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Quantitative analysis of metronidazole in intravenous admixture with ciprofloxacin by first derivative spectrophotometry

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Abstract

Metronidazole in parenteral admixture with ciprofloxacin was analysed by first-derivative spectrophotometry using the zero-crossing technique of measurement. The procedure did not require prior separation steps. The method was found to be linear ($r^2 > 0.999$) in the range of 2.5–10 μg ml^{-1} for metronidazole in absence or presence (at constant concentration) of ciprofloxacin. The first-derivative method was applied for the analysis of intravenous admixture of metronidazole and ciprofloxacin and proved to be rapid, accurate and reproducible.

Keywords: Metronidazole; Ciprofloxacin; Intravenous infusion; Derivative spectrophotometry; Parenteral admixture

1. Introduction

The parenteral admixture of ciprofloxacin with metronidazole is nowadays well known as a valuable tool for antibiotic therapy when anaerobic organisms are involved. Compatibility of metronidazole with ciprofloxacin has been scarcely studied and findings have failed to define its compatibility [1–4]. In a previous work we reported the development and validation of a reversed phase LC method for quantifying ciprofloxacin in presence of metronidazole [5]. That method and the subsequent work conditions tested were not stability indicating to separate and quantify metronidazole.

On account that none of the conditions tested by employing reversed phase LC was helpful, in this work derivative spectrophotometry on the basis of zero-crossing was assayed.

Derivative spectrophotometry presents greater selectivity than the normal does and overcomes the problem of resolving spectral overlap in the analysis of a multicomponent system [6]. A characteristic of this technique is that the differentiation discriminates against broad bands, emphasising sharper features to an extent that they increase with increasing derivative order.

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This analytical technique has been employed for the simultaneous determination of two or more components in admixtures of pharmaceutical interest [7–10] as well as in stability studies [11,12].

Derivative spectrophotometry on the basis of zero-crossing measurements involves the measurement of the absolute value of the total derivative spectrum at an abscissa value corresponding to the zero-crossing wavelengths of the derivative

