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Application of the bivariate spectrophotometric method for the determination of metronidazole, furazolidone and di-iodohydroxyquinoline in pharmaceutical formulations

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Abstract

The bivariate calibration algorithm was applied to the spectrophotometric determination of metronidazole, furazolidone and di-iodohydroxyquinoline in pharmaceutical dosage forms. The results obtained were compared with the results of derivative spectrophotometry. The statistical evaluation of method bias was carried out, and it was shown that the proposed procedure may be competitive with commonly used first-derivative spectrophotometry. The advantage of the bivariate calibration is its simplicity, and the fact that there is no need to use the derivatization procedures. © 1997 Elsevier Science B.V.

Keywords: Bivariate spectrophotometric method; Di-iodohydroxyquinoline; Furazolidone; Metronidazole

1. Introduction

Metronidazole, furazolidone and di-iodohydroxyquinoline are pharmaceuticals having a wide anti-bacterial and antiprotozoal spectrum. There exist commercial formulations containing one of these compounds, but usually the two component mixtures are more effective in the therapy of bacterial infections, different amoebas and protozoarial diseases. For example, the mixture metronidazole-di-iodohydroxyquinoline is specific against *Entamoeba histolitica* [1].

Several analytical procedures, namely spectrophotometry [2], chromatography [3] and polarography [4,5], have been reported for the individual determination of these compounds in formulations. The resolution of metronidazole and furazolidone in their mixtures was achieved using high-performance liquid chromatography [6,7] and mixtures of metronidazole and di-iodohydroxyquinoline were analysed by thin-layer chromatography [8]. The spectrophotometric determination in respective mixtures requires previous separation of the analytes, or the use of pretreatment procedures, enabling differentiation of spectral signals [9–11]. On the other hand, derivatization of spectral data and several multi-

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variate calibration techniques have been used in spectrophotometry for the resolution of two or more components whose spectra were partly overlapped [12–14]. Thus, metronidazole and furazolidone were determined by direct spectrophotometry using a least squares method [15].

Recently, we have proposed the bivariate calibration method for the resolution of two components in spectrophotometry [16]. This method is based on the simple mathematic algorithm, in which the data are used from four linear regression calibration equations: two calibrations for each component at two wavelengths selected using the method of Kaiser [17]. In work carried out on dyes, the statistical evaluation of method bias was performed, and it was concluded that the proposed methodology may be competitive to derivative spectrophotometry. In the present work, the bivariate method was applied for the resolution of metronidazole–furazolidone and metronidazole–di-iodohydroxyquinoline mixtures in the pharmaceutical formulations.

2. Outline of the bivariate method

The linear regression function for spectrophotometric determination of an analyte, A, at one selected wavelength (λ_i) is given by:

$$A_{Ai} = m_{Ai} C_A + e_{Ai}$$

where: m_{Ai}, is the slope of linear regression; C_A is the concentration of analyte (for practical reasons the concentration units of mg l^{-1} were used in this work); and e_{Ai} is the intercept value, which reflect the differences between the model and real system.

If the measurements of the binary mixture (A, B) are performed at two selected wavelengths (1 and 2), we obtain two equations:

$$A_{AB1} = m_{A1} C_A + m_{B1} C_B + e_{AB1}$$

$$A_{AB2} = m_{A2} C_A + m_{B2} C_B + e_{AB2}$$

where e_{AB1}, e_{AB2} are the sum of the intercepts of linear calibration at two wavelengths ($e_{ABi} = e_{Ai} + e_{Bi}$). The resolution of such equations set allows the evaluation of C_A and C_B values:

$$C_B = \frac{m_{A2}(A_{AB1} - e_{AB1}) + m_{A1}(e_{AB2} - A_{AB2})}{m_{A2} m_{B1} - m_{A1} m_{B2}}$$

$$C_A = \frac{A_{AB1} - e_{AB1} - M_{B1} C_B}{m_{A1}}$$

This simple mathematic algorithm allows the resolution of the binary mixture by measuring the absorbance of the mixture at two selected wavelengths and using the parameters of the linear regression functions evaluated individually for each component at these same wavelengths. The method of Kaiser [17] was used for the selection of the optimum wavelength set, which assured the

