Available online at www.sciencedirect.com
SCIENCE DIRECT®
Talanta 66 (2005) 952–960
Talanta
www.elsevier.com/locate/talanta

Response surface methodology for the optimisation of flow-injection analysis with in situ solvent extraction and fluorimetric assay of tricyclic antidepressants

María-Isabel Acedo-Valenzuela, Teresa Galeano-Díaz*, Nielene Mora-Díez, Antonio Silva-Rodríguez

Departamento de Química Analítica, Facultad de Ciencias, Universidad de Extremadura, Av. de Elvas s/n, 06071-Badajoz, Spain

Received 30 September 2004; received in revised form 24 November 2004; accepted 17 December 2004
Available online 21 January 2005

Abstract

A semiautomatic extraction-fluorimetric method for the determination of tricyclic antidepressant drugs (TCAs) based in the formation of ion pairs with 9,10-dimethoxyanthracene-2-sulphonate (DMAS) has been developed. The aqueous solutions of the TCAs (imipramine, desipramine, amitriptyline, nortriptyline, clomipramine or doxepine) are injected into a carrier composed by DMAS in an acid medium and the ion pair formed is extracted into dichloromethane where the fluorescence is measured. An experimental design (Central Composite Design) together with the Response Surface Methodology has been used to find the optimal instrumental FIA and chemical variables. We have considered as the response function the product of the peak height by the sampling frequency. The calibration curves were linear over the working range (0.25–3.00 mg L^{-1}). The limits of detection were lower than 0.30 mg L^{-1}. The method has been satisfactorily applied to the determination of imipramine, amitriptyline, clomipramine and doxepin in pharmaceutical preparations.

Keywords: Tricyclic antidepressant drugs; 9,10-Dimethoxyanthracene-2-sulphonate; Flow injection; Pharmaceuticals

1. Introduction

The tricyclic antidepressants (TCAs) are one of the largest groups of drugs for the treatment of psychiatric disorders such as depression, mainly endogenous major depression [1]. For this group of drugs, distinct ranges of optimum plasma concentration for therapy are required (100–300 μg L^{-1} for the most of the TCAs). At high concentrations, severe adverse effects and toxicity can appear. Therefore, the analysis of these compounds is important for obtaining optimum therapeutic concentrations and for quality assurance in pharmaceutical preparations.

A variety of techniques have been used to determine TCAs in diverse biological fluids and pharmaceuticals, including radioimmunoassay, gas chromatography, liquid chromatography (LC), capillary electrophoresis, spectrofluorimetry, spectrophotometry, chemiluminiscence, titrations, etc. [2–9].

Some of the TCAs are not fluorescent and different reagents have been used for their derivatization. Reactions with oxidants [8], methyl orange [10], erythrosine B [11], OPA (*o*-phthalaldehyde) and NAC (*N*-acetylcysteine) [13], etc., have been used. Subsequent extraction in an organic solvent improves both sensitivity and selectivity of the methods. Conventional batch solvent extraction process is a tedious and time-consuming procedure, and many efforts have been directed towards the automation of this procedure. Flow-injection technique has advantages that makes it suitable also for this purpose [10,11,14].

* Corresponding author. Tel.: +34 924289671; fax: +34 924289375.
E-mail addresses: miacedo@unex.es (M.-I. Acedo-Valenzuela), tgaleano@unex.es (T. Galeano-Díaz), nielene@unex.es (N. Mora-Díez), asilvaro@unex.es (A. Silva-Rodríguez).

0039-9140/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2004.12.044

In this paper we investigate the formation and extraction of the ion pairs formed between TCAs (dialkyl and trialkylamines) with the fluorophore 9,10-dimethoxyanthracene-2-sulfonate (DMAS), in order to develop a sensitive and automatic fluorimetric method for the determination of six TCAs in pharmaceuticals. In the bibliography, papers referred to FIA procedures for the analysis of TCAs describe methods to determine, separately, three or less of them [7,8,10–15].

The DMAS have been yet used as counter-ion in analysis [16,17]. It presents native fluorescence and forms hydrophobic ion pairs with amines extractable in organic solvents. Westerlund and Borg [16] studied the extraction of the ion pair formed between DMAS and the amitriptyline and protriptyline allowing quantitative extraction in dichloromethane. The TCAs studied here have been: imipramine (IM), amitriptyline (AM), doxepin (DO) (as tertiary amines) and desipramine (DE), nortriptyline (NO) and clomipramine (CLO) (as secondary amines).

Chemometrics have been shown to be successful for the optimisation of variables in flow-injection procedures [12,13,18,19]. In the development of this FI system we have used experimental design approaches (fractional factorial design and central composite design) and the response surface analysis methodology to elucidate the manner in which the FI and chemical variables affect the response function [20,21]. For the latter, we have considered separately the peak height and the sampling rate or a simple combination of both.

2. Experimental

2.1. Instrumentation and software

Fluorescence measurements were made on a SLM Aminco-Bowman, Series 2, spectrofluorimeter, equipped with a 150 W continuous Xenon lamp, and with an Hellma 176.752QS (Z: 8.5 mm; light path 1.5 mm) flow through compact cell. The spectrofluorimeter was interfaced by a GPIB card and driver with a Pentium PC microcomputer. Data acquisition and data analysis were performed by the use of AB2 software, Version 5.00, running under Windows 98. The excitation and emission slits were 4 nm for both.

