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Notes

Simultaneous quantitative determination of metronidazole and diloxanide furoate in a tablet preparation by difference spectroscopy

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Difference spectrophotometry has proved particularly useful in the determination of medicinal substances by eliminating specific interference from degradation products, co-formulated drugs and also the non-specific irrelevant absorption from the formulation matrix. Its advantages for selective analysis have been described (Doyle and Fezzari, 1974; Davidson, 1976; Parimoo, 1987; Parimoo et al., 1993). The technique involves the reproducible alteration of the spectral properties of the analyte in equimolar solutions and the measurement of the absorbance difference (δA) between two solutions, provided the absorbances of the other absorbing interferants are not affected by the reagents used for the spectral property alteration. Simple aqueous acids, alkali's and buffers are most frequently used for inducing spectral alterations since many drugs are weak acids or bases whose state of ionisation and absorptivity depends on the pH of the solution. In our earlier work in this area we described the successful application of difference spectroscopy for some related drug combinations (Parimoo and Umapathi, 1994; Parimoo et al., 1995). A combination of MDZ and DF in the form of a tablet is widely used for acute and chronic amoebiasis and giardiasis.

Some methods have been reported for the assay of MDZ (Florey, 1976) and DF individually (Sanghavi and Kulkarni, 1978; Shah and Mehta, 1981). Official monographs describe the procedure for the individual assay of MDZ and DF (British Pharmacopoeia, 1980). A reversed-phase HPLC determination of MDZ and DF in single and combined pharmaceutical formulations in concentration ranges of 1 mg/ml has also been reported (Ray, 1994). In this study we describe the pH induced difference spectrophotometric method for the simultaneous quantitation of MDZ and DF in the presence of each other as well as the excipients hitherto not reported before.

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The stock solution of MDZ was prepared by dissolving 25 mg of pure MDZ in 50 ml of methanol. Appropriate aliquots of the stock solution were transferred to 25 ml volumetric flasks in duplicate. The volume was made up with 0.1 N HCl and 0.1 N NaOH to give a series of equimolar solutions of 25 ml each in 0.1 N HCl and 0.1 N NaOH containing 10–30 μg/ml of MDZ. The stock solution of DF was prepared by dissolving 25 mg of pure DF in 50 ml of methanol. Appropriate aliquots were used as for MDZ to prepare 25 ml series of equimolar solutions of DF in O.1 N HCl and 0.1 N NaOH containing 20–40 μg/ml DF. Similarly, two series of equimolar solutions of mixtures of 25 ml MDZ and DF in 0.1 N HCl and 0.1 N NaOH were also prepared using the stock solutions. The first series contained a constant concentration of DF (25 μg/ml) and a varying concentration of MDZ (10–30 μg/ml). The second series contained a constant concentration of MDZ (20 μg/ml) and a varying concentration of DF (20–40 μg/ml). The drugs were protected from light throughout the study and the absorbance of the solutions of pure MDZ, DF and their mixtures were taken between 30 and 90 min after preparation. All reagents used were of analytical grade.

Twenty tablets were accurately weighed, well powdered and a weight of the powder equivalent to 5 mg of MDZ (and 6.25 mg of DF) was dissolved in 50 ml of methanol by thorough mixing and made up to volume in a 50 ml volumetric flask. The extract was filtered through a Whatman filter paper No. 1. The first and last 5 ml of the filtrate were discarded. The sample solutions of 25 ml of each in 0.1 N HCl and 0.1 N NaOH were prepared using 5 ml aliquots of the filtrate so as to obtain equimolar solutions containing approximately 20 μg/ml of MDZ and 25 μg/ml of DF.

The absorbance difference (δA) between the acidic solution and equimolar 0.1 N NaOH solutions of pure drugs and samples were measured from 230 to 350 nm on a Jasco 7800 UV–visible double beam autoscan spectrophotometer by placing the acidic solutions in the reference compartment and the 0.1 N NaOH solutions in the sample compartment. The absorbance difference of the analytes at 284 and 292 nm was corrected for the absorbance difference, if any, of 0.1 N NaOH solution relative to 0.1 N HCl at these wavelengths.

The difference absorption spectrum of a solution of MDZ in 0.1 N HCl solution in the reference cell and an equimolar solution of MDZ in 0.1 N NaOH solution in the sample cell compartment showed a maximum value of δA at 320 nm and a minimum value of δA at 272 nm. An isosbestic point (a wavelength of zero δA due to equal absorptivities of the two species) occurred at 292 nm (Fig. 1). The difference absorption spectrum of solutions of DF showed maximum values of δA at 297 and 243 nm and a minimum value of δA at 268 nm. The isosbestic points of the DF spectrum were obtained at 284 and 252 nm (Fig. 2).