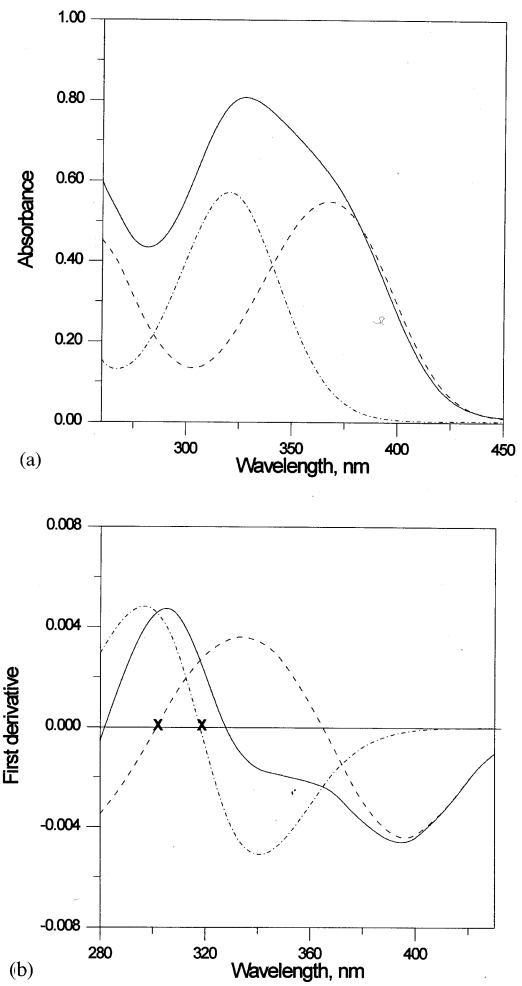

Fig. 1. Absorption spectra of: (– · –) 10.5 mg l^{-1} metronidazole; (- - - -) 7.5 mg l^{-1} furazolidone; and (——) their mixture. (a) Zero order; (b) first derivative.

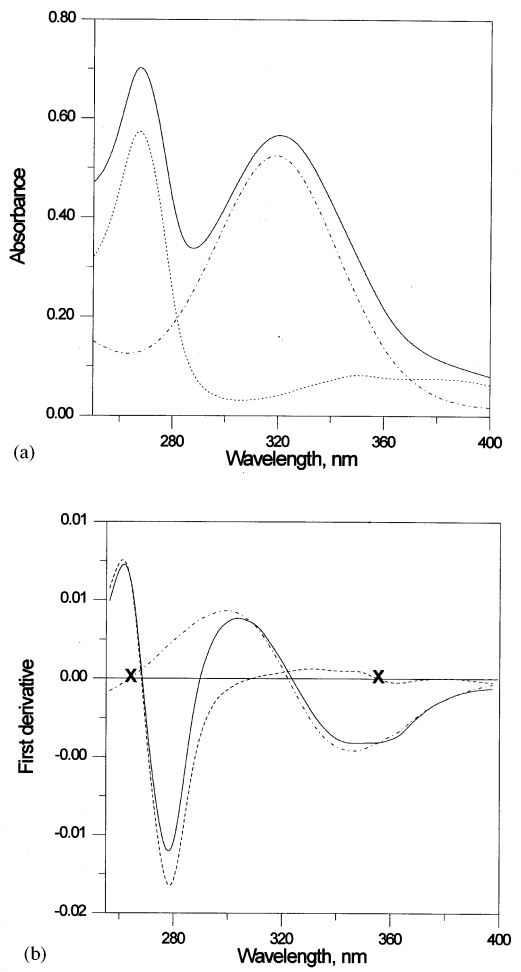

Fig. 2. Absorption spectra of: (– · – · –) 10 mg l^{-1} metronidazole; (- - - -) 8 mg l^{-1} di-iodohydroxyquinoline; and (——) their mixture. (a) Zero order; (b) first derivative.

best sensitivity and selectivity for the determination. A series of sensitivity matrices, **K**, was created for each binary mixture and for every pair of pre-selected wavelengths:

$$\mathbf{K} = \begin{bmatrix} m_{A2} & m_{B1} \\ m_{A2} & m_{B2} \end{bmatrix}$$

where m_{A1}, m_{A2} are the sensitivity parameters of the component, A, at two selected wavelengths (1, 2), and m_{B1}, m_{B2} are these parameters for the component B. It was decided to use the values of the linear regression calibration slope evaluated for one component at λ_i as the sensitivity factor. The determinants of these matrices were calculated, and the obtained values were used as the optimization criterion. The wavelength set was selected for which the highest matrix determinant value was obtained.

