Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis
32 (2003) 71–84
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Optimised procedures for the reversed-phase liquid chromatographic analysis of formulations containing tricyclic antidepressants

M.J. Ruiz-Angel [a], S. Carda-Broch [b], E.F. Simó-Alfonso [a], M.C. García-Alvarez-Coque [a,*]

[a] *Facultat de Química, Departament de Química Analítica, Universitat de València, Dr. Moliner 50, Burjassot, Valencia 46100, Spain*
[b] *Area de Química Analítica, Universitat Jaume I, Cra. Borriol s/n, Castelló 12080, Spain*

Received 3 September 2002; received in revised form 14 January 2003; accepted 15 January 2003

Abstract

The chromatographic behaviour (retention, selectivity, peak shape and resolution) of seven tricyclic antidepressants (TCAs), amitryptiline, clomipramine, doxepin, imipramine, maprotiline, nortryptiline and trimipramine, was examined. Conventional unendcapped C_8 and C_{18} columns and an endcapped XTerra MS C_{18} column recommended for the analysis of basic compounds were used together with acetonitrile–water and micellar sodium dodecylsulfate (SDS)–pentanol mobile phases. The two best combinations were XTerra C_{18}/acetonitrile, which yielded the largest efficiencies and resolution, and C_8/SDS–pentanol, which eliminated the peak tails that were still observed with the XTerra C_{18} column. Both the systems were used to develop simple chromatographic procedures for the control of TCAs in pharmaceutical formulations using UV detection. The selected mobile phase compositions were 35% (v/v) acetonitrile (XTerra C_{18} column) and 0.075 M SDS–6% (v/v) pentanol (C_8 column), both at pH 3. Satisfactory recoveries were achieved in both cases, with intra- and inter-day relative standard deviations (RSDs) always below 0.6 and 2.0%, respectively. The preparation of the samples was simple in both modes, since a previous extraction of the drugs was not needed. The micellar mode has, however, the advantage of using a smaller amount of organic solvent, which is retained in the micellar SDS solution. The C_8 column is also less expensive.

Keywords: Tricyclic antidepressants; Formulations; Reversed-phase liquid chromatography; Acetonitrile; Sodium dodecylsulfate; Pentanol

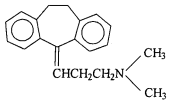
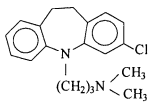
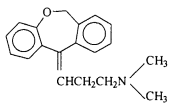
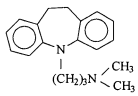
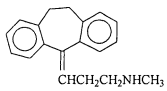
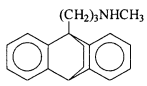
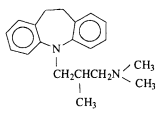
1. Introduction

Tricyclic antidepressants (TCAs) are commonly used for the treatment of depressive disorders owing to their efficiency in elevating the mood of patients by interfering the reuptake of norepinephrine or serotonin [1]. Chemically, the compounds possess three-ring structures in which the middle ring is alicyclic and contains seven atoms, except in some cases for which the middle ring is

* Corresponding author.

0731-7085/03/$ - see front matter
doi:10.1016/S0731-7085(03)00048-7

Table 1
Structures, dissociation constants and octanol–water partition coefficients of TCAs [2]

Compound	Structure	pK_a	log $P_{o/w}$
Amitryptiline		9.4	4.64
Clomipramine		9.4	5.30
Doxepin		9.0	3.88
Imipramine		9.5	4.41
Nortryptiline		9.7	4.32
Maprotiline		NA	4.22
Trimipramine		NA	4.73

NA, not available.

heterocyclic (Table 1). The side chain is derived from *N*-alkylmethylamine or *N*-alkyldimethylamine. Pharmacological action of these drugs shows high dependence on the structure. Tertiary amines are preferred in prescriptions since they are metabolised and excreted in the human body more rapidly than secondary amines [3].

Routine analysis methods for TCAs have been usually developed for the determination of the drugs in serum and plasma. Reversed-phase liquid chromatography (RPLC) with aqueous-organic mobile phases has commonly been the employed technique [4–8]. TCAs have also been examined among other basic compounds, with the objective of finding new stationary phases to resolve the problematic poor efficiencies and peak shapes found for these compounds in RPLC analysis [9–11]. However, a complete study of the chromatographic behaviour of TCAs considering retention, peak shape and resolution is not available in

