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Simultaneous quantitative determination of metronidazole and nalidixic acid in tablets by difference spectroscopy

P. Parimoo*, C.V.N. Prasad, R. Vineeth

Department of Pharmacy, Birla Institute of Technology and Science, Pilani, Rajasthan 333031, India

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Abstract

A difference spectrophotometric procedure has been developed for simultaneous determination of metronidazole (MDZ) and nalidixic acid (NA) in tablets. The method comprised the measurement of the absorbance of a solution of the tablet extract in 0.1 M NaOH relative to that of an equimolar solution in 0.1 M HCl at 292 nm for NA and 325 nm for MDZ. The presence of identical isosbestic points for pure drug solutions and tablet extracts indicated the non-interference of excipients in the absorption at these wavelengths. Compliance with Beer's law was observed in the concentration ranges 5–25 μg ml^{-1} for MDZ and 15–35 μg ml^{-1} for NA at these wavelengths.

Keywords: Metronidazole; Nalidixic acid; Difference spectroscopy; Simultaneous quantitative determination

1. Introduction

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 by several workers [1–4]. The technique involves the reproducible alterations of the spectral properties of the absorbance difference (δA) between two solutions, provided that the absorbance of the other absorbing substances is not affected by the reagents used to alter the spectral properties. Simple aqueous acids, alkalis and buffers are most frequently used for inducing spectral alteration since many drugs are weak acids or bases whose state of ionisation and absorptivity depend on the pH of the solution. In earlier work the present authors have described the successful application of difference spectroscopy to some of the related drug combinations [5,6]. The present research describes the pH-induced difference spectrophometric method for the simultaneous determination of metronidazole (MDZ) and nalidixic acid (NA) in the presence of each other as well as the excipients.

* Corresponding author.

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A combination of MDZ and NA in the form of tablets is widely used for diarrhoea and dysentery of mixed infective origin. The official monographs describe procedures [7,8] for the individual assay of MDZ and NA; a high performance thin layer chromatography (HPTLC) method has been reported [9].

2. Experimental

2.1. Standard solutions

The stock solution of MDZ was prepared by dissolving 25 mg of pure MDZ in 50 ml of methanol. Appropriate volumes of aliquots of the stock solution were transferred to 25 ml volumetric flasks in duplicate. The volume was adjusted with 0.1 M HCl and 0.1 M NaOH to give a series of equimolar solutions of 25 ml each in 0.1 M HCl and 0.1 M NaOH containing 5–25 μg ml^{-1} of MDZ. The stock solution of NA was prepared by dissolving 25 mg of pure NA in 50 ml of methanol. Appropriate volumes of aliquots were used as for MDZ, to prepare 25 ml each of a series of equimolar solutions of NA in 0.1 M HCl and 0.1 M NaOH containing 15–35 μg ml^{-1} of NA. Similarly two series of 25 ml each of an equimolar solution of a mixture of MDZ and NA in 0.1 M HCl and 0.1 M NaOH were prepared from the stock solutions. The first series contained a constant concentration of NA (15 μg ml^{-1}) and a varying concentration of MDZ (5–25 μg ml^{-1}). The second series contained a constant concentration of MDZ (10 μg ml^{-1}) and a varying concentration of NA (15–35 μg ml^{-1}). The drugs were protected from light throughout the study.

2.2. Sample preparation

Twenty tablets were accurately weighed, powdered and a weight of the powder equivalent to 30 mg of NA (20 mg of MDZ) was dissolved in 50 ml of methanol by thorough mixing and diluted to volume in a 50 ml volumetric flask. The extract was filtered through Whatman No. 1 filter paper. The first and last 5 ml of the filtrate were discarded. The sample solutions of 50 ml of NA and MDZ in 0.1 M HCl and 0.1 M NaOH respectively were prepared by using 10 ml aliquots of the filtrate to obtain equimolar solutions containing approximately 15 μg ml^{-1} of NA and 10 μg ml^{-1} of MDZ respectively.

The absorbance differences (δA) between the acidic solution and equimolar 0.1 M NaOH solutions of pure drug and samples were measured in the range 250–350 nm with a Jasco 7800 UV–visible double beam spectrophotometer by placing the acidic solutions in the reference compartment and the 0.1 M NaOH solutions in the sample compartment. The absorbance difference of the analytes at 292 nm and 325 nm was corrected for the absorbance difference, if any, of 0.1 M NaOH solution relative to 0.1 M HCl at these wavelengths.