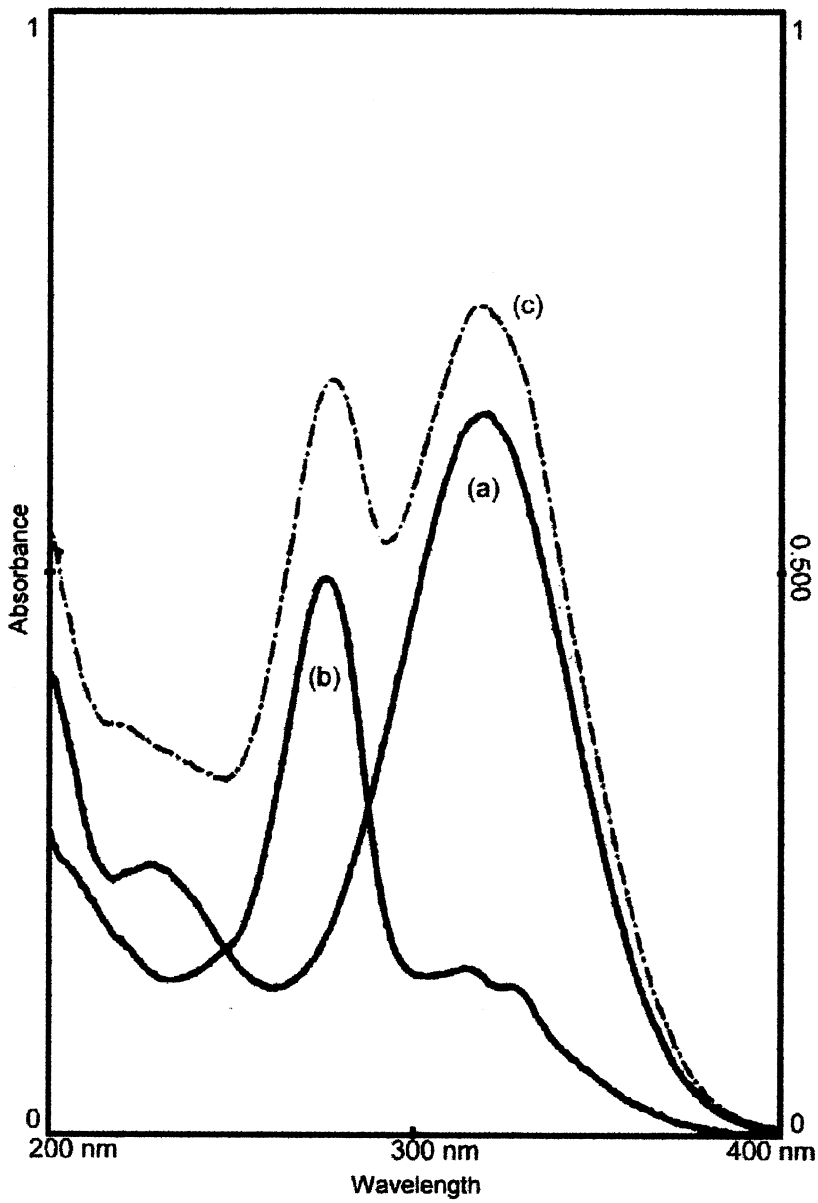

Fig. 1. Absorption spectra of: (a) metronidazole (10 μg ml^{-1}); (b) ciprofloxacin (4 μg ml^{-1}); and (c) a mixture of metronidazole and ciprofloxacin.

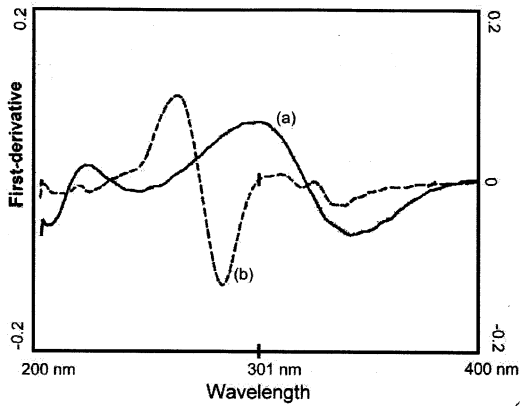

Fig. 2. First-order derivative spectra of: (a) metronidazole (5 μg ml^{-1}); and (b) ciprofloxacin (2 μg ml^{-1}).

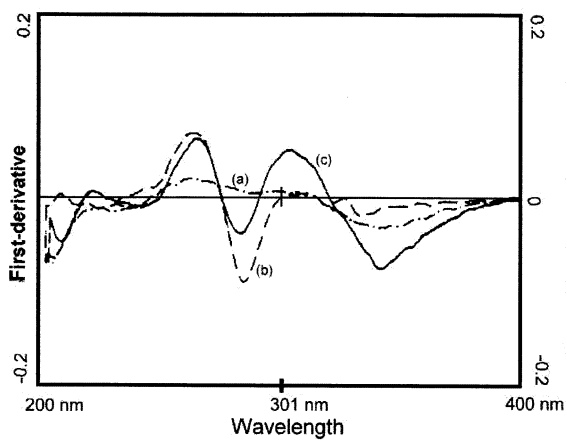

Fig. 3. First-order derivative spectra of: (a) solution of metronidazole intentionally decomposed; (b) solution of ciprofloxacin intentionally decomposed; and (c) mixture of the intact drugs.

spectra of the individual components. Measurements made at the zero crossing of the derivative spectrum of one of the two components should be a function only of the concentration of the other component [13].

The purpose of this work was to develop and validate a method that allows the determination and quantification of metronidazole in presence of ciprofloxacin without prior separation, and that facilitates the study of the compatibility of the admixture ciprofloxacin–metronidazole.

2. Experimental

2.1. Apparatus

Absorption and derivative spectra were recorded using a Shimadzu UV 260 spectrophotometer over the wavelength range 200–400 nm in 1 cm quartz cells.

The pH measurements were made with an Orion SA 520 and a Corning semi-micro combination pH electrode, model 91-03.

2.2. Materials and reagents

Ciprofloxacin and metronidazole were obtained as previously described [5].

The commercial formulations used for preliminary studies were:

Ocefax: ciprofloxacin lactate 2 mg ml^{-1}, lot N° 5711005 (kindly supplied by Laboratorio Roux Ocefa).

Flagyl: metronidazole 5 mg ml^{-1}, lot N° 096/1. (Laboratorio Rhône Poulenc Rorer).