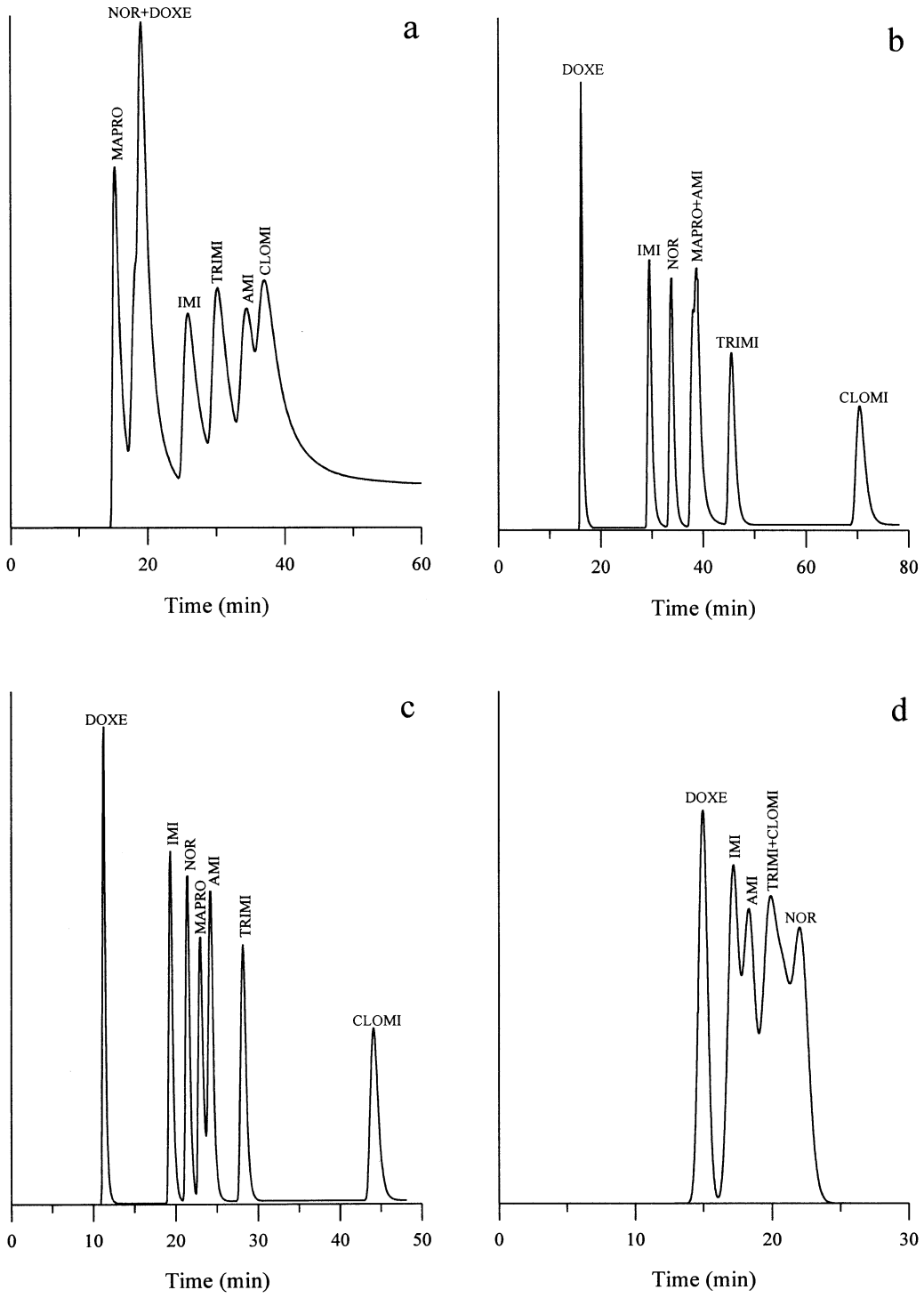


Fig. 1. Optimal chromatograms of mixtures of seven TCAs eluted from different systems. Chromatographic systems: (a) unendcapped C_{18}/60% acetonitrile, (b) C_8/25% acetonitrile, (c) XTerra C_{18}/29% acetonitrile and (d) C_8/0.10 M SDS–3.4% pentanol. Compounds: AMI = amitryptiline, CLOMI = clomipramine, DOXE = doxepin, IMI = imipramine, MAPRO = maprotiline, NOR = nortryptiline and TRIMI = trimipramine.

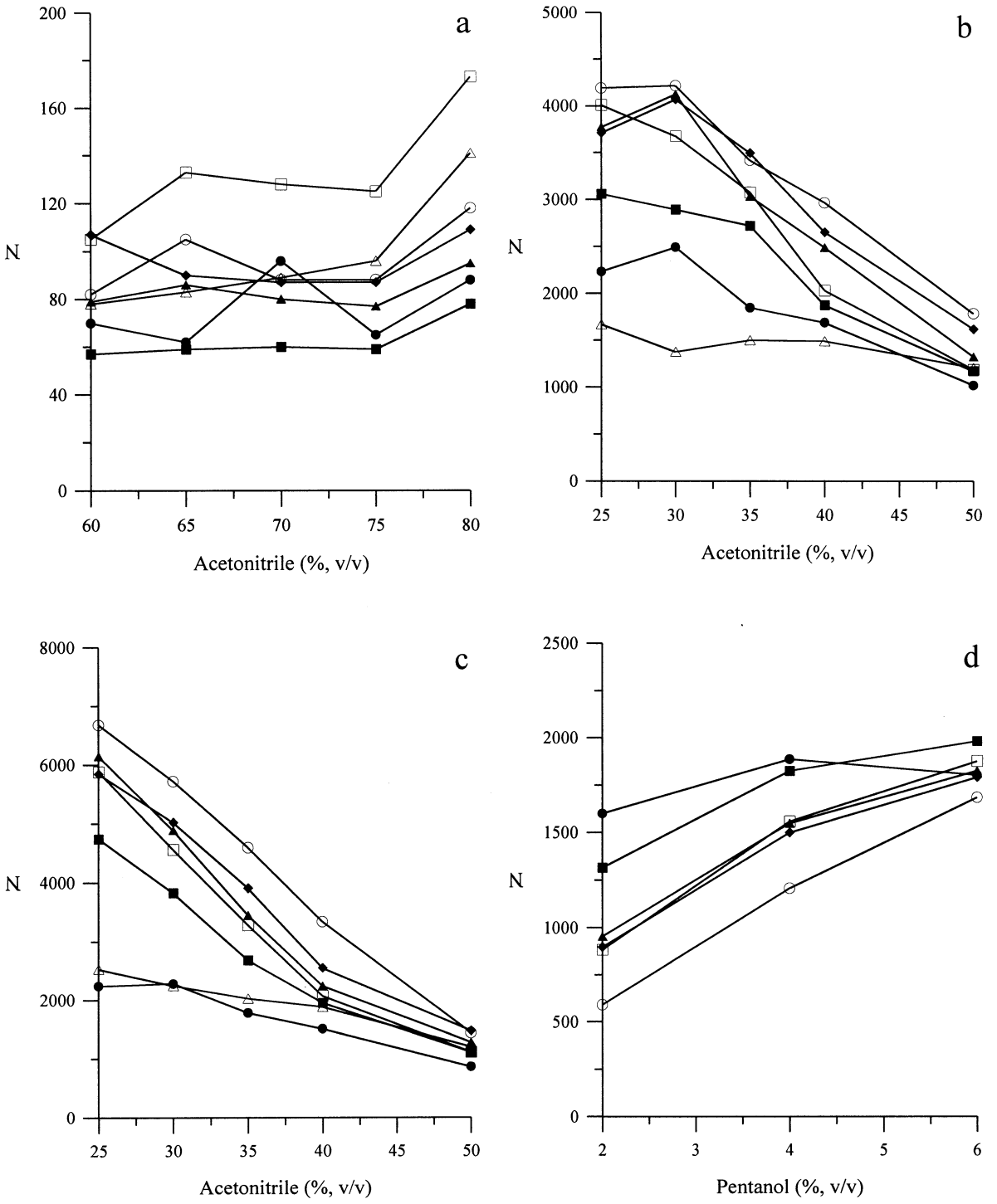


Fig. 2. Effect of organic solvent on the efficiency in the aqueous-organic system using unendcapped C_{18} (a), C_8 (b) and endcapped XTerra C_{18} (c) columns, and in the micellar system using the unendcapped C_8 column (d). Compounds: imipramine (■), doxepin (●), amitryptiline (▲), trimipramine (◆), clomipramine (○), maprotiline (△) and nortryptiline (□).