3. Results

The difference absorption spectrum of a solution of MDZ in 0.1 M HCl in the reference cell and an equimolar solution of MDZ in 0.1 M NaOH in the sample cell compartment showed a maximum value of δA at 320 nm and a minimum value at 272 nm. Isosbestic point (at wavelengths of zero δA due to equal absorptivities of the two species) occurred at 292 nm (Fig. 1). The difference absorption spectrum of the solution of NA showed a maximum value of δA at 335 nm and a minimum value of 307 nm. The isosbestic point of the NA spectrum was obtained at 325 nm (Fig. 2).

The δA values of the difference spectrum of MDZ at 325 nm (where the δA value of NA is zero) and that of NA at 292 nm (where the δA value of MDZ is zero) have been used for the determination of the two drugs. Thus at 325 nm the δA value of the mixture will be due to the contribution of MDZ alone and at 292 nm the contribution will be only that of NA.

The proportionality of the δA value and concentration of MDZ was found by measuring the δA values of the 10 pairs of solutions containing 5–25 μg ml^{-1} of MDZ at 325 nm. The linear regression equation calculated by using the method of least squares was

$$y = 24.25x - 0.635 \quad (1)$$

The correlation coefficient r was 0.998. The proportionality of δA and the concentration of NA was found by measuring the δA values of solutions of NA containing 15–35 μg ml^{-1} of NA at 292 nm. The calculated linear regression equation was

$$y = -85.95x - 1.210 \quad (2)$$

The correlation coefficient r was -0.997.

To evaluate further the specificity of the method in samples containing both MDZ and NA, two series each of 10 solutions were examined at the isosbestic wavelengths. The solutions of the first series gave a regression equation of

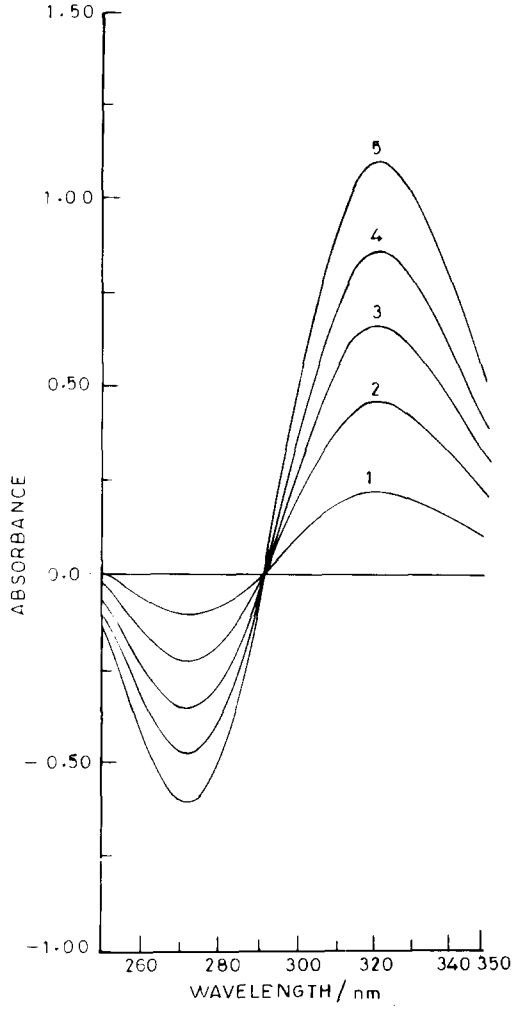

Fig. 1. Difference absorption spectra of metronidazole in 0.1 M HCl vs. 0.1 M NaOH of concentration 5–25 μg ml^{-1} in curves 1, 2, 3, 4 and 5 respectively.

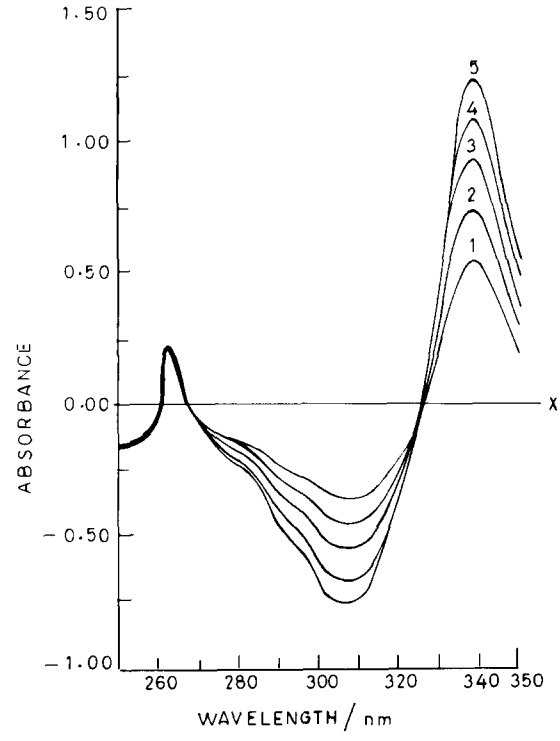

Fig. 2. Difference absorption spectra of nalidixic acid in 0.1 M HCl vs. 0.1 M NaOH of concentration 15–35 μg ml^{-1} in curves 1, 2, 3, 4 and 5 respectively.