All calculation were performed using the simple GWBASIC programme.[1]

3. Experimental

3.1. Apparatus

A Milton Roy (Rochester, NY, USA) Spectronic 3000 Diode Array spectrophotometer with 0.35 nm resolution was used, which was coupled to a 486 PC and User data Version 2.01 (Milton Roy) software for spectral data acquisition, storage and manipulation. All data treatment operations were carried out using a Hewlett Packard Vectra 486/66 VL microcomputer equipped with the GRAMS/386 tm software package, version 3.01A (Galactic, Salem, MA, USA).

3.2. Reagents

All chemicals were of analytical-reagent grade. Metronidazole, furazolidone and di-iodohydroxyquinoline were obtained from Sigma (Mexico), dimethylformamide (DMF) was from J.T. Baker (Glen Ellyn, IL, USA). The Tris/HCl buffer (0.1 mol l^{-1}, pH 7) and ammonium buffer (0.1 mol l^{-1}, pH 9.5) solutions were prepared from Sigma reagents.

Stock solutions contained respectively 1.000 g l^{-1} of metronidazole, furazolidone and di-iodohydroxyquinoline in DMF. These solutions were stored at 4°C and protected against light. Working solutions were prepared daily by appropriate dilution.

The 'Flaganese 400' in capsules (0.4 g metronidazole and 0.2 g di-iodohydroxyquinoline, Laboratorios Liomont, Mexico), 'Flagyl' in tablets (0.5 g metronidazole, Rhone-Poulenc Pharma de Méx-

[1] The GWBASIC programme is available by request to the corresponding author.

Table 1
Analytical characteristics for the determination of metronidazole, furazolidone and di-iodohydroxyquinoline (evaluated at respective λ_{max})

Compound	pH	Detection limit (mg l^{-1})	Working range (mg l^{-1})	r^2 ($P<0.05$)
Metronidazole	7.0	0.19	3.0–16.5	0.9995
Furazolidone	7.0	0.12	2.0–12.4	0.9996
Metronidazole	9.5	0.07	1.5–20.0	0.9999
Di-iodohydroxyquinoline	9.5	0.06	1.0–16.0	0.9997

ico) and 'Fuxol' in tablets (0.1 g furazolidone, Laboratorios Columbia, Mexico) were analysed.

Pure water of Milli-Q class (Labconco, Kansas City, MO, USA) was used throughout.

3.3. Procedures

Two series of solutions containing metronidazole (3.0–16.5 mg l^{-1})–furazolidone (2.0–12.4 mg l^{-1}), and metronidazole (1.5–20.0 mg l^{-1})–di-iodohydroxyquinoline (1.0–16.0 mg l^{-1}) were prepared for the bivariate calibration. To do so, the accurate volumes of stock solutions of metronidazole and furazolidone, or metronidazole and di- iodohydroxyquinoline, were introduced to the 25 ml volumetric flasks, then 2.5 ml of DMF and 2.5 ml of Tris/HCl buffer solution (pH 7) were added to each flask and the volume was completed with Milli-Q water. Calibration solutions for the mixture of metronidazole-di- iodohydroxyquinoline were prepared in the same way, but ammonium buffer was used to attain pH 9.5.

Table 2
Application of the method of Kaiser for the selection of the wavelengths set for the mixture metronidazole–furazolidone: the absolute values of determinants of sensitivity matrices ($\mathbf{K}\times10^{-6}$)

λ/λ	312	317	323	328	355	360	**365**	370
312	0	550	551	968	3319	3555	3672	3650
317		0	335	779	3336	3598	**3735**	3722
323			0	462	3186	3472	3632	3633
328				0	2808	3114	3296	3317
355					0	366	654	788
360						0	295	441
365							0	153
370								0

The contents of three 'Flagenase 400' capsules were weighted, 5 ml DMF was added and the mixture was ultrasonicated for 5 min. After centrifugation (3500 rpm, 5 min) the sample was filtered, and the volume made up to 10 ml with DMF. The aliquots of 0.5 ml were taken and prepared in the same way as calibration samples.The analysis was done in triplicate.

Ten tablets of 'Fuxol' and five tablets of 'Flagyl' were exactly weighed, shaken with 50 ml of DMF for 1 h and centrifuged (3500 rpm, 15 min). The supernatant was introduced to a 250 ml volumetric flask, and diluted to volume with DMF. The aliquots of 0.8 ml were taken and prepared in the same way as the calibration samples. The analysis was done in triplicate.