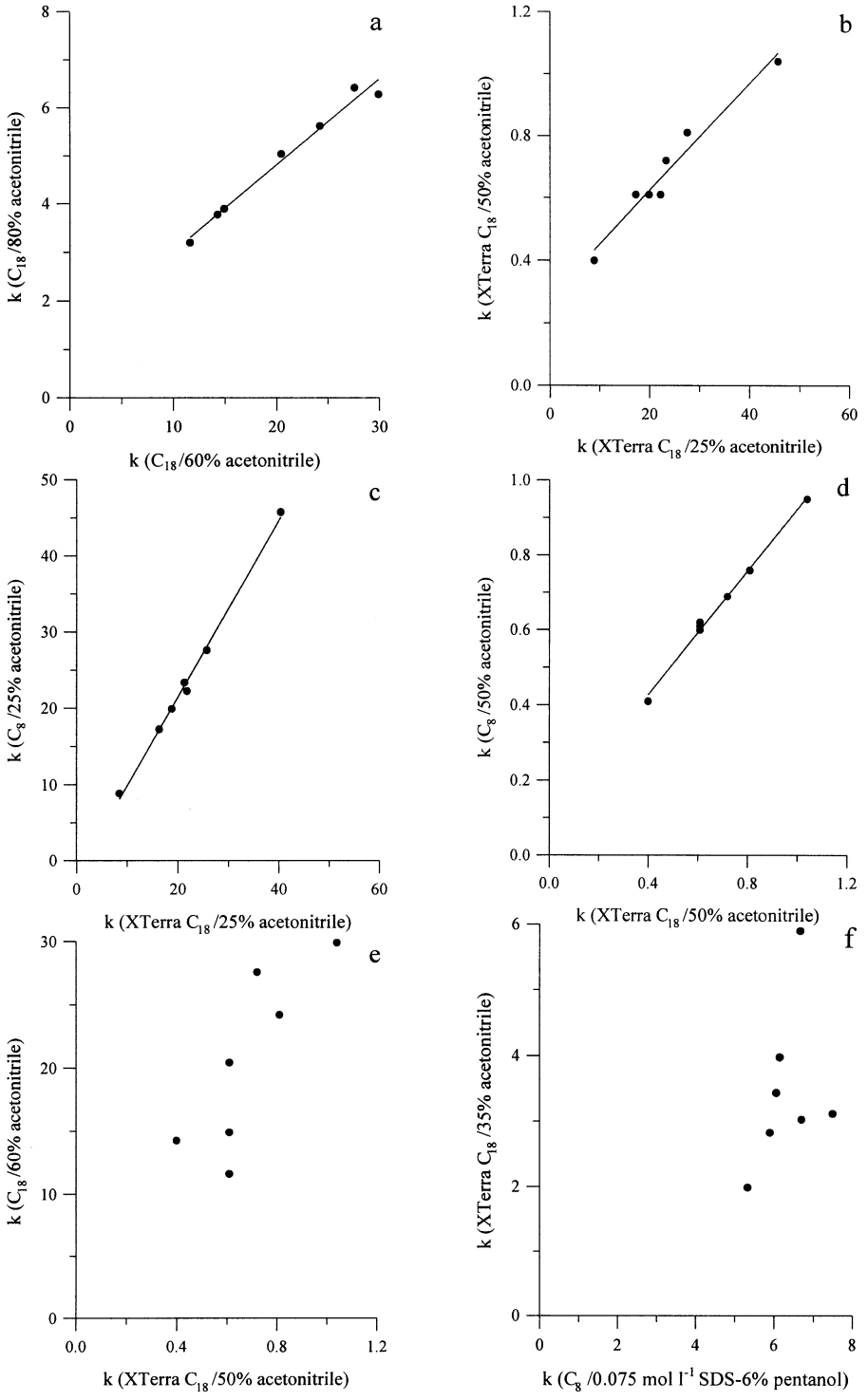


Fig. 3. Selectivity of the chromatographic systems (retention factors are plotted).

the literature. Also, procedures to determine these compounds in commercialised formulations are scarce and refer only to amitriptyline-, nortriptyline- and imipramine-derived drugs [12–14].

In this work, a detailed study of the chromatographic behaviour of several TCAs is presented using three different stationary phases: unendcapped C_8 and C_{18} columns and a special C_{18} column that contains bonded hybrid particles, where methylsiloxane units replace one-third of the silica units (XTerra C_{18}). This permits a significant improvement in the peak shape. The study considers aqueous- and micellar-organic mobile phases. With the achieved information, simple RPLC procedures for the determination in pharmaceutical formulations of commonly prescribed TCAs were established. Even though these drugs are not found together in the formulations, they were analysed using the same column and mobile phase composition. A single procedure suitable for the control of several drugs is very useful for the pharmacological laboratory, since the maintenance of separate chromatographic conditions for each drug increases the cost of analysis.

2. Experimental

2.1. Reagents

Stock solutions containing 100 μg/ml of TCAs, amitryptiline, clomipramine, doxepin, imipramine, maprotiline, nortryptiline and trimipramine (Sigma, St. Louis, MO; Table 1) were prepared. For the aqueous-organic mode, TCAs were dissolved in ethanol (Prolabo, Paris, France) with the aid of an ultrasonic bath (model 617, Selecta, Barcelona, Spain) and made up to the mark in a volumetric flask with water. For the micellar mode, the stock solutions were prepared following the same procedure but dilution for analysis was made with 0.1 M sodium dodecylsulfate (SDS) (Merck, Darmstad, Germany). All drug solutions remained stable during at least 2 months at 4 °C as checked by measurement of the area of the chromatographic peaks.

Acetonitrile (HPLC grade, Scharlab, Barcelona, Spain) was used in the aqueous-organic mixtures. The micellar mobile phases were prepared with SDS and pentanol (Scharlab). Sodium dihydrogenphosphate (Panreac, Barcelona, Spain) was used as buffer system and the pH was set at 3 with HCl (Panreac). Mobile phases and standard solutions were filtered through 0.45 μm nylon membranes (Micron Separations, Westboro, MA). Nanopure water (Barnstead, Sybron, Boston, MA) was used throughout.

2.2. Apparatus and chromatographic conditions

Chromatography was performed with an Agilent chromatograph (Palo Alto, CA) provided with an isocratic pump (Series 1100, Model G1310A), an autosampler and a UV–visible detector (Model HP1050). The signal was monitored at 254 nm. Data acquisition was carried out with the Agilent Peak 96 software (Avondale, PA). The chromatographic data were treated with MICHROM [15], a software for optimisation in micellar liquid chromatography (MLC), which was modified to process the data in the aqueous-organic mode [16].