$$y = 23.16x - 0.296 \quad (3)$$

The correlation coefficient r was 0.999 at 325 nm. This equation is similar to Eq. (1), suggesting that the presence of NA did not affect the absorptivity of MDZ at 325 nm. The δA values of the second series of solutions gave a regression equation of

$$y = -84.98x - 2.081 \quad (4)$$

The correlation coefficient r was -0.999 at 292 nm.

The similarity of Eq. (4) to Eq. (2) suggested the non-interference of the absorptivity of MDZ with that of NA 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 measure-

Table 1
Selectivity of the method for the determination of metronidazole by difference spectroscopy

Composition of mixture (μg ml^{-1})		Mean[a] (δA) (at 325 nm)	95% confidence limits[b]
MDZ	NA		
5	15	0.220 ± 0.006	±0.004
10	15	0.445 ± 0.003	±0.002
15	15	0.654 ± 0.005	±0.004
20	15	0.874 ± 0.007	±0.004
25	15	1.085 ± 0.007	±0.004

[a] Ten replicate measurements.
[b] Based on Student's t-test distribution.

ment of the absorbance values at these wavelengths. The absorbance values of the two series of solutions are given in Tables 1 and 2.

The δA values of standard solutions of MDZ (10 μg ml^{-1}) and NA (15 μg ml^{-1}) relative to the δA value for the tablet sample solution were used for the determination of MDZ and NA in the tablet preparation.

The concentrations of MDZ (C_{MDZ}) and NA(C_{NA}) in the tablets of average weight (AW) as a percentage of the stated quantity of drug (C_t) were calculated from the equations

$$C_{MDZ} = \frac{A_{325}^{Sam} \times C_{MDZ}^{Std} \times AW \times 100}{A_{325}^{Std} \times WT_{Sam} \times C_t}$$

$$C_{NA} = \frac{A_{292}^{Sam} \times C_{NA}^{Std} \times AW \times 100}{A_{292}^{Std} \times WT_{Sam} \times C_t}$$

Table 2
Selectivity of method for the determination of nalidixic acid by difference spectroscopy

Composition of mixture (μg ml^{-1})		Mean[a] (δA) (at 292 nm)	95% confidence limits[b]
MDZ	NA		
10	15	−0.200 ± 0.002	±0.001
10	20	−0.260 ± 0.001	±0.001
10	25	−0.322 ± 0.004	±0.003
10	30	−0.375 ± 0.005	±0.003
10	35	−0.436 ± 0.001	±0.001

[a] Ten replicate measurements.
[b] Based on Student's t-test distribution.

where the concentrations of the standard solutions are in mg ml^{-1}, WT_{Sam} denotes the weight of tablet powder (mg ml^{-1}) taken for the preparation of sample solution and the weights are in milligrams. The results are given in Table 3.

4. Discussion

Difference spectroscopy has been used for the quantitation of only a few drug mixtures, mainly for two reasons. First, the application of difference spectroscopy to the analysis of two-component formulations depends on the fortuitous juxtaposition of the isosbestic points. The positioning should be such that the isosbestic point of one component is suitable for the measurement of the δA value of the difference spectrum of the other component and vice versa. In addition, the δA values of the components at the isosbestic points must be in the range 0.2–1.2 for minimum relative error.

The proposed method meets these requirements. The non-interference of the excipients in the determination is demonstrated by the identical isosbestic points in standard and sample solutions. The rectilinearity of δA values at the isosbestic points is shown by the regression equations. The corresponding correlation coefficients confirm the proportional relationship between δA values and concentration at the isosbestic points and indicate the precision and reproducibility of the method. The drugs were protected from light throughout the study and the absorbance of the solutions of pure MDZ, NA and their mixtures were measured within 3 h. Hence, in the absence of an official method for the simultaneous determination of MDZ and NA, the proposed method is suitable for the simultaneous determintion of these drugs in drug formulations.

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Table 3
Assay results of metronidazole and nalidixic acid in commercial formulations by difference spectroscopy

Sample	MDZ		NA	
	mg per tablet	%w/w stated[a]	mg per tablet	%w/w stated[a]
Brand A	199.24	99.62 ± 0.48	297.57	99.19 ± 0.75
Brand B	194.50	97.25 ± 0.55	299.07	99.69 ± 0.90
Brand C	200.90	100.49 ± 1.30	297.00	99.00 ± 0.79

[a] Five replicate measurements.

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