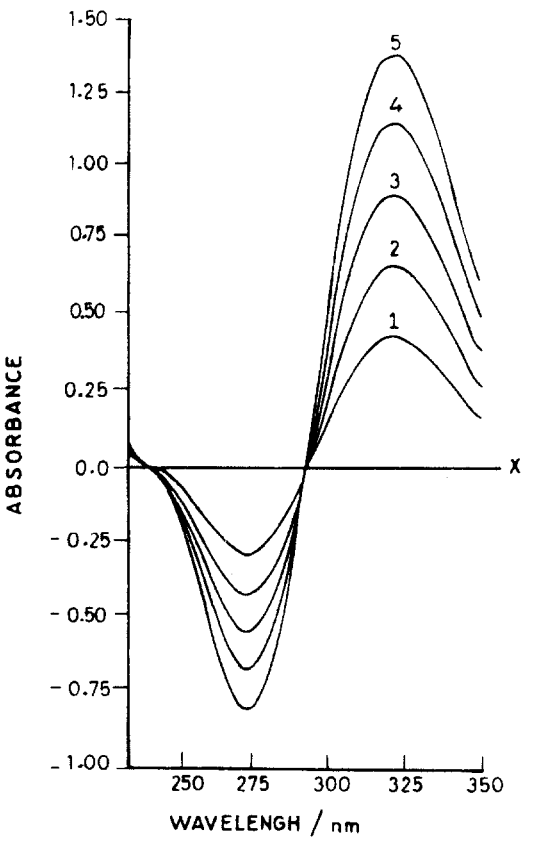

Fig. 1. Difference absorption spectra of metronidazole in 0.1 N HCl vs 0.1 N NaOH of concentration 10–30 μg/ml.

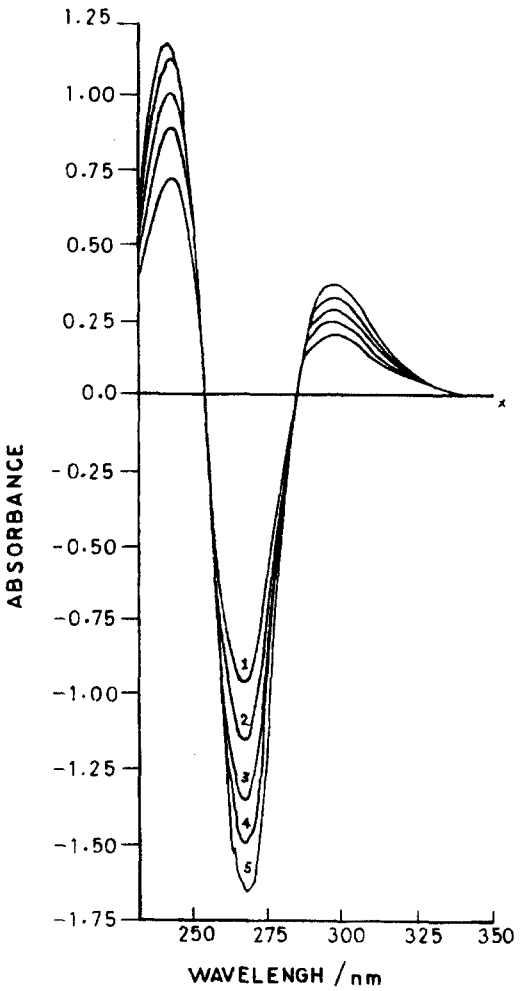

Fig. 2. Difference absorption spectra of diloxanide furoate in 0.1 N HCl vs 0.1 N NaOH of concentration 20–40 μg/ml.

The wavelength of 284 nm was chosen for the estimation of MDZ since the δA values of the MDZ difference spectrum were more optimal and linear for accurate measurements of different concentrations of MDZ than the values at 252 nm. For the wavelength of 284 nm, at which the δA value of the MDZ difference spectrum was about -0.274 for a concentration of 20 μg/ml, the absorbance value of the DF difference spectrum was about 0.213 at 292 nm for a concentration of 25 μg/ml. These concentrations were chosen on the basis of the proportions of MDZ and DF in commercial formulations as well as to have absorbance values between 0.2 and 1.2 which would provide a minimum relative error (Connors, 1982).

The proportionality of the δA value and concentration of MDZ was found by measuring δA of the 10 pairs of solutions containing 10–30 μg/ml of MDZ at 284 nm. The linear regression equation calculated using the method of least squares was

$$y = -79.11x - 1.978 \quad (1)$$

with a correlation coefficient of $r = -0.9999$. The proportionality of δA and the concentration of DF was found by measuring the δA values of solutions of DF containing 20–40 μg/ml at 292 nm. The calculated linear regression equation was

$$y = 108.4x - 1.844 \quad (2)$$

with a correlation coefficient of $r = 0.9998$.

To evaluate further the specificity of the method for samples containing MDZ and DF, two series each of 10 solutions (mentioned under standard preparation) were examined at the isosbestic wavelengths. The solutions of the first series gave a regression equation of

$$y = -79.36x - 1.810 \quad (3)$$

with a correlation coefficient of $r = -0.9998$ at 284 nm, which was similar to that of Eq. (1), suggesting that the presence of DF did not affect the absorptivity of MDZ at 284 nm. The δA values of the second series of solutions gave a regression equation of

$$y = 108.9x - 1.830 \quad (4)$$

with a correlation coefficient of $r = 0.9997$ at 292 nm.

Its similarity to Eq. (2) suggests no interference of the absorptivity of MDZ with that of DF at 292 nm. The identical isosbestic points of the two components in the standard and sample difference spectra confirmed the non-interference of the excipients in the measurement of the absorbance values at these wavelengths.

The δA values of standard solutions of MDZ (20 μg/ml) and DF (25 μg/ml) relative to the δA of the tablet sample solution was used for the determination of MDZ and DF in the tablet preparations. The results are given in Table 1.

Table 1
Assay results of metronidazole and diloxanide furoate in commercial formulations by difference spectroscopy

Sample	MDZ		DF	
	mg/tablet	% w/w stated[a]	mg/tablet	% w/w stated[a]
Brand A	199.70	99.85 ± 0.55	249.27	99.71 ± 0.91
Brand B	199.70	99.85 ± 0.48	246.47	98.59 ± 0.46
Brand C	399.68	99.92 ± 0.36	500.45	100.09 ± 0.69

[a]Five replicate measurements.

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