The commercial formulations used for compatibility studies were:

Ocefax: ciprofloxacin lactate 2 mg ml^{-1}, lot N° 5910141 (kindly supplied by Laboratorio Roux Ocefa).

Flagyl: metronidazole 5 mg ml^{-1}, lot N° 107/1. (Laboratorio Rhône Poulenc Rorer).

2.3. Procedure

A calibration curve was constructed with metronidazole standard [5] in distilled water.

Stock solutions of ciprofloxacin (0.10 mg ml^{-1}) and metronidazole (0.25 mg ml^{-1}) were prepared in distilled water. Further dilutions were made by using the same diluent to give final concentrations of metronidazole in the range 2–10 μg ml^{-1}.

Each measurement was carried out in triplicate.

In order to choose the best work conditions, the zero order and first and second derivative spectra of each drug and mixtures of both drugs were recorded.

To measure metronidazole the zero-crossing values of ciprofloxacin were selected from the first derivative spectra.

The commercial solution Flagyl was evaluated to assess the content of metronidazole. This assay was done by duplicate. These Flagyl solutions were employed to validate the method and to carry out the compatibility studies.

2.4. Validation of the analytical method [14]

2.4.1. Specificity

The specificity of the method for determining metronidazole in presence of ciprofloxacin and

Table 1
Linear regression data for the first-derivative spectrophotometric method

Parameters	Curve 1		Curve 2
Range (μg ml^{-1})	0–10		0–10
Ciprofloxacin (μg ml^{-1})	–		2
Slope ± CI [a]	$(6.0 \pm 0.30) \times 10^{-3}$		$(6.3 \pm 0.32) \times 10^{-3}$
Intercept ± CI [a]	$(-0.067 \pm 1.83) \times 10^{-3}$		$(0.051 \pm 2.03) \times 10^{-3}$
Correlation coefficient	0.9996		0.9996
Limit of detection (μg ml^{-1})	0.047		0.049
Limit of quantitation (μg ml^{-1})	0.156		0.162
t experimental[b]	0.12		0.08
t theoretical[b]	3.182		3.182
F experimental[c]		4.74	
F theoretical [c]		5.99	
N	5		5

[a] CI: Confidence interval ($P = 0.05$).
[b] Student's t-test for intercept. ($P = 0.05$ and 3 degrees of freedom).
[c] By applying analysis of variance ($P = 0.05$, 3 and 3 degrees of freedom).

Table 2
Precision of the system for the determination of metronidazole

Concentration of ciprofloxacin (mg ml^{-1})	Concentration of metronidazole (mg ml^{-1})	RSD [a] (within-day)	RSD (between day)
2	5	1.79	1.74
4	5	1.90	1.68
5	5	1.90	1.35

[a] Average of three determinations.

Table 3
Recovery percentage of metronidazole in presence of ciprofloxacin

Nominal concentration of ciprofloxacin (μg ml^{-1})	Nominal concentration of metronidazole (μg ml^{-1})	Found concentration of metronidazole (μg ml^{-1})[a]	Recovery percentage
2	4.17	4.16 ± 0.061	99.7
2	5.03	4.95 ± 0.028	98.4
2	6.05	5.96 ± 0.043	98.4

[a] Average of three determinations ± confidence interval.

