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KINETIC DETERMINATION OF METHOTRIMEPRAZINE, THIORIDAZINE AND THEIR MIXTURE BY USE OF A MODULAR STOPPED-FLOW/DIODE-ARRAY DETECTION SYSTEM

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Summary—The oxidation reactions of methotrimeprazine and thioridazine by iron(III) were used to develop kinetic methods based on measurements of the formation rate of the corresponding coloured phenothiazinyl free radicals. The calibration graphs were linear over the range 0.8–60 μg/ml for methotrimeprazine and 1.1–60 μg/ml for thioridazine. The relative standard deviation was 1.1–3.4%. The method was used to determine the analytes in commercially available pharmaceutical preparations. The different absorption maxima of the oxidation products formed and also the different, high initial rates of the reactions for both analytes allow their simultaneous determination by using a stopped-flow module coupled to a diode-array spectrophotometric detector. Reaction rate and absorbance increment measurements of the kinetic curves obtained were used to resolve mixtures of both phenothiazines by using the proportional-equation method and establishing three different equation systems that allowed the results obtained in each case to be compared. Mixtures of methotrimeprazine and thioridazine in ratios between 8:1 and 1:8 were satisfactorily resolved.

The joint use of a diode-array detector and the stopped-flow mixing technique has given rise to few, but very interesting applications to the simultaneous kinetic resolution of mixtures[1] as it enables implementation of chemical systems involving fast reactions, whatever the relative values of the rate constants involved. In the simplest situation, no spectral overlap between the reaction products is involved, so only simultaneous measurements of the initial rate at the maximum absorption wavelength of each product are required for their direct determination.[2] However, in the event of spectral overlap between the chemical systems involved, simultaneous resolution can also be readily achieved by using an appropriate mathematical method.[3]

In order to extend the application scope of this approach to other simultaneous determinations, we studied the resolution of a mixture of two phenothiazine derivatives whose oxidation products exhibit different absorption maxima but overlapped absorption spectra. These systems allow the simultaneous determination of both analytes using the kinetic data obtained at both wavelengths; since the reaction rates are different, a combination of kinetic and equilibrium data obtained at each wavelength can be also used. For this purpose, the proportional-equation method was used to establish three different systems of two equations each.

The two phenothiazines chosen were methotrimeprazine (levomepromazine) [*N*,*N*-dimethyl-3-(2-methoxyphenothiazin-10-yl)-2-methylpropilamine] and thioridazine [10-(2-(1-methyl-2-piperidyl)ethyl)-2-methylthiophenothiazin], which are commonly used as tranquilizers. No reaction-rate methods have so far been reported for either. Several individual determinations for the two involve oxidation reactions and equilibrium photometric measurements. Thus, potassium periodate was used as reagent for the determination of methotrimeprazine over the range 20–120 μg/ml and thioridazine from 30 to 170 μg/ml.[4] The absorbance was measured after shaking the solution containing the analyte and the oxidant in the presence of sulphuric and phosphoric acid (4*M* each) for 5 min. Iron(III) nitrate can also be used to determine methotrimeprazine hydrochloride (12–30 μg/ml) in the presence of 5%

nitric acid 2–3 min after the reagent is added.[5] An individual flow injection determination for both analytes (10–250 μg/ml in each) was developed by using iron(III) perchlorate in a strongly acidic medium (10*M* perchloric acid)[6] that called for acid-proof pump tubing. Methotrimeprazine and thioridazine have also been determined individually over the ranges 1–8 and 1–10 μg/ml, respectively, by oxidative coupling of both analytes with 2-methylbenzothiazolin-2-one hydrazone in the presence of iron(III).[7] Measurements are performed against a reagent blank ca. 15 min after the reactants are mixed. The proposed method reported in this paper allows the individual and simultaneous determinations of both phenothiazines from very fast kinetic measurements, which allows application to routine analyses.

EXPERIMENTAL

Reagents

Stock solutions of methotrimeprazine (200 μg/ml) and thioridazine (200 μg/ml) were prepared by dissolving in each case the appropriate amount of the corresponding maleate and hydrochloride salt (Sigma), respectively, in 2 ml of ethanol and diluting to 25 ml with distilled water. A $5 \times 10^{-3}M$ solution of ammonium iron(III) sulphate dodecahydrate (Merck) in 2*M* hydrochloric acid was also prepared.