The analytical separations were accomplished by using the following columns, all of them with 5 μm particle size: 125×4.6 mm i.d. ODS-2 C_{18} (Scharlab), 150×4.6 mm i.d. Eclipse XDB C_8 (Agilent), both connected to a 30×4.6 mm i.d. Nucleosil C_{18} guard column (Scharlab) and 150×4.6 mm i.d. endcapped XTerra MS C_{18} (Waters, Barcelona, Spain) connected to a 20×3 mm i.d. XTerra MS C_{18} guard column. The chromatographic columns were periodically washed with appropriate solvents depending on the nature of the mobile phase and type of the column.

The mobile phases used in the analysis of the pharmaceutical preparations were 35% (v/v) acetonitrile (endcapped XTerra C_{18} column) and 0.075 M SDS–6% (v/v) pentanol (C_8 column), both buffered at pH 3. The pH was adjusted before the addition of the organic solvent. The chromatographic runs were carried out at room temperature. The flow rate was 1.0 ml/min and the injection volume was 20 μl. Duplicate injections were made.

Table 2
Retention times, efficiencies and peak asymmetries for TCAs chromatographed in different systems

Compound	[3]C_{18}/80% acetonitrile			C_8/35% acetonitrile			XTerra C_{18}/35% acetonitrile			C_8/0.075 M SDS–6% pentanol		
	t_R (min)	N	B/A	t_R (min)	N	B/A	t_R (min)	N	B/A	t_R (min)	N	B/A
Amitryptiline	9.2	95	4.7	8.8	3035	2.7	8.5	3450	1.7	8.6	1825	1.2
Clomipramine	9.0	120	4.3	13.1	3420	3.0	13.6	4595	1.7	9.3	1685	1.1
Doxepin	5.9	90	4.7	5.1	1850	2.8	5.1	1780	2.0	7.8	1805	1.4
Imipramine	7.4	80	5.4	7.4	2720	2.5	7.5	2680	2.1	8.5	1985	1.2
Maprotiline	5.1	140	5.6	8.3	1500	3.8	8.3	2025	2.6	10.4	1870	1.1
Nortryptiline	6.1	175	4.5	8.0	3080	2.6	7.9	3285	1.7	9.6	1880	1.3
Trimipramine	8.3	110	4.7	9.8	3495	2.4	10.1	3910	1.6	8.7	1795	1.3

2.3. Procedure

The pharmaceuticals analysed were tablets and capsules. The average weight per tablet or capsule was calculated from 10 units. The tablets and capsules contents were ground and reduced to a homogeneous fine powder in a mortar. Several portions of the powder were taken and sonicated in the presence of a small amount of ethanol for the aqueous-organic mode and with 1:1 ethanol–SDS for the micellar mode. Dilution was made with water or 0.10 M SDS. The excipients were not soluble in the assayed media; hence the sample solutions should be filtered through 0.45 μm nylon membranes before injection into the chromatograph.

3. Results and discussion

3.1. Chromatographic behaviour of TCAs

Several reports have been published where TCAs are determined using RPLC with conventional unendcapped C_{18} and C_8 columns, and mobile phases of acetonitrile, methanol or mixtures of both, although acetonitrile is usually preferred [4–8]. The addition of an amine (buty-

Table 3
Calibration parameters for the TCAs obtained in different days

Compound	XTerra C_{18}/acetonitrile			C_8/SDS–pentanol		
	Intercept	Slope	r	Intercept	Slope	r
Amitryptiline	0.11 ± 0.11^a	0.137 ± 0.004^a	0.9990^a	0.04 ± 0.04^a	0.0391 ± 0.0010^a	0.9997^a
	-0.34 ± 0.20^b	0.143 ± 0.004^b	0.9990^b	0.019 ± 0.003^b	0.0397 ± 0.00006^b	0.9999^b
Clomipramine	-0.40 ± 0.35^a	0.204 ± 0.005^a	0.9998^a	0.005 ± 0.013^a	0.0519 ± 0.0004^a	0.9998^a
	-0.72 ± 0.21^b	0.205 ± 0.004^b	0.9994^b	0.02 ± 0.02^b	0.0519 ± 0.0004^b	0.9999^b
Doxepin	0.01 ± 0.13^a	0.1976 ± 0.0012^a	0.9999^a	0.005 ± 0.011^a	0.0498 ± 0.0002^a	0.9999^a
	0.05 ± 0.05^b	0.2013 ± 0.0009^b	0.9999^b	-0.018 ± 0.009^b	0.0507 ± 0.0002^b	0.9999^b
Maprotiline	-0.033 ± 0.015^a	0.0250 ± 0.0007^a	0.9995^a	-0.001 ± 0.005^a	0.0057 ± 0.0012^a	0.9995^a
	-0.03 ± 0.05^b	0.0260 ± 0.0009^b	0.9995^b	0.003 ± 0.006^b	0.00560 ± 0.00011^b	0.9999^b
Nortryptiline	-0.17 ± 0.13^a	0.1460 ± 0.0017^a	0.9998^a	0.01 ± 0.02^a	0.0397 ± 0.0008^a	0.9998^a
	0.11 ± 0.27^b	0.136 ± 0.005^b	0.9997^b	0.002 ± 0.004^b	0.0403 ± 0.00008^b	0.9999^b
Trimipramine	-0.14 ± 0.13^a	0.1789 ± 0.0004^a	0.9998^a	-0.02 ± 0.02^a	0.0455 ± 0.0005^a	0.9997^a
	-0.41 ± 0.17^b	0.181 ± 0.003^b	0.9998^b	0.05 ± 0.01^b	0.0439 ± 0.0002^b	0.9999^b

[a] Mean value of the parameters of three calibration straight lines obtained in 3 consecutive days.
[b] Values from calibration straight lines obtained 1–3 months later.