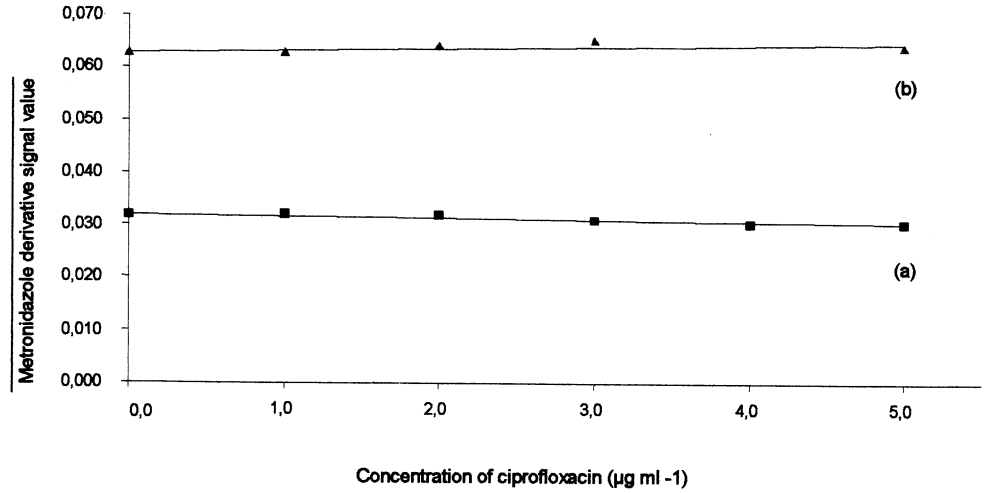

Fig. 4. Derivative signal value at the zero-crossing wavelength of ciprofloxacin (301 nm), obtained for metronidazole in presence of ciprofloxacin: (a) metronidazole 5 μg ml^{-1}; and (b) metronidazole 10 μg ml^{-1}.

their degradation products is demonstrated by ciprofloxacin, Ocefax, metronidazole and Flagyl undergoing acidic conditions and intense heating, without protection from light in order to decompose them deliberately [5].

The derivative spectra of the degraded solutions were obtained under conditions described in Section 2.3

2.4.2. Linearity

Solutions of metronidazole between 2 and 10 μg ml^{-1} in the absence of ciprofloxacin and in the presence of 2 μg ml^{-1} of ciprofloxacin were prepared for two calibration curves. The significance of the intercept of the y-axis of both regression lines was evaluated by applying Student's t-test at 95% confidence level [15]. The analysis of variance assumes that the residual error variance does not change from one calibration graph to another. The variance ratio (F experimental) was calculated and compared with a theoretical value.

2.4.3. Accuracy

For recovery assays of metronidazole in presence of ciprofloxacin, artificial matrices of the commercial formulations similar to those reported by the laboratory were prepared. Intravenous infusion Flagyl contains citric acid 44 mg, sodium phosphate 150 mg, sodium chloride 740 mg, and distilled water to make 100 ml.

Intravenous infusion Ocefax contains lactate

Table 4
Results from the determination of metronidazole concentration in a mixture with ciprofloxacin under room lighting

Time	Concentration (%) ± SD	pH
0	100.00 ± 0.00	4.24
30 min	103.30 ± 3.14	4.22
1 h	97.78 ± 4.67	4.23
3 h	99.96 ± 4.63	4.25
6 h	97.30 ± 3.82	4.25
12 h	100.56 ± 2.32	4.28
24 h	97.86 ± 6.14	4.32

Table 5
Results from the determination of metronidazole concentration in a mixture with ciprofloxacin protected from light

Time	Concentration (%) ± SD	pH
0 h	100	4.25
30 min	102.11 ± 4.49	4.23
1 h	95.77 ± 2.99	4.20
3 h	96.83 ± 5.98	4.23
6 h	94.71 ± 5.98	4.22
12 h	93.66 ± 1.50	4.28
24 h	97.89 ± 2.99	4.31

acid 95 mg, sodium chloride 900 mg, and distilled water to make 100 ml.

Synthetic mixtures, each containing 4.17, 5.03 and 6.05 μg of metronidazole per ml and 2.02 μg of ciprofloxacin per ml were analysed with the proposed method.

2.4.4. Precision

To evaluate precision of the analytical method three series of mixed solutions of metronidazole and ciprofloxacin were assessed. The mixtures contained metronidazole 5 μg ml^{-1} and ciprofloxacin 2, 4, 5 μg per ml each.

Admixtures of similar concentrations were analysed on different days.

2.4.5. Detection and quantification limits

Both limits were determined from the slope of the calibration curve and standard deviation of responses [16].

2.4.6. Robustness

Robustness of the method was tested by evaluating the derivative signal value of metronidazole at an abscissa value corresponding to zero-crossing wavelength of ciprofloxacin (301 nm), under varying concentrations of ciprofloxacin.

2.5. Determination of metronidazole in admixtures with ciprofloxacin

2.5.1. Compatibility study

Equal volumes of the commercial products [17] were mixed in an erlenmeyer flask and kept closed in a bath at controlled temperature (25°C) under room light simulating clinical conditions of administration. The mixture was immediately stirred, two aliquots of 2 ml each were taken, placed in vials of polypropylene (Nalgene Cryoware) and frozen with liquid air. These aliquots were considered 'zero time', other aliquots were taken at 30 min and at 1, 3, 6, 12, 24 h. The samples were stored at −20°C until analysis. The mixture was prepared and the procedure was performed in a laminar airflow hood type A (Labconco Model 36208). The pH of the commercial formulations was measured separately and the pH of the mixture was assessed at each time. During analysis, the samples were left to reach room temperature and were diluted conveniently in distilled water for spectrophotometric analysis.