Apparatus

A Hewlett-Packard 8451A diode array spectrophotometer equipped with an HP-9121 floppy-disk drive and an HP-98155A keyboard was used. A stopped-flow module supplied by Quimi-Sur Instrumentation,[8] with an observation cell of 1 cm path length, was fitted to the spectrophotometer for kinetic measurements. The solutions in the stopped-flow module and cell compartment were kept at a constant temperature (50°C) by circulating water from a thermostatted tank.

Procedures

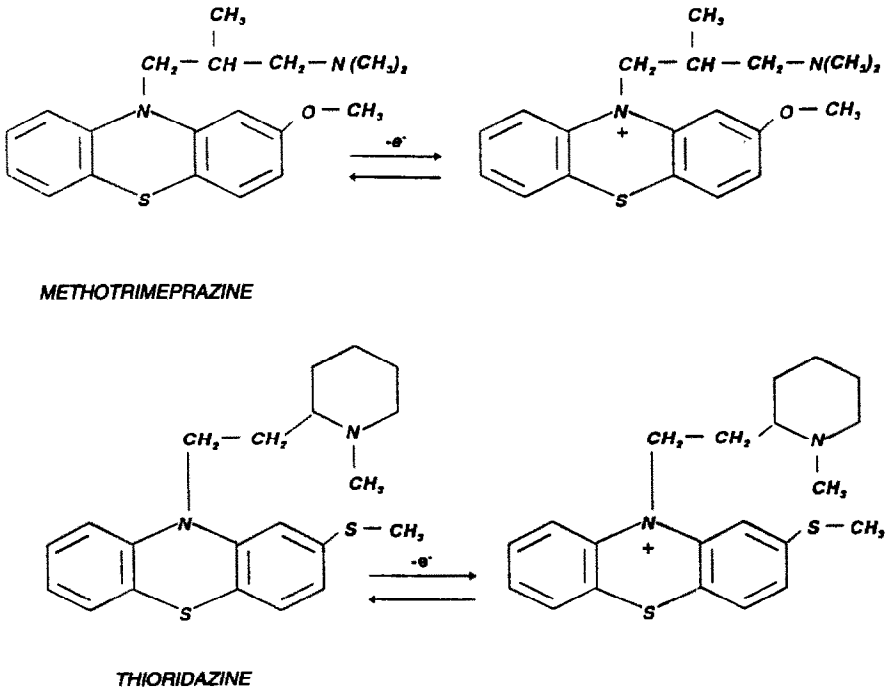
Individual determination of methotrimeprazine and thioridazine. One of the two 10-ml reservoir syringes of the stopped-flow module was filled with 10 ml of an aqueous solution containing 0.1*M* hydrochloric acid and $2.25 \times 10^{-3}M$ iron(III). The other syringe was filled with 10 ml of a previously prepared aqueous solution containing 0.1*M* hydrochloric acid and methotrimeprazine or thioridazine standard or sample in a final concentration range of 0.8–60 and 1.1–60 μg/ml, respectively. After the two 2-ml drive syringes were filled, 0.15 ml of each solution from the syringes was mixed in the mixing chamber in each run. The absorbance increment during the reaction was monitored at 564 nm for methotrimeprazine and 634 nm for thioridazine, and subsequently processed by linear regression using the microcomputer, furnished with a program for application of the initial-rate method. The reaction rate for each analyte was determined within *ca.* 5 sec and each sample was assayed in triplicate. The blank signal was found to be negligible. All measurements were made at 50°C.

Determination of methotrimeprazine and thioridazine in pharmaceutical samples. No sample pretreatment was needed for the analyses other than appropriate dilution to obtain a concentration level within the linear working range of the calibration graph. Diluted samples were treated as described above.

Simultaneous determination of methotrimeprazine and thioridazine. The procedure used was the same as that described, but the syringe containing the analyte was filled with appropriate concentrations of both phenothiazines to obtain a final concentration level of each analyte within the corresponding calibration graph. Four calibration graphs (initial rate and absorbance increment at 564 and 634 nm against analyte concentration) were run for methotrimeprazine and thioridazine. The data thus obtained were processed by using different forms of the proportional equation method described below.