Table 4
Analysis of several formulations containing TCAs

Formulation (laboratory)	Composition (mg)	XTerra C_{18}/acetonitrile		C_8/SDS–pentanol	
		Found (mg)	Label claim (%)	Found (mg)	Label claim (%)
Tryptizol (Merck-Sharp & Dohme)	Per tablet: amitryptiline chlorhydrate (50), lactose and other excipients	50.1	100.2	49.0	98.0
Mutabase (Shering-Plough)	Per tablet: amitryptiline (25), perfenazine (2), lactose (67), starch (18) and sacarose	26.5	106.0	24.5	98.0
Anafranil (Novartis)	Per tablet: clomipramine chlorhydrate (25), glycerol and other excipients	24.5	98.0	24.8	99.2
Sinequan (Farmasierra)	Per capsule: doxepin chlorhydrate (25), excipients	28.5	112.2	27.5	110.0
Ludiomil (Novartis)	Per tablet: maprotiline chlorhydrate (25), wheat starch (27), lactose and other excipients	26.4	105.6	25.4	101.6
Norfenazin (Reig Jofré)	Per tablet: nortryptiline chlorhydrate (25), perfenazine (2), lactose and other excipients	21.9	87.6	24.4	97.6
Surmontil (Rhône-Poulenc Rorer)	Per tablet: trimipramine maleate (25), wheat starch and other excipients	25.5	102.0	24.9	99.6

lamine, diethylamine or triethylamine) is a usual practice to improve the peak shape. However, even in the presence of an amine, the efficiencies remain low. For this reason, special columns are used to improve the results [7,9–11].

MLC with the non-ionic surfactant Brij-35 was used to examine the correlations between the retention factor of some drugs and several pharmacokinetic parameters and biological responses, since this technique seems to emulate in vitro the partitioning process in biomembranes [17]. In previous work, MLC also appeared as a suitable technique for the determination of some groups of basic drugs using unendcapped C_{18} columns without the need of adding amine modifiers, solving at least partially the problem related with the low efficiencies and tailing peaks obtained for these compounds with aqueous-organic mobile phases. Several interesting examples that employ hybrid micellar mobile phases containing the anionic surfactant SDS, and propanol, butanol or pentanol as modifiers, have been published for β-blockers [18], phenethylamines [19], diuretics [20] and tetracyclines [21].

On view of these results, we performed a comparative study of the chromatographic behaviour of TCAs using classical RPLC and MLC, with unendcapped C_8 and C_{18}, and endcapped XTerra C_{18} columns. The mobile phases were buffered at pH 3 to enhance peak shapes by protonation of free silanol groups on the columns. In order to follow with detail the variation of the behaviour of the drugs with mobile phase composition, the chromatographic data (retention factors, efficiencies and asymmetry factors) were modelled according to previously established equations [18,22], using experimental designs that involved four mobile phases for the optimisation of one factor (acetonitrile in classical RPLC) and six mobile phases for two factors (SDS and organic modifier in MLC).

As observed in Table 1, octanol–water coefficients (log $P_{o/w}$) of TCAs are in the range 3.9–5.3, which means that the drugs are strongly associated with the alkyl chains of a conventional C_{18} stationary phase. The optimal mobile phase contained accordingly a rather high amount of acetonitrile (60%). Fig. 1(a) depicts a chromatogram of the mixture of TCAs showing the maximal resolution attained for the unendcapped C_{18}/acetonitrile system. As observed, the compounds are poorly resolved, the analysis time is also too long, the efficiencies are low (in the range 60–110 plate counts per column) and the asymmetry factors high ($B/A = 4.7$–6.7, where B and A are the distances from the centre to the tailing and leading

Table 5
Intra- and inter-day assays for the TCAs formulations

Compound	Added (μg/ml)	Intra-day RSD (%)[a]		Inter-day RSD (%)[b]		Inter-day RSD (%)[c]	
		XTerra C_{18}/acetonitrile	C_8/SDS–pentanol	XTerra C_{18}/acetonitrile	C_8/SDS–pentanol	XTerra C_{18}/acetonitrile	C_8/SDS–pentanol
Amitryptiline	30	0.20	0.27	0.26	1.1	2.6	0.94
	50	0.14	0.17	0.45	1.3	2.3	1.1
	70	0.13	0.13	0.57	0.54	0.98	0.44
Clomipramine	30	0.29	0.40	0.78	1.2	0.99	1.1
	50	0.50	0.25	0.87	1.1	0.93	1.3
	70	0.16	0.63	0.58	0.54	1.4	0.46
Doxepin	30	0.28	0.18	1.5	1.4	0.55	0.79
	50	0.07	0.09	0.27	1.6	0.20	1.1
	70	0.20	0.09	0.31	0.58	0.67	0.33
Maprotiline	30	0.33	0.19	1.4	0.65	2.1	1.8
	50	0.15	0.16	1.7	0.90	1.0	1.9
	70	0.15	0.29	1.1	0.98	1.3	1.3
Nortryptiline	30	0.30	0.30	1.2	0.59	1.5	0.46
	50	0.33	0.20	0.36	1.3	3.1	1.2
	70	0.24	0.14	1.5	0.38	0.65	0.23
Trimipramine	30	0.20	0.30	0.72	0.96	0.86	1.5
	50	0.19	0.10	0.38	1.1	0.95	1.8
	70	0.24	0.10	0.53	0.36	0.44	0.66

[a] Sixfold replicates.
[b] Sixfold replicates made in consecutive days.
[c] Fivefold replicates made in different days along 2 months.