Two Gilson Minipuls3 peristaltic pumps, an Omnifit six-way manual injection valve and a homemade phase separator of solid Teflon (containing a PTFE hydrophobic membrane with a 0.5 μm pore size) were also used.

The software package THE UNSCRAMBLER® 6.11_b. CAMO ASA, running under Windows XP, was used for the application of chemometrics.

2.2. Reagents

The TCAs hydrochlorides (≥98%) were obtained from Sigma (St. Louis, MO, USA) and standard solutions of 100 μg mL^{-1} of these compounds were prepared by dissolving the appropriate amounts in water or in hydrochloric acid. These solutions were kept refrigerated and in the dark, and working solutions of lower concentrations were freshly prepared by appropriate dilution. Sodium 9,10-dimethoxyantracene sulfonate was supplied by Fluka (Fluka, Riedel-de Haën, Germany). All reagents were of analytical-reagent grade or better.

2.3. Pharmaceuticals

Tofranil (per tablet: 50 mg of imipramine hydrochloride, lactose and exc.); *Tofranil Pamoato* (per capsule: 75 mg of imipramine pamoate and exc.); *Anafranil* (per tablet: 25 mg of clomipramine hydrochloride, lactose and exc.) from Norvatis Farmacéutica, SA; *Nobritol* (per capsule: 12.5 mg of amitriptyline hydrochloride, 5 mg of medazepam, lactose and exc.) from Kern Pharma, SL; *Mutabase* (per tablet: 10 mg of amitriptyline hydrochloride, 2 mg of perphenazine and exc.) from Schering Plough, SA; *Tryptizol* (per tablet: 25 mg of amitriptyline hydrochloride and exc.) from Laboratorios Neurogard, SA and *Sinequan* (per capsule: 25 mg of doxepin hydrochloride and exc.) from Farma Sierra, were acquired in a local pharmacy.

In all cases, the treatment of the sample was the same, namely: 20 tablets (or the content of 20 capsules) were exactly weighed and grounded to fine powder. Accurately weighted aliquots were transferred to volumetric flasks, and dissolved in purified water (except for imipramine pamoate that was dissolved in 1 M HCl) by sonicating for about 30 min and diluting to the mark. A portion of the solutions were filtered and used to prepare other diluted solutions in water (except for imipramine pamoate that was in 0.5 M HCl). The results, expressed as mg of drug per tablet or capsule, are shown in Table 6.

2.4. Manifold and procedure for the analysis of TCAs

The manifold used in this work is shown in Fig. 1. A two channel-configuration incorporating a pump each one of them was used. Each pump incorporates Tygon tubes to impel aqueous solutions and the flow of organic solvent (line 2) occurs by displacement in a glass bottle containing water (upper liquid) and the organic solvent (lower liquid). All the connections as well as the coil were made of PTFE (0.5 mm i.d.) tubings.

The sample (aqueous solutions containing between 0.2 and 3.0 μg mL^{-1} of the TCA to be analysed) was injected (I) into a carrier consisting in 2.9×10^{-4} M of DMAS in 0.2 M HCl (line 1), to acquire positive charge and to form the ion pair in the reaction coil. After that, the carrier merged with the organic stream of the line 2 (dichloromethane) and the mixture passed through the extraction coil (R_2), where the extraction of the ion pair takes place. After crossing the phase separator (PS), the organic stream arrived to the detector. The fluorescence signal was obtained at 265 nm with an excitation slit of 4 nm, and measured at 448 nm with an emission slit of 4 nm.

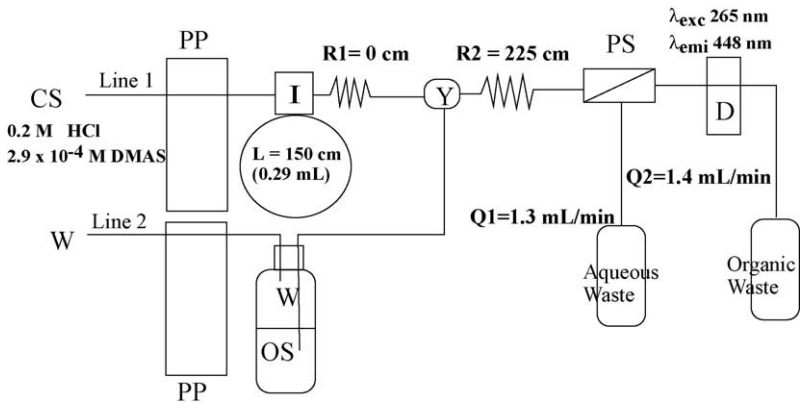

Fig. 1. Schematic diagram of the proposed flow-injection method. CS: carrier solution; W: water; I: injection valve; PP: peristaltic pumps; *L*: loop; Y: PTFE "Y" connector; R_1: reaction coil; R_2: extraction coil; PS: phase separator; Q_1: aqueous flow rate; Q_2: organic flow rate; D: fluorimetric detector; OS: organic solvent.