RESULTS AND DISCUSSION

The oxidation of phenothiazine derivatives occurs in two steps. First, coloured phenothiazinyl free radical intermediates are formed (Scheme 1) that are subsequently transformed into their corresponding colourless sulphoxides.[9,10] Powerful oxidants such as cerium(IV) yield transient redox absorbing products, whereas milder oxidants such as iron(III) give the coloured free radicals only since their formal reduction potentials do not allow further oxidation to the corresponding sulphoxides. Thus, cerium(IV) has been used for the individual photometric determination of some



Scheme 1.

phenothiazines including chlorpromazine and trifluoperazine by a transient redox effect,[9] and iron(III) has been used to study the kinetics and mechanism of promethazine and promazine oxidation to the corresponding first-step oxidation products with the aid of the stopped-flow mixing technique.[10] We carried out a kinetic study of the oxidation of methotrimeprazine and thioridazine in order to investigate their behaviour in the presence of different oxidants and develop a simultaneous photometric method for resolution of their mixtures combining use of the stopped-flow mixing technique and diode-array spectrophotometric detector.

The maximum absorption wavelengths of the intermediate oxidation products of methotrimeprazine and thioridazine obtained in the presence of iron(III) were 564 and 634 nm, respectively. As can be seen in Fig. 1, each phenothiazine derivative absorbed at the maximum wavelength of the other. The use of a diode-array detector allows one to obtain simultaneously the oxidation rate of these compounds and the absorbance increment corresponding to the oxidation products formed at both wavelengths, and hence to resolve their mixtures by using mathematical methods such as those described below. The use of the stopped-flow mixing technique is essential in order to obtain kinetic data since the oxidation reactions involved are very fast. This is one of the most useful approaches to applying kinetic methodology to routine analyses, as shown elsewhere.[1]

Effect of reaction variables

Variables affecting the systems were optimized by applying the univariate method to reaction-rate and absorbance amplitude measurements in order to develop individual and simultaneous kinetic methods for the determination of methotrimeprazine and thioridazine. All concentrations given here are initial concentrations in the syringes (twice the actual concentrations in the reaction mixture at time zero after mixing).

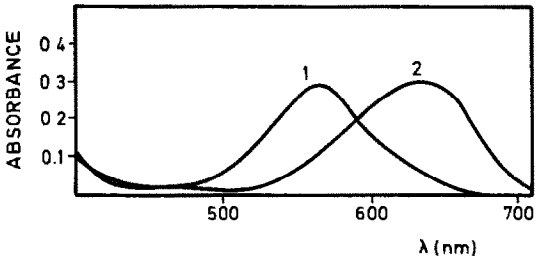


Fig. 1. Absorbance spectra of methotrimeprazine (1) and thioridazine (2) in the presence of iron(III) ($2.5 \times 10^{-3} M$) and hydrochloric acid ($0.1 M$). [Methotrimeprazine] = [thioridazine] = 10 μg/ml.

The two most important experimental variables were the oxidizing agent and the acid medium used, which had a marked effect on the reaction rates. Figure 2 illustrates the kinetic behaviour of the two compounds at their maximum absorption wavelengths in the presence of various oxidants, as well as the kinetic curves obtained for each phenothiazine derivative at the maximum absorption wavelength of the other. As can be seen, in the presence of a powerful oxidant such as cerium(IV), the transient signal of the corresponding free radical intermediate (curves 4, 8, 4′ and 8′) was obtained for the two analytes at both wavelengths. Although the reaction rates are very high and provide good analytical results provided an appropriate instrument with a high response time is used,[9] the time constant of the diode-array spectrophotometer used here does not allow one to measure the initial rate of such fast reactions. However, the reaction rates obtained in the presence of other oxidants such as iron(III), vanadium(V) and periodate, are somewhat lower and allow one to monitor the formation of the free radical intermediates. The best reaction rate values were obtained by using iron(III), which was chosen for subsequent experiments. Figure 3 shows the variation of the initial rate for each analyte with the iron(III) concentration at both wavelengths; as can be seen, the parameter was constant above a $2 \times 10^{-3}M$ iron(III) concentration. The variation of the absorbance increment with this variable was quite similar.

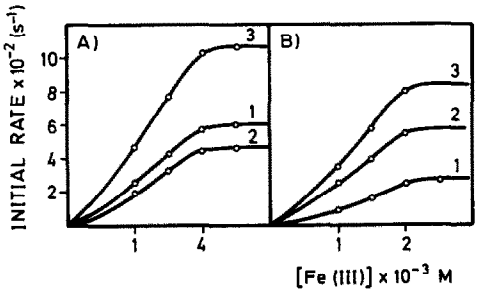


Fig. 3. Effect of iron(III) on the oxidation rate of methotrimeprazine (1), thioridazine (2) and a mixture of both (3) at 564 nm (A) and 634 nm (B). [Methotrimeprazine] = [thioridazine] = 20 μg/ml.

