Formulation	Diloxanide furoate Claimed (mg)	Found* (mg) ±S.D.	Added (mg)	Recovery (%)	Tinidazole Claimed (mg)	Found* (mg) ±S.D.	Added (mg)	Recovery (%)
1	250	236.97 ± 0.76	25	100.12	150	145.91 ± 0.58	25	99.36
2	250	248.94 ± 0.56	40	99.77	300	294.93 ± 0.59	40	99.15
3	250	248.77 ± 0.66	50	99.14	300	300.84 ± 0.59	50	98.80
Mean ± S.D.				99.67 ± 0.50				99.10 ± 0.28
4	50	49.52 ± 0.18	—	—	25	24.79 ± 0.08	—	—
5	25	24.71 ± 0.11	—	—	50	49.65 ± 0.15	—	—
6	50	49.48 ± 0.16	—	—	50	50.14 ± 0.14	—	—

*Average of five determinations.
1–3, tablets; 4–6, standard mixtures.

Table 2
Assay results of diloxanide furoate and tinidazole formulations by the difference spectrophotometric method

Formulation	Diloxanide furoate Claimed (mg)	Found* (mg) ±S.D.	Added (mg)	Recovery (%)	Tinidazole Claimed (mg)	Found* (mg) ±S.D.	Added (mg)	Recovery (%)
1	250	236.22 ± 0.38	25	98.40	150	145.24 ± 0.43	25	99.72
2	250	249.23 ± 0.56	40	98.25	300	294.85 ± 0.75	40	101.05
3	250	248.68 ± 0.47	50	99.68	300	300.85 ± 0.87	50	100.82
Mean ± S.D.				98.77 ± 0.78				100.53 ± 0.71
4	50	49.67 ± 0.15	—	—	25	24.57 ± 0.09	—	—
5	25	25.16 ± 0.10	—	—	50	49.83 ± 0.17	—	—
6	50	49.67 ± 0.17	—	—	50	49.82 ± 0.15	—	—

*Average of five determinations.
1–3, tablets; 4–6, standard mixtures.

wavelengths of measurement. The measurement of absorptivity of the compounds in aqueous solvents of different pH (Fig. 3) showed that at 267 nm, TDZ has constant absorptivity from neutral (water) to alkaline media (0.1 M NaOH) and DLF has constant absorptivity in alkaline media. Therefore, DLF can conveniently be assayed by measuring the absorbance of its solution in water (neutral) relative to that in alkaline media of any strength between 0.02 and 0.05 M NaOH. Similarly, at 320 nm the absorptivity of DLF is constant from neutral pH to 0.2 M HCl, whereas TDZ shows almost constant absorptivity in acidic media between 0.1 and 0.2 M HCl. Therefore, TDZ may be estimated by measuring the absorbance of its solution in water relative to that in acidic media of any strength between 0.1 M and 0.2 M HCl. Thus water, (pH $\simeq$ 7), 0.02 M NaOH (pH $\simeq$ 12.5) and 0.1 M HCl (pH $\simeq$ 1) were chosen. Variation up to $\pm$10% in the strength of acid or alkali does not affect the absorptivity values. As a result of these spectral changes of the compounds in solutions of different pH, the components were assayed selectively without prior separation.

Three commercial dosage forms were analysed by both of the proposed methods. The analytical results (Tables 1 and 2) show that the relative standard deviation was less than 1%. The methods were also subjected to recovery studies by adding known amount of the drugs to the pre-analysed samples. The mean per cent recoveries ($\pm$S.D) varied from 99.10 $\pm$ 0.28 to 100.53 $\pm$ 0.71 (Tables 1 and 2). To prove the validity and applicability of the proposed methods, three synthetic mixtures of the drugs in different concentration ratios were analysed by both of the methods. The results (Tables 1 and 2) confirmed the applicability of the proposed methods. The presence of degradation products as potential co-extractive are likely to interfere in both of the methods. However, common excipients, such as aerosil 400, starch, lactose, acacia mucilage, talc, magnesium stearate and dibasic calcium phosphate, do not interfere. The proposed methods have useful application in the quality control of formulations containing TDZ and DLF.

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