3. Results and discussion

Some preliminary studies about the reaction between the TCAs and the reagent DMAS were realized, in batch conditions. From the studies of Westerlund and Borg [16] and selecting one of the antidepressant, the amitriptyline, we checked the optimal conditions for the formation of the ion pair and its extraction. It was found that the formation of the ion pair is favoured in strongly acid medium and does not occur at pH values higher than 6 (pK_a of amitriptyline 9.42). A rate of concentration between the DMAS and the antidepressant of almost 10 times, and dichloromethane as solvent, were found to be the more convenient conditions. Thus, the formation and extraction of the ion pair was rapid (shaking time from 0.5 to 3 min did not produce any change in the fluorescence intensity) and reproducible and the differences between the fluorescence of the ion pair and reagent coextracted were maxima. In Fig. 2, the excitation and emission spectra of the ion pair and the DMAS, after the extraction in dichloromethane are shown. Both, the ion pair and the DMAS, present the same excitation (265 and 381 nm) and emission (448 nm) wavelengths. The spectra of the ion pairs formed by the other TCAs (CLO, DO, NO, IM and DE) show the same shape, but different intensity of emitted fluorescence. Actually, CLO and DO present the highest intensity of fluorescence for the same concentration.

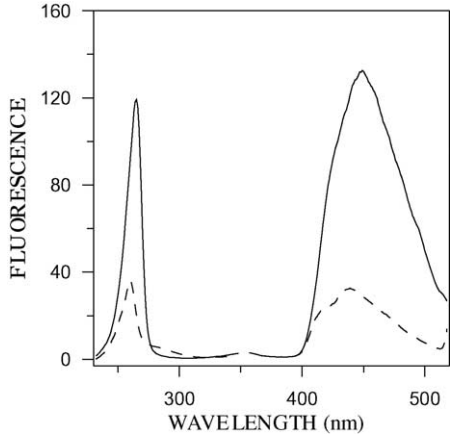

Fig. 2. Excitation (λ_{emi} = 448 nm) and emission (λ_{exc} = 265 nm) spectra of DMAS (- - - - -) and ion pair AM-DMAS (—) extracted in dichloromethane. Initial aqueous concentrations: 5.5×10^{-6} M DMAS and 5.5×10^{-7} M AM.

On the other hand, imipramine and desipramine possess a weakly native fluorescence (λ_{exc} = 270 nm and λ_{em} = 405 nm) in basic media, but their fluorescence strongly increases when the ion pair is formed.

For the optimisation of the FI system, the FI parameters and the chemical variables were investigated separately. This approach was chosen because a combined evaluation of both the FI and chemical parameters creates a complex situation generating a too large number of factors.

On the other hand, since the objective of the optimisation is to find conditions where the response is best (maximal or minimal), it was necessary to define the response to consider. In FI, one of these criteria could be the residence time which should be minimized so that the number of samples to be examined in a given time (sampling rate, SR) is increased. Also, the peak height (*H*) for a given concentration of sample should be maximized. These two criteria we used in this work.

3.1. Optimization of FI variables

From the FI system, several parameters must be examined (Fig. 1) such as the length of the reaction coil (R_1) and the extraction coil (R_2), the flow of the carrier solution (Q_1) and the organic solvent (Q_2), and the loop size (*L*). Given the great number of variables to optimize (five variables) a preliminary screening was made, to eliminate those which less influence the fluorimetric signal. With this purpose a fractional factorial design which implies a total of 18 experiments was used, and the peak height (response function I) and the sampling rate, number of peaks in an hour (response function II), were obtained. The effect of each variable (as percentage), and of the interactions between them, over both response functions

was evaluated. It was found that the length of the reaction coil (R_1) had a negative effect, as it could be expected since the reactions are very fast, and the increase of R_1 only produces a greater dispersion of the sample plug. Q_1, Q_2, and also Q_1/Q_2 had positive effects over both response functions, being less important the effect of Q_1. In consequence we opted for fixing the value of this variable at 1.3 mL min^{-1}. Lower values cause a decrease of repeatability, and greater values imply a high consumption of the very expensive reagent. The other variable examined, L, had an important positive effect over the peak height, and a negative effect over sampling rate.

From these results it was dediced to eliminate the reaction coil ($R_1 = 0$ cm) and the three variables optimized were: R_2 or R from now on, length of the reaction coil; Q_2, flow of the organic solvent measured at the end of line 2; L, length of the loop. A central composite design was used with the aim of calculating simultaneously the effect of the changes in each one of them and their possible interactions. Five levels were fixed for each variable. There are also three central samples, giving rise to a total of 17 experiments (Table 1). This design is rotatable and therefore the precision in the calculation of the response is uniform over the whole experimental field. From the shape of the excitation and emission spectra of the ion pairs, the fiagrams could be obtained selecting in the detector two different wavelengths pairs, 265/448 or 380/448 nm. The first of them offers greater sensitivity but the stability of the fluorescence signal is poor, probably due to photodegradation of the compounds. However, repeatability of the FI signals is very good even at 265/448 nm. In consequence this pair has been selected. Peak height and sampling rate were combined in order to be optimised in different response functions (RFs):

$$\mathrm{RF1} = H \times \mathrm{SR}, \qquad \mathrm{RF2} = H + \mathrm{SR}$$

where H is the peak height and SR the sampling rate.