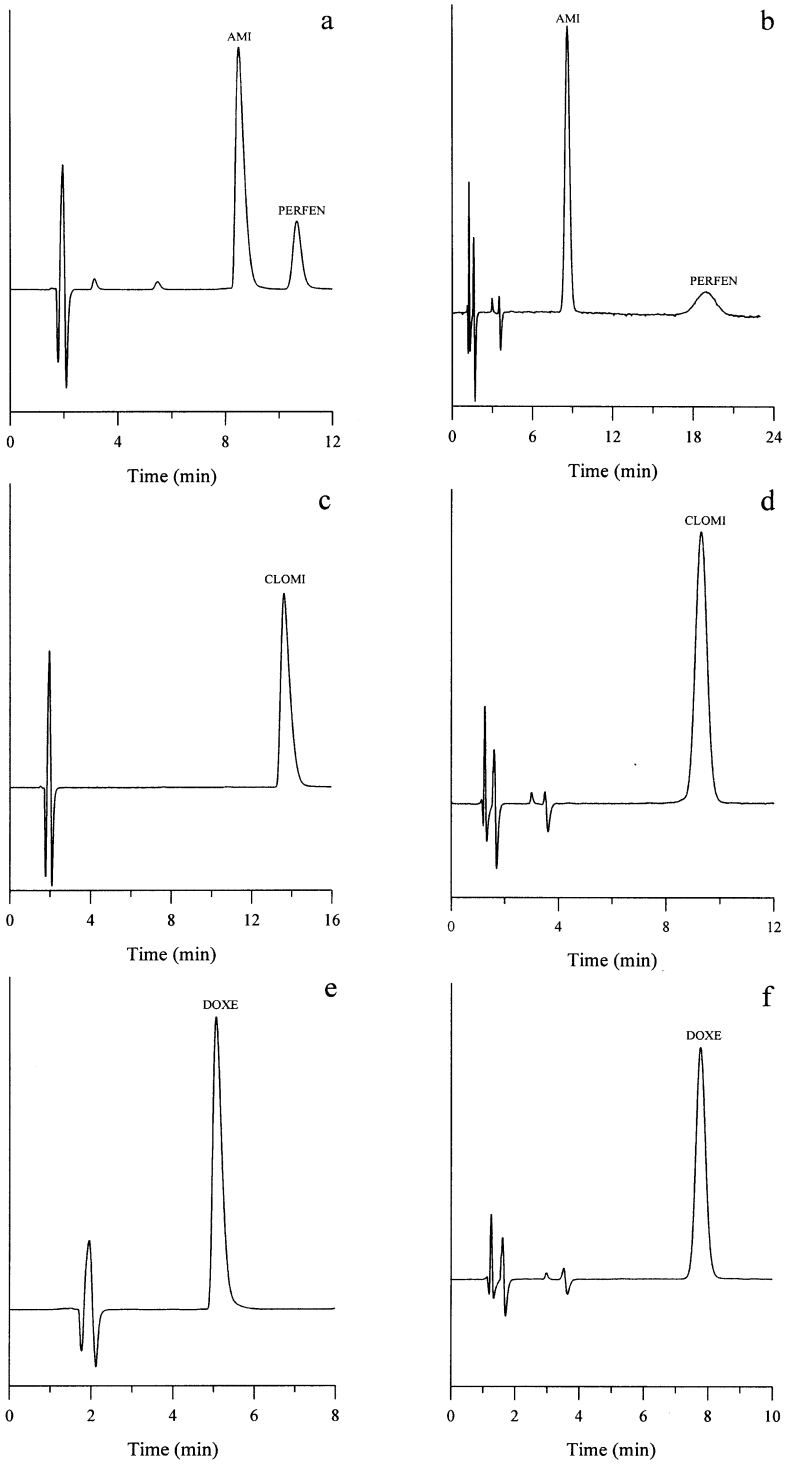


Fig. 4. Chromatograms of several formulations with the aqueous-organic (left) and micellar (right) systems: (a, b) Mutabase, (c, d) Anafranil and (e, f) Sinequan. PERFEN = perfenazine. See Fig. 1 for other abbreviations.

edge of the chromatographic peak, respectively, measured at 10% of the peak height). Similar efficiencies and asymmetries were obtained with mobile phases at other acetonitrile contents (Fig. 2(a)).

A similar study was performed with the C_8 column and acetonitrile–water mixtures, but a smaller amount of acetonitrile was needed owing to the weaker association to the column (25%, Fig. 1(b)). The quality of the chromatograms improved appreciably with respect to the C_{18} column, with efficiencies and peak asymmetries in the range 1700–4200 plate counts and $B/A = 2.0$–3.4, respectively. However, amitriptyline and maprotiline overlapped in the whole experimental range, and the analysis time was still too long. On the other hand, a general decrease in the efficiencies was observed at increasing acetonitrile concentration (Fig. 2(b)).

The composition of the optimal mobile phase for the XTerra C_{18} column was similar to the C_8 column (29%, Fig. 1(c)). This column gave the best results in terms of efficiencies and resolution. The peaks appeared well resolved, but the total analysis time was still 45 min. The efficiencies were 2200–6700 plate counts for the optimal mobile phase, but again deteriorated at increasing amount of acetonitrile (Fig. 2(c)).

The selection of the column and organic modifier for the micellar system took also into account the low polarity of the TCAs. Consequently, a strong organic solvent, pentanol and a C_8 column were used to obtain the best results in terms of resolution and analysis time. In fact, the retention times were checked to be above 50 min for all compounds using the unendcapped C_{18} column and mobile phases of SDS–acetonitrile. Pentanol shortened the retention to more acceptable values (15–25 min with the strongest mobile phases), but yielded tailing peaks. This was the reason of changing to the C_8 column.

Fig. 1(d) shows the chromatogram for the optimal conditions (0.10 M SDS–3.4% pentanol). The resolution was poor and the retention time of maprotiline (not shown) was too high. It is interesting to note that, in contrast to the aqueous-organic mode, the efficiencies increased at greater amount of organic solvent in the micellar mobile phase (Fig. 2(d)). Although the efficiencies were smaller compared with the aqueous-organic mode (1000–1800 at intermediate SDS and pentanol concentrations in the studied ranges), peak tails almost disappeared, being B/A usually below 1.2. This value must be compared with the best values obtained in classical RPLC with the XTerra C_{18} column, which were always $B/A > 1.6$. Achievement of symmetric peaks for basic compounds, when chromatographed with micellar mobile phases in unendcapped columns has been related to the adsorption of SDS monomers on the column, which prevents the interaction of solutes with free silanol groups. Also, the association kinetics between the charged solutes and the anionic surfactant seems to be more facile than the ion-exchange processes involving the silanol groups on the silica surface [19].

The elution order of TCAs in the C_8 and XTerra C_{18} columns with acetonitrile–water correlated well with $\log P_{o/w}$ of the drugs: doxepin (3.88), imipramine (4.41), nortriptyline (4.32), maprotiline (4.22), amitryptiline (4.64), trimipramine (4.73) and clomipramine (5.30) (Fig. 1(b) and (c)). The correlation coefficients of the retention factor (k) vs. $\log P_{o/w}$ plots were $r = 0.943$ and 0.960, respectively. In contrast, for the unendcapped C_{18} column using acetonitrile–water, the secondary amines (maprotiline and nortryptiline) eluted before the tertiary amines (amitryptiline, clomipramine, doxepin, imipramine and trimipramine), showing a poorer correlation between retention and polarity inside this group (Fig. 1(a), $r = 0.872$ for the tertiary amines). This behaviour was opposite to that obtained in MLC: tertiary amines eluted before secondary amines, according to their $\log P_{o/w}$ values (Fig. 1(d), $r = 0.973$ for the tertiary amines). Therefore, in RPLC using the unendcapped C_{18} column or MLC with the C_8 column, the nature of the amine was an important factor governing the retention.