Spectra of the obtained solutions were registered in the range 260–450 nm for metronidazole–furazolidone, and 200–400 nm for metronidazole–di-iodohydroxyquinoline. The respective buffer solution (2.5 ml of the buffer solution + 2.5 ml of DMF diluted to 25 ml with Milli-Q water) was used as the reference. Absorbance of the reference solutions at 268 nm did not exceeded 0.05, as measured against water.

First derivative spectra were calculated from the smoothed spectra (35 experimental points for metronidazole–furazolidone and 19 for metronidazole–di-iodohydroxyquinoline) using the Savitsky–Golay procedure [18].

4. Results and discussion

The effect of pH on the absorption spectra of metronidazole, furazolidone and di-iodohydroxyquinoline was studied, and for the metronida-

Table 3
Application of the method of Kaiser for selection of the wavelengths set for the mixture metronidazole–di-iodohydroxyquinoline: the absolute values of determinants of sensitivity matrices ($\mathbf{K} \times 10^{-6}$)

λ/λ	252	260	268	280	290	300	**320**	330	340	350
252	0	256	484	200	922	1452	2069	1893	1393	863
260		0	206	494	1306	1996	2838	2575	1945	1236
268			0	773	1725	2663	**3702**	3366	2556	1642
280				0	760	1245	1779	1591	1169	701
290					0	216	65	238	94	50
300						0	29	63	179	282
320							0	128	283	420
330								0	169	325
340									0	164
350										0

Table 4
Linear regression calibration formulae used for the bivariate algorithm ($A_i = m_i C + e_i$)

Binary mixture	Component	Calibration equations	
		$\lambda = 317$ nm	$\lambda = 365$ nm
MTZ–FZ	MTZ	$A = 0.0536C + 0.0028$ ($r^2 = 0.9995$)	$A = 0.0075C + 0.0026$ ($r^2 = 0.9989$)
	FZ	$A = 0.0262C + 0.0036$ ($r^2 = 0.9999$)	$A = 0.0740C - 0.0018$ ($r^2 = 0.9999$)
		$\lambda = 268$ nm	$\lambda = 320$ nm
MTZ–DHQ	MTZ	$A = 0.0120C + 0.0004$ ($r^2 = 0.9983$)	$A = 0.0523C - 0.0013$ ($r^2 = 0.9999$)
	DHQ	$A = 0.0718C - 0.0033$ ($r^2 = 0.9997$)	$A = 0.0048C + 0.0014$ ($r^2 = 0.9997$)

zole–furazolidone system, a pH value of 7.0 was selected. For metronidazole–di-iodohydroxyquinoline, a pH value of 9.5 assured better precision. Spectral overlapping of metronidazole ($\lambda_{max} = 317$ nm at pH 7.0 and $\lambda_{max} = 320$ nm at pH 9.5), furazolidone ($\lambda_{max} = 365$ nm at pH 7.0) and di-iodohydroxyquinoline ($\lambda_{max} = 268$ nm at pH 9.5) can be observed in Fig. 1(a) and Fig. 2(a), where their individual and two-component spectra are presented. The analytical characteristics for a one-component determination at the wavelength corresponding to the absorption maximum were evaluated for the three compounds and the obtained results are given in Table 1. The values for working ranges were obtained from the Ringbom graph. For the binary mixtures studies, the concentration range for each analyte was taken according to the working range of the individual calibration function.

The two wavelength sets for these mixtures were selected using the method of Kaiser [17]. For this purpose, the eight wavelengths were taken for the metronidazole–furazonidole and 10 for the metronidazole–di-iodohydroxyquinoline systems. The slope values of the linear regression (m_{Ai}: A, component; i, wavenumber) were estimated for the respective compounds at the selected wavelengths (see Tables 2 and 3). Using the obtained data, the sensitivity matrices were created for each mixture, and the respective determinants were calculated. The sensitivity results obtained are presented in Tables 2 and 3. For the bivariate determination of metronidazole and furazolidone, 317 and 365 nm were used for the analysis, and for metronidazole–di-iodoiodohydroxyquinoline 268 and 320 nm were used. At these selected wavelengths, the one-component calibration

Table 5
The calibration formulas for the metronidazole (MTZ), furazolidone (FZ) and di-iodohydroxyquinoline (DHQ) in the binary mixtures obtained using the zero-crossing method for the derivative spectra