The optimization of the models was made with THE UNSCRAMBLER software package [22], and making the corresponding ANOVA, in which a second grade quadratic model is assumed. We found that the best statistical parameters for the optimized model were obtained with RF1, which coincides with the ratio between the peak height and the residence time, and therefore is greater for the higher and more narrow peaks: The ANOVA for the RF1 is in Table 2. On the other hand it is proven that the considered effects contribute significantly to the model except the main effect R whose linear contribution is not significant for the established 95% confidence level. Also the interaction L–L is significant. Fig. 3 shows the response surfaces estimated by the model, for each pair of components. In these diagrams, it can be appreciated the variations in the response function when only the two specified factors are considered. In these surfaces the maximum value of response function is located at the following values of the variables: $R = 225$ cm; $Q_2 = 1.4$ mL min^{-1}; $L = 150$ cm. Other way to visualize the results of the optimization is the Pareto

Table 1
Optimization of the FI variables

Experiments[a]		R_2 (cm)	Q_2 (mL min^{-1})	L (cm)
1	Cent-c	163	2.45	113
2	Cube006a	245	1.35	165
3	*H: A-a	300	2.45	113
4	Cube002a	245	1.35	61
5	Cube003a	81	2.92	61
6	*L: A-a	25	2.45	113
7	*L: C-a	163	2.45	25
8	Cube001a	81	1.35	61
9	*H: C-a	163	2.45	200
10	Cent-a	163	2.45	113
11	*H: B-a	163	3.30	113
12	Cube008a	245	2.92	165
13	*L: B-a	163	0.70	113
14	Cube007a	81	2.92	165
15	Cube004a	245	2.92	61
16	Cube005a	81	1.35	165
17	Cent-b	163	2.45	113

Experiments resulting of a central composite design. (*) Experiment is a star point in the model.
[a] The experiments are numbered according the randomized sequence followed.

Table 2
Optimization of FI variable

Effect	Sum of squares	d.f.[a]	Mean square	F-ratio	P-value[b]
R	6.672E+06	1	6.672E+06	3.082	0.1226
Q_2	6.810E+07	1	6.810E+07	31.452	0.0008
L	8.180E+07	1	8.180E+07	37.777	0.0005
R–Q_2	6.029E+06	1	6.029E+06	2.705	0.1391
R–L	2.002E+06	1	2.002E+06	0.925	0.3683
Q_2–L	5.624E+06	1	5.624E+06	2.598	0.1511
R–R	2.061E+07	1	2.061E+07	9.521	0.0177
Q_2–Q_2	2.446E+07	1	2.446E+07	11.296	0.0121
L–L	7.530E+07	1	7.530E+07	34.775	0.0006
Total error	1.516E+07	7	2.165E+06		
Total (corrected)	2.430E+08	16			

Results from ANOVA of effects contributing to the model (by response surface methodology). R^2 (adjusted for d.f.) = 0.938; model check quadratic = 0.0097 (<0.05); lack-of-fit = 0.5756 (>0.05).
[a] Degrees of freedom.
[b] The effect is declared significant if $P < 0.05$.