The effect of the acid medium used on both systems is shown in Fig. 4. Of the three acids tested (hydrochloric, nitric and perchloric), the first gave the best reaction rate values. The reaction rate was independent of the concentration of hydrochloric acid in the solutions held in both syringes of the stopped-flow module over the range 0.05–0.15M. Regarding temperature, the best reaction rate values were obtained between 48 and 52°C.

Under the optimum experimental conditions a pseudo-zero-order kinetic dependence on the iron(III) and hydrochloric acid concentration was found. Also, the initial slopes of the

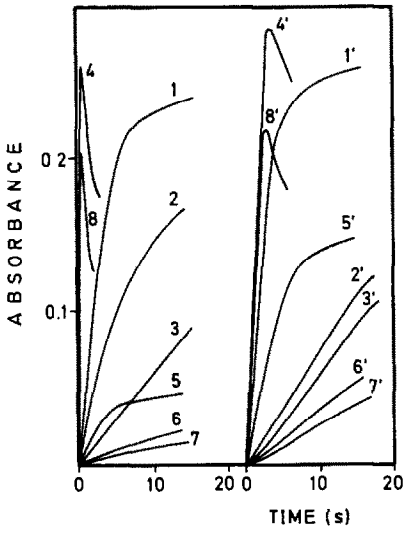


Fig. 2. Kinetic curves obtained for methotrimeprazine (1–8) and thioridazine (1′–8′) at 564 nm (1–4, 5′–8′) and 634 nm (5–8, 1′–4′) in the presence of hydrochloric acid (0.1M) and iron(III) (1,5,1′,5′), vanadium(V) (2,6,2′,6′), periodate (3,7,3′,7′) and cerium(IV) (4,8,4′,8′). [Oxidant] = $2.25 \times 10^{-3}M$.

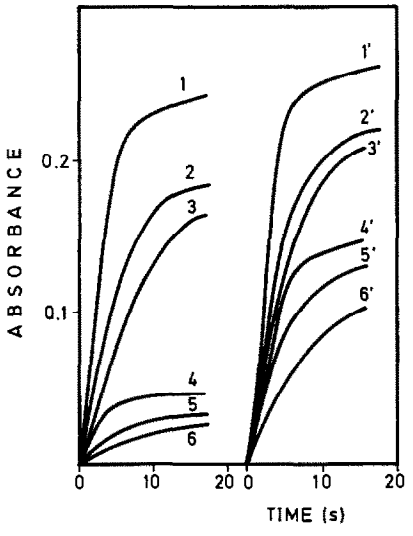


Fig. 4. Kinetic curves obtained for methotrimeprazine (1–6) and thioridazine (1′–6′) at 564 nm (1–3, 4′–6′) and 634 nm (4–6, 1′–3′) in the presence of iron(III) ($2.25 \times 10^{-3}M$) and HCl (1,4,1′,4′), HNO_3 (2,5,2′,5′) and $HClO_4$ (3,6,3′6′). [Acid] = 0.1M.

Table 1. Quantitative performance of the proposed method

Analyte	Methotrimeprazine		Thioridazine	
Wavelength (*nm*)	564	634	564	634
Linear range ($\mu g/ml$)	0.8–60	3.8–60	2.2–60	1.1–60
Slope ± SD ($\times 10^{-3}$)				
V ($ml \cdot \mu g^{-1} \cdot sec^{-1}$)	1.2 ± 0.1	0.69 ± 0.05	0.51 ± 0.05	1.0 ± 0.1
ΔA ($A\ ml/\mu g$)	4.1 ± 0.2	1.1 ± 0.1	3.0 ± 0.3	5.6 ± 0.1
Intercept ± SD ($\times 10^{-3}$)				
V ($1/sec$)	0.7 ± 0.1	0.42 ± 0.08	2.3 ± 0.4	2.2 ± 0.1
ΔA (A)	1.2 ± 0.2	2.0 ± 0.3	4.1 ± 0.5	3.8 ± 0.4
Correlation coefficient (r)				
V	0.998	0.999	0.998	0.998
ΔA	0.998	0.999	0.999	0.995
Detection limit ($\mu g/ml$)	0.2	1.1	0.6	0.2
Precision (RSD%)*				
4 $\mu g/ml$	3.2	3.3	1.9	2.6
10 $\mu g/ml$	3.4	2.3	2.2	1.1

V = Reaction-rate measurements.
ΔA = Absorbance increment measurements.
A = Absorbance.
*Relative standard deviation ($n = 10$).

absorbance *vs.* time curves obtained at 564 and 634 nm were consistent with a first-order dependence on the methotrimeprazine and thioridazine concentration.