Binary mixture	Component	λ (nm)	Calibration equation	r^2 ($\alpha = 0.05$)
MTZ–FZ	MTZ	301.33	$^1D = 4.337 \cdot 10^{-4}C - 4.52 \cdot 10^{-5}$	0.9997
	FZ	318.30	$^1D = 3.703 \cdot 10^{-4}C - 5.55 \cdot 10^{-5}$	0.9999
MTZ–DHQ	MTZ	355.85	$^1D = -4.084 \cdot 10^{-4}C + 2.49 \cdot 10^{-5}$	0.9999
	DHQ	263.58	$^1D = 8.169 \cdot 10^{-4}C + 1.79 \cdot 10^{-5}$	0.9997

Table 6
Recovery results for metronidazole (MTZ), furazolidone (FZ) and di-iodohydroxyquinoline (DHQ) in the binary mixtures obtained using the bivariate method and derivative spectrophotometry

Mixture	Analyte	Average recovery, % ± R.S.D. ($n = 10$, $P < 0.05$)	
		Bivariate method	Derivative spectrophotometry
MTZ–FZ	MTZ	98.2 ± 1.2	98.9 ± 0.9
	FZ	100.4 ± 0.8	98.6 ± 2.1
MTZ–DHQ	MTZ	95.9 ± 1.1	98.7 ± 0.6
	DHQ	100.3 ± 1.9	103.7 ± 2.2

curves were obtained. For the linear response range, the linear regression calibration function was calculated ($r^2 > 0.9990$), and m_i, e_i values were taken for the bivariate algorithm (Table 4).

These same binary mixtures were resolved using the first derivative spectra (shown in Fig. 1(b) and Fig. 2(b)). The zero-crossing measurement method was applied. The selected wavelengths, and the formula of calibration function for each component in 10 mixtures studied, are presented in Table 5.

Finally, two sets of 10 synthetic mixtures were prepared (concentrations in the working range of each component) for resolution of the two systems studied. In each mixture the recovery experiments were carried out using the binary and the first derivative calibration equations (Tables 4 and 5). Mean recovery results obtained are presented in Table 6. The evaluation of method bias was carried out using statistical tests (F- and T-tests, $P < 0.05$), and no statistically significant differences were detected for recoveries and precisions of metronidazole in the synthetic samples. For furazolidone and for di-iodohydroxyquinoline, the bivariate procedure gave better results.

4.1. Application

The bivariate calibration models were applied for the direct simultaneous determination of two mixtures (metronidazole–furazolidone and metronidazole–di-iodohydroxyquinoline) in pharmaceutical formulations. The results obtained for the 'Flaganese 400' and the 'Flagyl'–'Fuxol' mixture are presented in Table 7. As can be observed in Table 7, the results obtained using the proposed bivariate calibration and using derivative spectrophotometry were in good agreement, and also in agreement with the approximate composition of the formulation.

5. Conclusions

In this work, the bivariate calibration algorithm was applied for the resolution of two-component mixtures in pharmaceutical dosage forms. Satisfactory results were obtained in recovery experiments carried out in two sets of 10 binary mixtures: one set for metronidazole–furazolidone (mean recoveries 98.2 ± 1.2% and 100.4 ± 0.8%)

Table 7
Determination of metronidazole (MTZ), furazolidone (FZ) and di-iodohydroxyquinoline (DHQ) in pharmaceuticals using the bivariate method and derivative spectrophotometry

Pharmaceutical	Analyte	Approximate content (g)	Average content (mg ± R.S.D.) ($n = 3$, $P < 0.05$)	
			Bivariate method	Deriv. spectrophotom.
Flagyl–Fuxol	MTZ	0.5	502.5 ± 7.8	500.3 ± 6.3
	FZ	0.1	106.1 ± 2.1	104.9 ± 3.1
Flagenase 400	MTZ	0.4	397.9 ± 5.3	404.7 ± 3.5
	DHQ	0.2	183.6 ± 4.2	172.1 ± 4.1

and a second set for metronidazole–di-iodohydroxyquinoline (mean recoveries 95.9 ± 1.1% and 100.3 ± 1.9%). These results were compared with those obtained by derivative spectrophotometry. The evaluation of method bias was carried out using *F*- and *T*-tests, and no statistically significant difference was detected for the metronidazole determination, while for furazolidone and di-iodohydroxyquinoline the bivariate calibration gave better results. The results of real sample analysis obtained using the bivariate calibration and derivative spectrophotometry were in good agreement. The advantage of the bivariate calibration is its simplicity, and the fact that derivatization procedures are not necessary.

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