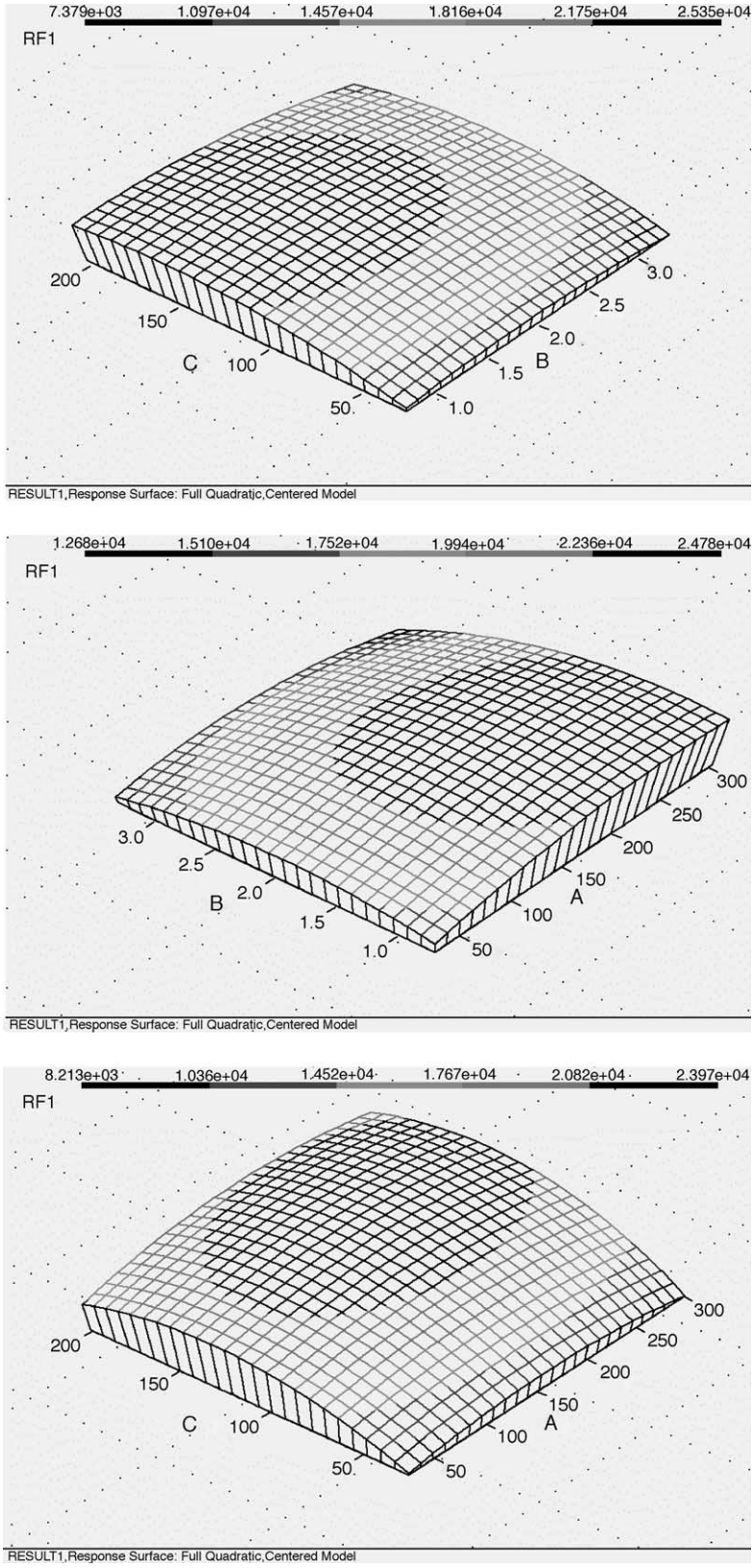

Fig. 3. Estimated response surfaces for each pair of FI variables. (A) R_2; (B) Q_2; (C) L.

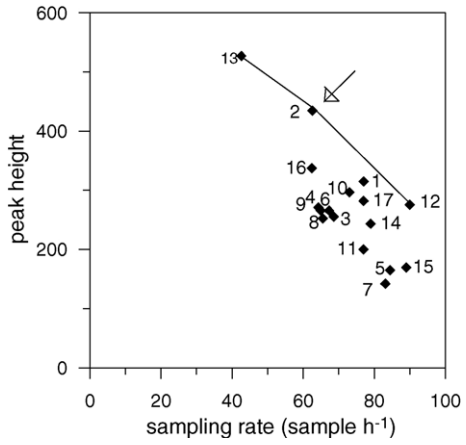

Fig. 4. Pareto diagram for visualizing the results of the optimization of the FI variables. Optimal points are connected for the situation in which peak height and sampling rate are considered optimal. The arrow indicates the best compromise.

approach [23,24] in which an experiment is said to be Pareto optimal if there is no other experiment which has better result for one criterion without having a worst result for another. For two criteria this method can be visualized plotting the results of both criteria (in our case response function I, peak height, and response function II, sampling rate) for the experiments of the design, with the two criteria as coordinates (Fig. 4). The experiments 13, 2, and 12 situated at the border of the cloud of points, can be connected with a line, and they dominate all the other experiments below them. The user usually decides which experiment among the points along this line will offer the best compromise. In our case, experiment 2 was selected. The conditions corresponding to it are $R = 245$ cm; $Q_2 = 1.3$ mL min^{-1}; $L = 165$ cm. We can observe the great coincidence with the results obtained from the response surface methodology, and therefore those were maintained below. This selection is reflected in the manifold of Fig. 1.

3.2. Optimization of chemical variables

The above described experiments were made with a carrier (line 1, Q_1) consisting of DMAS 1.47×10^{-4} M at pH 2.4 (with chloroacetic/chloroacetate buffer) and injecting aliquots of a 1 μg mL^{-1} solution of amitriptyline in the system. Preliminary experiments indicated that strong acid media is most appropriate to form the ion pair. Besides we proved that it was more convenient to use hydrochloric acid to adjust the acidity than other acids such as perchloric acid. In the presence of the latter the fluorescence signals obtained were lower, probably due to the competition of perchlorate ion with the derivatising fluorescent anion to form ion pairs with the cationic analytes. Therefore, it was decided to optimize the hydrochloric acid concentration [HCl] in the carrier. The other chemical variables optimized were the concentration of derivatising agent [DMAS], and the ionic strength, varied with the addition of sodium chloride, since the latter may influence the extraction yield. In previous experiments it was observed that, in the case of chemical variables, the optimal conditions could be different depending on the TCAs, and thus, it was decided to optimize the assay by studying them separately and try to find compromise conditions. Similarly to the optimization of FI variables, the experiments were the result of a central composite design, and their conditions are reported in Table 3.