The different elution order in the studied column/mobile phase combinations indicates different selectivities. A better evidence can be achieved by comparing the retention factors in the different systems. Fig. 3(a) and (b) shows that the selectivity did not change with mobile phase composition for the aqueous-organic systems (a

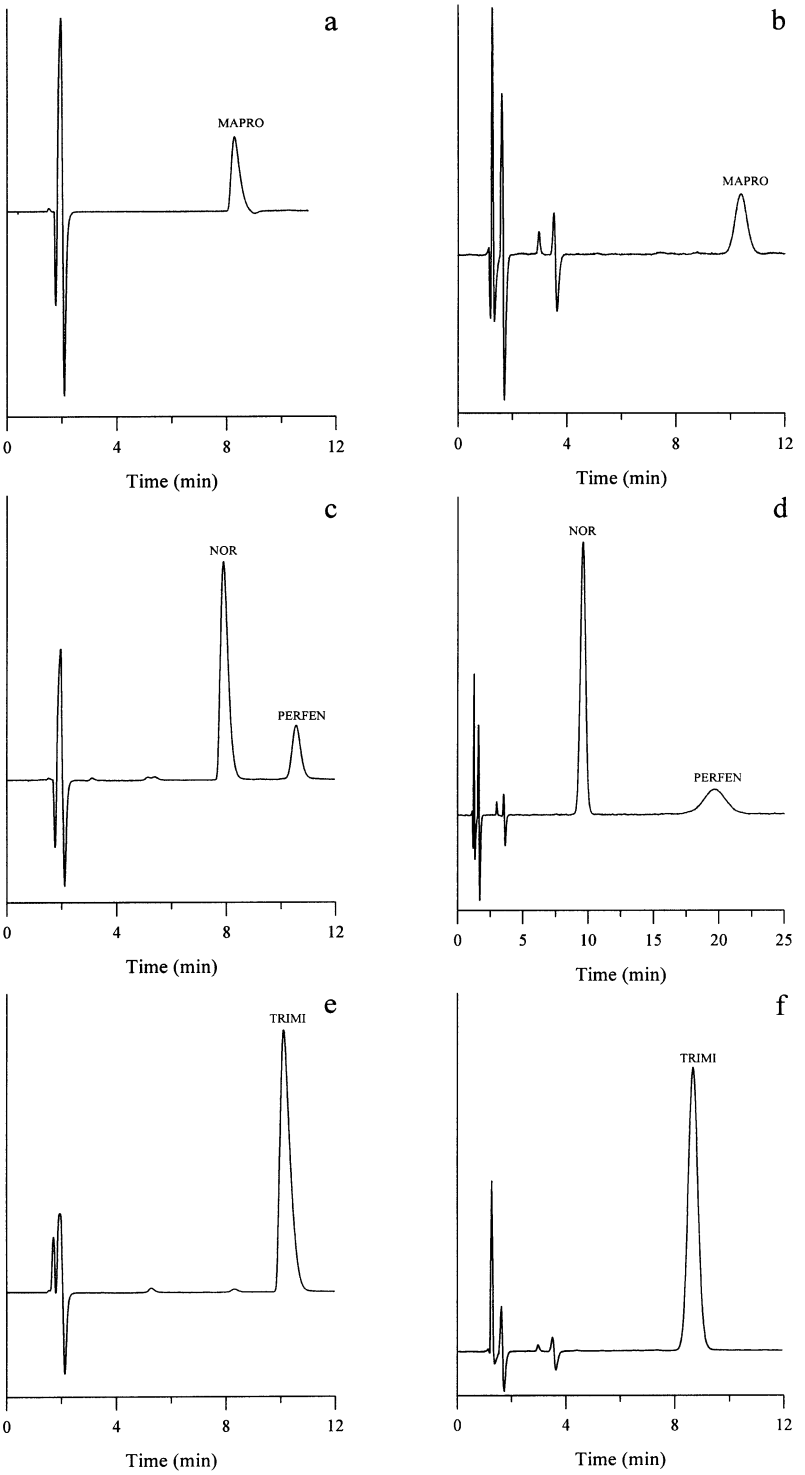


Fig. 5. Chromatograms of several formulations with the aqueous-organic (left) and micellar (right) systems: (a, b) Ludiomil, (c, d) Norfenazin and (e, f) Surmontil. See Figs. 1 and 4 for abbreviations.

similar plot was obtained for the C_8 column). Also, the selectivity was similar for the C_8 and XTerra C_{18} columns (Fig. 3(c) and (d)), but appreciably different for the unendcapped C_{18} column using acetonitrile–water (Fig. 3(e)), or the micellar C_8 system (Fig. 3(f)).

3.2. Figures of merit and analysis of pharmaceutical formulations

The study shown above indicates that the best column for screening purposes is the XTerra C_{18}. However, commercialised pharmaceutical formulations contain only one TCA. Therefore, the retention of the drugs was again examined in mobile phases of stronger elution strength to select new conditions in which the analysis times were shorter. Table 2 shows the retention times for the mobile phases finally selected. It is again evident that although the efficiencies are smaller for the C_8/micellar system compared with C_8/acetonitrile or XTerra C_{18}/acetonitrile, peak symmetry in MLC is appreciably improved. The retention time of clomipramine in the XTerra C_{18} column could be decreased by increasing the amount of acetonitrile, but if a single mobile phase composition is used to analyse the whole set of TCAs, the retention of doxepin would decrease excessively, making its resolution from the perturbations at the head of the chromatograms difficult.

Due to the good performance of the XTerra C_{18} column, the possibility of adding an amine modifier was left out of consideration. On the other hand, the MLC procedure with the C_8 column and SDS–pentanol requires a smaller amount of organic solvent than classical RPLC (even with the XTerra C_{18} column), which was attractive. For this reason, we compared further the performance of both aqueous-organic and micellar systems in the analysis of TCAs formulations.

Formulations of only six of the seven TCAs studied in this work were analysed: amitryptiline, clomipramine, doxepin, maprotiline, nortryptiline and trimipramine, which are prescribed in Europe. Parallel analyses of all formulations were carried out using the combinations XTerra C_{18}/35% acetonitrile and C_8/0.075 M SDS–6% pentanol.