The same working procedure was repeated with the metronidazole–ciprofloxacin mixture, which was protected from light.

3. Results and discussion

Fig. 1 shows the zero-order absorption spectra of metronidazole and ciprofloxacin separated and in a mixture. The complete overlap of both spectra is observed at the working wavelength range.

The first derivative spectra of ciprofloxacin and metronidazole are shown in Fig. 2. The zero-crossing value of ciprofloxacin appears at 301 nm and it was selected as the working wavelength.

Various values of $\Delta\lambda$ were tested and $\Delta\lambda = 2$ nm was chosen as the optimum working parameter.

The specificity of the method in the work conditions used in this study is demonstrated with the spectra of the degradation products of ciprofloxacin and metronidazole obtained under extreme acid conditions. The solution of the degradation products of ciprofloxacin showed a zero-crossing point at 301 nm whereas solution of the degradation products of metronidazole showed little absorption at 301 nm. Spectra of the degradation products recorded under work conditions are shown in Fig. 3.

The linear response of the method for metronidazole without ciprofloxacin and metronidazole in mixtures with ciprofloxacin was evaluated. Good correlation coefficients ($r^2 \geq 0.999$) were obtained with the calibration curve. The linear regression data for both calibration curves are shown in Table 1.

As can be seen in Table 1, the calculated 't' values do not exceed the theoretical values and hence the intercept on the ordinate is negligible. The experimental value of 'F' is smaller than the theoretical value of 'F' so at 95% confidence level the source of variation is not significant. Therefore, the amplitude of the derivative signal of the mixture measured at the zero-crossing point of the

derivative spectrum of ciprofloxacin may be a function only of the concentration of metronidazole.

The intravenous infusion, Flagyl, contained $99.89 \pm 1.43\%$ of metronidazole in accordance with the USP 24 [18] and with the concentration reported by the manufacturer.

The accuracy of the method was defined by applying the analytical method to a mixture of sample matrices of both drugs to which known amounts of ciprofloxacin and metronidazole were added. The accuracy is expressed as percentage of recovery of metronidazole in presence of ciprofloxacin. Recovery assays of the method are listed in Table 2.

The precision of the system was assessed from the dispersion of three determinations of metronidazole in each series of mixed solutions. The relative standard deviation for each mixture within-day and between-day are listed in Table 3.

An interaction graph plotting the metronidazole derivative signal value at zero-crossing wavelength of ciprofloxacin is shown in Fig. 4. All the samples which contain the same concentration of metronidazole show a similar derivative signal value at different concentrations of ciprofloxacin.

The results obtained from the determination of the concentration of metronidazole in the compatibility study for the mixture under room light are shown in Table 4, and the results obtained for the mixture protected from light are shown in Table 5. The first aliquot taken at the moment of the mixture was determined as time zero and was defined as 100%. The values of metronidazole are expressed as percentage of the concentration at time zero.

The pH of the commercial formulations was determined separately before mixing to observe changes once the mixture was prepared. According to some authors, a variation of pH in one unit is indicative of incompatibility, [4] however in this study such changes were not observed in the pH value. The pH of each product is 4.87 for Flagyl and 4.07 for Ocefax. The pH measured at each time for both samples is shown in Table 4 and Table 5.

Trissel refers to 'compatibility' when a minimum 90% of the drug remains unchanged and available for administration [19]. From the results obtained, metronidazole can be compatible with ciprofloxacin in a mixture for 24 h at room temperature. However, taking into account the photolability of metronidazole [20] and results obtained previously for ciprofloxacin [5], it would be convenient to protect the admixture from light.

4. Conclusions

The first-derivative spectrophotometry method was validated by linearity in the range of concentrations employed as well as by the accurate and reproducible quantification of metronidazole in presence of ciprofloxacin. It offers adequate specificity without the requirement of prior separation of the compounds. The simplicity of this method and short analysis time are also important advantages.

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