It was found that the considered effects contribute significantly to the model, except the main effect [NaCl] whose linear contribution was not significant for the established 95% confidence level. Fig. 5 shows, as an example, the response surfaces estimated by the model, for each pair of

Table 3
Optimization of the chemical variables

Experiments[a]		[HCl] (M)	[NaCl] (M)	[DMAS] (M)
1	Cube004a	0.39	0.39	1.59×10^{-4}
2	Cube005a	0.11	0.11	5.30×10^{-4}
3	*L: B-a	0.25	0.01	3.45×10^{-4}
4	Cube006a	0.39	0.11	5.30×10^{-4}
5	Cube008a	0.39	0.39	5.30×10^{-4}
6	*H: C-a	0.25	0.25	6.60×10^{-4}
7	Cube002a	0.39	0.11	1.59×10^{-4}
8	*L: A-a	0.01	0.25	3.45×10^{-4}
9	Cube003a	0.11	0.39	1.59×10^{-4}
10	*H: A-a	0.49	0.25	3.45×10^{-4}
11	Cent-b	0.25	0.25	3.45×10^{-4}
12	*H: B-a	0.25	0.49	3.45×10^{-4}
13	Cent-c	0.25	0.25	3.45×10^{-4}
14	Cube001a	0.11	0.11	1.59×10^{-4}
15	*L: C-a	0.25	0.25	0.33×10^{-4}
16	Cent-a	0.25	0.25	3.45×10^{-4}
17	Cube007a	0.11	0.39	5.30×10^{-4}

Experiments resulting of a central composite design. (*) Experiment is a star point in the model.

[a] The experiments are numbered according the randomized sequence followed.

Table 4
Opimization of chemical variables

Analyte	R^2	Check model quadratic	Lack-of-fit	[HCl] (M)	[DMAS] (M)
IM	0.913	0.006	0.22	0.17–0.31	2.67×10^{-4} to 4.22×10^{-4}
DE	0.902	0.003	0.17	0.13–0.27	2.67×10^{-4} to 4.48×10^{-4}
AM	0.903	0.009	0.38	0.17–0.30	2.41×10^{-4} to 3.96×10^{-4}
NO	0.910	0.007	0.35	0.17–0.31	2.41×10^{-4} to 3.96×10^{-4}
CLO	0.871	0.025	0.22	0.17–0.29	1.11×10^{-4} to 3.19×10^{-4}
DO	0.843	0.033	0.10	0.17–0.31	0.58×10^{-4} to 2.90×10^{-4}

Statistical parameters of the adjusted models and optimal regions for each one of the TCAs.

components, for amitriptyline. The statistical parameters of the adjusted models, as well as the intervals of [HCl] and [DMAS] corresponding to the zones of maximum response (with differences in the RF1 about 5%) are summarized in Table 4, for each of the TCAs. As already mentioned, [NaCl] had less effect on the fluorescent signal and, indeed, it was confirmed that this signal is almost the same when the sodium chloride is suppressed in the carrier. Because of that, this variable does not appear in Table 4 and was considered 0 thereafter. From the results shown in this table, the conditions selected as a compromise, were [HCl] = 0.2 M and [DMSA] = 2.9×10^{-4} M, as reflected in Fig. 1.