Figures of merit of the proposed method

The data obtained from the kinetic curves for methotrimeprazine and thioridazine at 564 and 634 nm were processed by using two quantitation methods involving initial-rate and absorbance-increment (ΔA) measurements. Table 1 summarizes the features of the four methods developed for the two analytes. The individual determination of each analyte is preferably carried out by using data obtained at its maximum absorption wavelength (564 nm for methotrimeprazine and 634 nm for thioridazine). The use of the other data shown in Table 1 lies in the simultaneous resolution of mixtures as is described below. The features of both determinations are very similar. The correlation coefficients suggest very good calibration linearity. The detection limits were calculated according to IUPAC's recommendations.[11] As can also be seen in Table 1, the precision (RSD) obtained at two concentrations of each analyte was 1.1–3.4%. One other salient features of the determinations is expeditiousness: initial rate measurements were performed in *ca.* 5 sec, which resulted in a very high sample throughput.

The selectivity of the methotrimeprazine and thioridazine determinations was studied by assaying other psychotropic drugs (Table 2). The maximum concentration tested for each potential interferent was 100-fold that of each analyte. As can be seen, the tolerated species/analyte ratio was better for methotrimeprazine than for thioridazine, although the selectivity was quite good for both since only acetopromazine interfered at the same concentration level as thioridazine.

Applications

The individual kinetic methods were applied to the determination of methotrimeprazine and thioridazine in the commercially available pharmaceutical preparations listed in Table 3. Recoveries were calculated by adding various amounts of each phenothiazine to each pharmaceutical and subtracting the results obtained for a pharmaceutical sample prepared in a

Table 2. Effect of various foreign species on the determination of 5 μg/ml methotrimeprazine and 5 μg/ml thioridazine

	Tolerated species/analyte ratio	
Foreign species	Methotrimeprazine	Thioridazine
Nortriptyline	100	50
Trimipramine	100	10
Amitriptyline	50	25
Fluphenazine	25	10
Imipramine	25	10
Promethazine	25	2
Perphenazine	10	2
Chlorpromazine	2	2
Acetopromazine	1	—

Table 3. Determination of methotriprazine and thioridazine in pharmaceutical preparations

Sample	Analyte	Content (*mg/ml*) Stated	Found*	Added (*mg/ml*)	Recovery Found* (*mg/ml*)	% Recovery
Sinogan (Rhone-Poulenc Roner, S.A.)	Methotrimeprazine	40	38.4	12	12.5	104.2
				16	16.6	103.7
				20	21.2	106
Meleril (Sandoz)	Thioridazine-HCl	30	30.0	9	8.55	95
				12	12.3	102.5
				15	15.6	104
Visergil (Sandoz)	Thioridazine-HCl	10	9.20	3	2.82	94
				4	3.75	93.7
				5	4.80	96

*Average of three determinations.

similar manner to which no analyte was added. The recoveries obtained are also given in Table 4. The mean recovery was 104.6% for methotrimeprazine and 97.5% for thioridazine.

Resolution of mixtures

The proportional-equation method was used for the simultaneous determination of methotrimeprazine and thioridazine. Three different possibilities were tested for this purpose. In one, two equations derived from the reaction rates obtained at the maximum absorption wavelengths of both analytes were established, namely: $v_1 = v_{1,M} + v_{1,T}$ and $v_2 = v_{2,M} + v_{2,T}$, where v_M and v_T are the contributions of methotrimeprazine and thioridazine to the overall reaction rate obtained at 564 nm (v_1) and 634 nm (v_2). The other two methods are based on the reaction rate and absorbance increment (ΔA) obtained for both analytes at each wavelength, namely: $v = v_M + v_T$ and $\Delta A = \Delta A_M + \Delta A_T$. The absence of synergistic effects in the three methods ensured that the parameters obtained from the kinetic curves for a mixture of the two analytes were the sum of the corresponding parameters obtained for each individual analyte separately.