Calibration curves were built using the areas of the chromatographic peaks from duplicate injections of standards, at five increasing concentrations in the range 30–70 μg/ml. The curves were obtained in 3 consecutive days and repeated after 1–3 months to study their variability. Table 3 shows the parameters of the fitted straight lines, where the concentrations are given in μg/ml. Coefficients of variation for the slopes were (aqueous-organic and micellar, %): amitryptiline (3.1, 2.1), clomipramine (1.9, 0.5), doxepin (1.0, 1.0), maprotiline (3.3, 2.0), nortryptiline (3.6, 1.8) and trimipramine (0.6, 2.0). The intercepts were usually statistically zero for MLC. The slopes were also smaller than for the aqueous-organic system, which was translated in poorer limits of detection (aqueous-organic and micellar, μg/ml): amitryptiline (0.02, 0.54), clomipramine (0.19, 0.18), doxepin (0.03, 0.24), maprotiline (0.21, 1.7), nortryptiline (0.22, 0.40) and trimipramine (0.04, 0.09). The inter-day repeatability obtained from the measurement of triplicates of 50 μg/ml standards carried out in 3 consecutive days was (aqueous-organic and micellar, %): amitryptiline (0.54, 0.17), clomipramine (0.43, 0.30), doxepin (0.23, 0.45), maprotiline (0.42, 1.70), nortryptiline (0.42, 0.20) and trimipramine (0.29, 0.60).

Six samples of each formulation were analysed making duplicate injections to obtain average values of the drug concentrations. For this purpose, an appropriate amount of each sample was weighed to prepare solutions containing 50 μg/ml of the drugs. Table 4 gives the declared and found contents, together with the label claim percentages, which were usually in the range 97–110%. The label claim was below 90% for norfenazin (nortryptiline) using the aqueous-organic system and above 110% for Sinequan (doxepin) with both systems. However, the reproducibility for both formulations was excellent.

The reproducibility was studied by measuring the signals from replicate solutions where the drugs were at three different concentrations: 30, 50 and 70 μg/ml. These were made by weighing for each measurement appropriate amounts of the formulations to reach values as close as possible to the indicated concentrations. This operation contributed thus to the total error. Intra-day reprodu-

cibility was calculated from sixfold assays, and inter-day reproducibility from triplicate assays performed during 6 consecutive days, and during 5 days along 2 months. The values are given in Table 5. Intra-day relative standard deviations (RSDs) were always below 0.6% and inter-day RSDs below 2%.

Figs. 4 and 5 show chromatograms of the formulations, where the elimination of peak tails in the micellar mode is evident. The excipients eluted at the dead time or did not absorb at the measuring wavelength. The peak of perfenazine, a drug also found in two formulations, appeared at longer retention times, showing no interference (Figs. 4(a) and (b) and 5(c) and (d)).

In both RPLC modes, the optimised procedures were applied to the control of commercialised formulations with satisfactory results. Since there was no interference from common additives, excipients or other drugs, previous extraction of TCAs was not needed. The preparation of the samples (solubilisation) was simple, requiring similar effort for both modes. The MLC procedure has, however, the advantage of using a smaller amount of organic solvent (6% pentanol against 35% acetonitrile for the aqueous-organic mode). Pentanol is also less toxic than acetonitrile, and is highly retained in the SDS micellar solution, which reduces the risk of evaporation. The C_8 column used in the micellar mode is also appreciably less expensive than the XTerra C_{18} column. However, for screening purposes, only the endcapped column with acetonitrile–water yielded adequate resolution for the studied drugs.

Acknowledgements

This work was supported by Project BQU2001-3047 (Ministerio de Ciencia y Tecnología of Spain and FEDER funds) and Project CTIDIB/2002/226 (Generalitat Valenciana). M.J.R.A. thanks MCYT for an FPI grant.

References

[1] T. Nogrady, Medicinal Chemistry, 2nd ed., Oxford University Press, Oxford, 1988, pp. 176–178.
[2] C.J. Drayton (Ed.), Comprehensive Medicine Chemistry, Vol. 6, Pergamon Press, Oxford, 1990.
[3] B.G. Reuben, H.A. Wittcoff, Pharmaceutical Chemicals in Perspective, Wiley/Interscience, New York, 1989, pp. 255–258.
[4] G. Carfagnini, A. Di Corcia, M. Marchetti, R. Samperi, J. Chromatogr. 530 (1990) 359–366.
[5] R. Theurillat, W. Thormann, J. Pharm. Biomed. Anal. 18 (1998) 751–760.
[6] L.P. Hackett, L.J. Dusci, K.F. Ilett, Ther. Drug Monit. 20 (1998) 30–34.
[7] Y.F. Cheng, D.J. Phillips, U. Neue, L. Bean, J. Liq. Chromatogr. Relat. Technol. 20 (1997) 2461–2473.
[8] T.W. Ryan, J. Liq. Chromatogr. 16 (1993) 1545–1560.
[9] K. Kallury, P. Shieh, R. Paschal, N. Cooke, Supelco. Rep. 17 (1998) 5.
[10] S.R. Needham, P.R. Brown, K. Duff, D. Bell, J. Chromatogr. A 869 (2000) 159–170.
[11] S.R. Needham, P.R. Brown, J. Pharm. Biomed. Anal. 23 (2000) 597–605.
[12] D. Burke, H. Sokoloff, J. Pharm. Sci. 69 (1980) 138–140.
[13] S.I. Sa'sa, I. Jalal, Microchem. J. 38 (1988) 181–187.
[14] J. Joseph-Charles, M. Bertucat, J. Liq. Chromatogr. Relat. Technol. 21 (1998) 3047–3064.
[15] J.R. Torres-Lapasió, MICHROM Software, Marcel Dekker, New York, 2000.
[16] J.R. Torres-Lapasió, M. Rosés, E. Bosch, M.C. García-Alvarez-Coque, J. Chromatogr. A 886 (2000) 31–46.
[17] C. Quiñones-Torrelo, S. Sagrado, R.M. Villanueva-Camañas, M.J. Medina-Hernández, J. Med. Chem. 42 (1999) 3154–3162.
[18] M.J. Ruiz-Angel, S. Carda-Broch, J.R. Torres-Lapasió, E.F. Simó-Alfonso, M.C. García-Alvarez-Coque, Anal. Chim. Acta 454 (2001) 109–123.
[19] M. Gil-Agustí, J.R. Torres-Lapasió, M.C. García-Alvarez-Coque, J. Esteve-Romero, J. Chromatogr. A 866 (2000) 35–49.
[20] S. Carda-Broch, J.R. Torres-Lapasió, J.S. Esteve-Romero, M.C. García-Alvarez-Coque, J. Chromatogr. A 893 (2000) 321–337.
[21] R.D. Caballero, J.R. Torres-Lapasió, M.C. García-Alvarez-Coque, G. Ramis-Ramos, Anal. Lett. 35 (2002) 687–705.
[22] J.R. Torres-Lapasió, J.J. Baeza-Baeza, M.C. García-Alvarez-Coque, Anal. Chem. 69 (1997) 3822–3831.