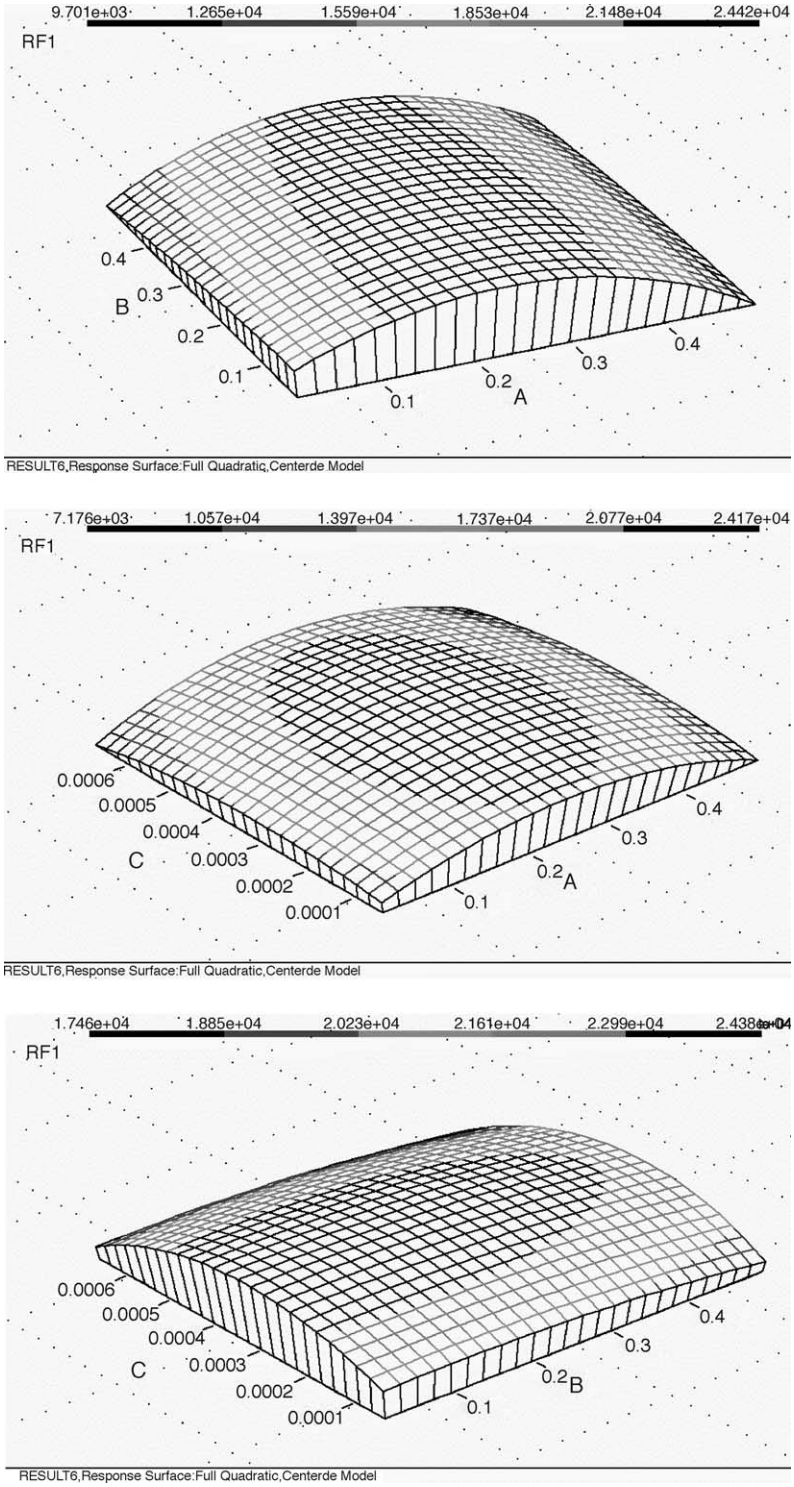

Fig. 5. Estimated response surfaces, corresponding to amitriptyline, for each pair of chemical variables. (A) [HCl]; (B) [NaCl]; (C) [DMAS].

Table 5
Summary table of the calibration parameters

Analyte	Calibration curves	$S_{y/x}$	R^2	LOD[a] (mg L^{-1})	LOD[b] (mg L^{-1})
IM	$H = 337.8(\pm5.4) \times C_{IM} - 11.5\ (\pm11.4)$	34.2	0.998	0.10	0.23
DE	$H = 311.0(\pm7.8) \times C_{DE} + 28.3\ (\pm13.1)$	32.9	0.996	0.13	0.26
AM	$H = 345.9(\pm3.8) \times C_{AM} - 45.6\ (\pm7.8)$	21.5	0.9992	0.07	0.18
NO	$H = 359.1(\pm8.4) \times C_{NO} + 3.61\ (\pm14.2)$	35.7	0.996	0.12	0.24
CLO	$H = 424.8(\pm14.3) \times C_{CLO} + 33.7\ (\pm24.0)$	60.3	0.993	0.17	0.35
DO	$H = 369.7(\pm7.7) \times C_{DO} - 10.8\ (\pm12.9)$	32.7	0.997	0.11	0.22

[a] LOD, detection limit calculated by Long and Winerfordner method.
[b] LOD, detection limit calculated by Clayton method ($\alpha = \beta = 0.05$); C, mg L^{-1} of analyte.

Table 6
Result of the analysis of TCAs in pharmaceuticals

Pharmaceutical	Drug	Nominal value (mg)	FIA[a] (mg)	Reference method (mg)
Tofranil	Imipramine	50	50.5 ± 0.9	50.6 ± 0.3[d]
Tofranil pamoate	Imipramine pamoate	75	74.5 ± 0.3[b]	74.7 ± 0.3[e]
			74.8 ± 0.6[c]	
Tryptizol	Amitriptyline hydrochloride	25	25.1 ± 0.2	25.5 ± 0.8[d]
Mutabase	Amitriptyline hydrochloride	25	25.8 ± 0.5	25.6 ± 0.2[d]
Nobritol	Amitriptyline hydrochloride	12.5	14.5 ± 0.3	14.3 ± 0.3[d]
Anafranil	Clomipramine	25	24.8 ± 0.6	25.7 ± 0.2[d]
Sinequan	Doxepin	25	25.4 ± 0.7	25.7 ± 0.2[d]

[a] Average ± S.D. for three determinations.
[b] Treatment of the sample recommended by the USP.
[c] Treatment of the sample proposed.
[d] LC method [5].
[e] USP photometric method [27].

3.3. Calibration parameters

To study the influence of the concentration of the TCAs on the peak height, aliquots of 0.29 mL of standards of TCAs freshly prepared concentrations in water, between 0.25 and 3 mg L^{-1} were injected into the manifold. Each standard was prepared by triplicate and each was injected by triplicate. The average of the heights of the three peaks (H) was used as analytical signal. A linear relationship was obtained between peak height and TCAs concentrations. In Table 5, the equations of the calibration curves obtained for each TCA are shown. Also, the detection limits calculated following Winefordner–Long [25] and Clayton et al. [26] methods are included. The repeatability was evaluated by performing 11 successive injections of solutions 1.5 mg L^{-1} of each analyte; R.S.D. values between 2.1% and 2.4% in the peak height were obtained. The day-to-day repeatability was assessed over 3 days performing each day 11 successive injections of the recently prepared solutions of the analytes; R.S.D. values between 2.3% and 5.8% were obtained.