Table 4. Resolution of methotrimiprazine (M) and thioridazine (T) mixtures

M-T ratio	Method	M (*μg/ml*) Taken	Found*	T (*μg/ml*) Taken	Found*
8:1	1	40	39.5	5	5.40
	2		39.7		4.92
	3		39.7		5.27
4:1	1	20	19.8	5	5.23
	2		19.8		5.17
	3		19.7		5.27
2:1	1	10	9.72	5	5.17
	2		9.94		4.64
	3		9.98		5.06
1:1	1	5	4.86	5	5.22
	2		4.83		5.29
	3		5.01		5.12
1:2	1	5	4.98	10	10.2
	2		5.13		9.89
	3		5.21		10.1
1:4	1	5	4.94	20	18.6
	2		4.69		19.1
	3		4.66		18.8
1:8	1	5	4.81	40	39.7
	2		4.47		40.5
	3		4.52		39.9

*Average of three determinations.
†See text.

By using the slopes (a, a') and intercepts (b, b') of the corresponding calibration graphs obtained for each analyte at both wavelengths (Table 1), the following equation systems were established and used for simultaneous mixture resolution:

Method 1:

$$v_1 = a_{1,M}[M] + a_{1,T}[T] + b_{1,M} + b_{1,T}$$
$$v_2 = a_{2,M}[M] + a_{2,T}[T] + b_{2,M} + b_{2,T}$$

Method 2:

$$v_1 = a_{1,M}[M] + a_{1,T}[T] + b_{1,M} + b_{1,T}$$
$$\Delta A_1 = a'_{1,M}[M] + a'_{1,T}[T] + b'_{1,M} + b'_{1,T}$$

Method 3:

$$v_2 = a_{2,M}[M] + a_{2,T}[T] + b_{2,M} + b_{2,T}$$
$$\Delta A_2 = a'_{2,M}[M] + a'_{2,T}[T] + b'_{2,M} + b'_{2,T},$$

where [M] and [T] are the concentrations (μg/ml) of methotrimeprazine and thioridazine, respectively. A simple BASIC program was used to solve the equations. Table 2 summarizes the results obtained by applying the three methods to solutions containing known concentrations of both analytes in different ratios. From the results it follows that the three methods can be satisfactorily applied to the simultaneous resolution of mixtures of methotrimeprazine and

thioridazine in ratios between 8:1 and 1:8 with very similar results.

CONCLUSIONS

The results obtained in this work clearly show the potential of coupling the stopped-flow mixing technique and a diode-array detector for the kinetic resolution of mixtures involved in fast reactions. Although the simultaneous determination of both analytes can be achieved by using the kinetic curves obtained at a single wavelength, using the data obtained at both wavelengths allows one to check the results as they are comparable for all three methods. In relation to the individual determination of these phenothiazines, the proposed reaction rate methods are faster and their quantification limits lower than those provided by equilibrium and flow injection methods.[4–7] Also, the acid concentration required by the kinetic methods is somewhat lower.

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REFERENCES

1. A. Gómez-Hens and D. Pérez-Bendito, *Anal. Chim. Acta*, 1991, **242**, 147.
2. M. C. Gutiérrez, A. Gómez-Hens and D. Pérez-Bendito, *Anal. Chim. Acta*, 1989, **225**, 115.
3. G. M. Ridder and D. W. Margerum, *Anal. Chem.*, 1977, **49**, 2098.
4. L. Kuzmicka, H. Puzanowska-Tarasiewicz and M. Tarasiewicz, *Pharmazie*, 1988, **43**, 288.
5. M. I. R. M. Santoro, S. Storpirtis, E. R. M. Hackmann and J. F. Magalhaes, *Anal. Lett.*, 1989, **22**, 929.
6. M. A. Koupparis and A. Barcuchova, *Analyst*, 1986, **111**, 313.
7. M. E. El-Kommos and K. M. Emara, *Analyst*, 1988, **113**, 1267.
8. A. Loriguillo, M. Silva and D. Pérez-Bendito, *Anal. Chim. Acta*, 1987, **199**, 29.
9. H. A. Mottola and A. Hanna, *Anal. Chim. Acta*, 1978, **100**, 167.
10. M. R. Gasco and M. E. Carlotti, *J. Pharmac. Sci.*, 1978, **67**, 168.
11. G. L. Long and J. D. Winefordner, *Anal. Chem.*, 1983, **55**, 712A.