3.4. Determination of TCAs in pharmaceuticals

A LC procedure [5] was used to validate the proposed FI method. The chromatographic method uses a C-18 column, acetonitrile: phosphate buffer of pH 6.8 (40:60) as mobile phase, a flow rate of 1 mL $min^{-1}$and UV detection at 210 nm. Also a reference method from the USP [27] was used to validate the determination of imipramine in the pharmaceutical *Tofranil pamoate*. The USP method uses two different successive liquid–liquid extractions in the treatment procedure of the sample while in our proposed FI method, these cleaning stages were not necessary, as it can be deduced from the results in Table 6.

Applying the F-test and t-test, we found that results for both methods (FI and LC) are comparable for all the pharmaceuticals. We can deduce that interferences from other compounds does not occur.

4. Conclusions

It has been demonstrated that non-fluorescent tricyclic antidepressant drugs display fluorescence by means of the formation of an ion pair with DMAS. It is interesting to develop a FI method for the determination of the tricyclic antidepressant drugs in routine analysis. The optimization of the FI and chemicals variables by an experimental design (Central Composite Design and Response Surface Methodology) gives good results. The accuracy of the proposed method has been validated by comparison with a reference method and it has been applied to different pharmaceuticals obtaining good results.

The proposed FI method has the advantages of the simple operation, high sampling rate, high sensitivity, repeatability and decreased exposure to organic solvent vapours. In addition, the proposed method can be widely applied for quality control of pharmaceutical preparations.

Acknowledgements

The authors wish to thank to Ministerio de Ciencia y Tecnología Español for the financial support (CTQ2004-02142). Antonio Silva Rodríguez acknowledges a fellowship from the Consejería de Educación, Ciencia y Tecnología-Junta de Extremadura.

References

[1] J. Wang, M. Bonakdar, C. Morgan, Anal. Chem. 58 (1986) 1024.
[2] P.A. Mason, K.M. Rowan, B. Law, A.C. Moffat, E.A. Kilner, L.A. King, Analyst 109 (1984) 1213.
[3] T.R. Norman, K.P. Maguire, J. Chromatogr. B 41 (1985) 173.
[4] R.N. Gupta, G. Molnar, Biopharm. Drug Dispos. 1 (1980) 259.
[5] T.A. Ivandini, B.V. Sarada, C. Terashima, T.N. Rao, D.A. Tryk, H. Ishiguro, Y. Kubota, A. Fujishima, J. Electroanal. Chem. 521 (2002) 117.
[6] G. Lunn, Capillary Electrophoresis Methods for Pharmaceutical Analysis, 1st ed., Wiley, New York, 2000.
[7] G.M. Greenway, S.J.L. Dolman, Analyst 124 (1999) 759.
[8] J.L.F.C. Lima, J.A.V. Prior, B.F. Reis, J.L.M. Santos, E.A.G. Zagatto, Anal. Chim. Acta 467 (2002) 75.
[9] M.P. San Andrés, D. Sicilia, S. Rubio, D. Pérez-Bendito, J. Pharm. Sci. 87 (1998) 821.
[10] T. Pérez-Ruiz, C. Martínez-Lozano, A. Sanz, C. Alonso, Talanta 41 (1994) 1523.
[11] T. Pérez-Ruiz, C. Martínez-Lozano, V. Tomás, C. Sidrach, Analyst 120 (1995) 1103.
[12] C. Vannecke, E. Van Gyseghem, M.S. Bloomfield, T. Coomber, Y. Vander Heyden, D.L. Massart, Anal. Chim. Acta 446 (2001) 413.
[13] C. Vannecke, M.S. Bloomfield, Y. Vander Heyden, D.L. Massart, Anal. Chim. Acta 455 (2002) 117.
[14] B. Starczewska, P. Halaburda, K. Anatol, J. Pharm. Biomed. Anal. 30 (2002) 553.
[15] S.J.L. Dolman, G.M. Greenway, Anal. Commun. 33 (1996) 139.
[16] D. Westerlund, K.O. Borg, Anal. Chim. Acta 67 (1973) 89.
[17] K. Kümmerer, A. Eitel, U. Braun, P. Hubner, F. Daschner, G. Mascart, M. Milandri, F. Reinthaler, J. Verhoef, J. Chromatogr. A 774 (1997) 281.
[18] S.M. Sultan, A.D. Walmsley, Talanta 46 (1998) 897.
[19] L.B.C. Álvares-Ribeiro, A.A.S.C. Machado, Analyst 122 (1997) 1435.
[20] G.E.P. Box, K.B. Wilson, J. R. Stat. Soc. B 13 (1951) 1.
[21] D.C. Montgomery, Design and Analysis of Experiments, 2nd ed., Wiley, New York, 1984.
[22] Unscrambler v. 6.11, CAMO A/S Olav Tryggvasonsgt, Trondheim, Norway.
[23] H.R. Keller, D.L. Massart, J.P. Brans, Chem. Intell. Lab. Syst. 11 (1991) 175.
[24] H.R. Keller, D.L. Massart, Trends Anal. Chem. 9 (8) (1990) 251.
[25] G.L. Long, J.D. Winefordner, Anal. Chem. 55 (1983) 712A.
[26] C.A. Clayton, J.W. Hines, P.D. Elkins, Anal. Chem. 59 (1987) 2506.
[27] USP24-NF19 Supplement2, The United States Pharmacopeial Convention Inc., 2000.