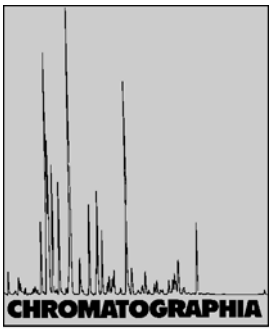
A Micellar Liquid Chromatographic Method For Quality Control of Pharmaceutical Preparations Containing Tricyclic Antidepressants



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Key Words

Column liquid chromatography
Micellar liquid chromatography
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Pharmaceutical preparations

Summary

Micellar liquid chromatography methods for quality control of pharmaceutical preparations (capsules, pills, tablets, injections) containing the tricyclic antidepressants amineptine, amitriptiline, clomipramine, doxepin, imipramine, melitracen and nortriptyline alone or together with other CNS drugs like diazepam, medazepam and perphenazine are described. The methods using micellar solutions of cetyltrimethylammonium bromide as mobile phases and UV detection are rapid and reproducible. Due to the versatility of interactions in micellar liquid chromatography, it is possible determine highly hydrophobic compounds such as TCAs in a short time using mobile phases containing low organic solvent concentrations and usual flow rates, in contrast with the RP-HPLC methods proposed for these compounds. Samples preparation only requires solution and adequate dilution with the mobile phase before injection into the chromatographic system.

Introduction

Tricyclic antidepressants (TCAs) are drugs used in the treatment of depression and other kinds of disorders such as phobias, obsessive syndromes, crisis of anguish, states of anxiety, etc. Depression is due to a decrease of neurotransmitter levels into the synaptic gap. In order to avoid this fact, TCAs can act in different ways: i) blocking the presynaptic receptor specific to each biogenic amine (serotonin, 5-HT, and noradrenaline, NA) which allows the release of neurotransmitters from the presynaptic cell to the postsynaptic one, ii) blocking the synaptic monoamine transporters and, consequently, inhibiting the re-uptake of the amines into the presynaptic neuron at level of the cell membrane [1].

TCAs can be classified attending to the structure [2] into: benzazepine derivates (amineptine, clomipramine and imipramine), benzocycloheptene derivates (amitriptyline, doxepin and nortriptyline) and others like melitracen.

The determination of TCAs in pharmaceutical preparations has been performed using several analytical techniques like volumetric analysis, potentiometry, polarography, infrared spectroscopy, spectrophotometry, chemiluminiscence, thin layer chromatography, gas chromatography and HPLC [3–9]. The United States Pharmacopeia, USP XXIII [10] recommends spectrophotometric and chromatographic methods. Spectrophotometric methods in the UV region require repetitive liquid-liquid extractions with chloroform or ether. Reversed phase chromatographic methods use mobile phases with high concentrations of organic solvents like acetonitrile-phosphate buffer (42:58), methanol-acetonitrile-ammonium carbonate solution (47.5:47.5:5), methanol-water (65:35; 80:20), methanol-ammonium acetate (4:1), and high mobile phase flow rate about 2–3.5 mL min^{-1}. The sample preparation steps are lengthy. The USP XXIII [10] recommends for the determination of amitriptyline and perphenazine in pharmaceuticals an ion-pair reversed phase chromatographic method using water-acetonitrile-methanol (49:31:20) with 2% methanosulphonic acid as mobile phase [10].

Micellar liquid chromatography (MLC) is a mode of reversed phase liquid chromatography, which uses aqueous solutions of surfactants above the critical micellar concentration. In MLC, electrostatic, hydrophobic and steric interactions between the solute and both the stationary and mobile phases exist, which allow the effective separation of compounds of different nature [11, 12]. The mobile phases are, almost non-flammable, biodegradable, cheaper and have a much lower pol-

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luting impact than other aqueous-organic phases. In addition the solvent power of micellar solutions facilitates the sample preparation step.

The retention of a solute in a micellar liquid chromatography system can be modified by changing the eluent composition in terms of the nature of the surfactant and its concentration, pH (in the case of ionizable compounds), ionic strength and/or by the addition of organic modifiers. MLC has been used in the determination of catecholamines, diuretics, amino acids, caffeine, theophyline, anabolic steroids, β-blockers, local anesthetics, sulfonamides and phenethylamines in pharmaceutical preparations [13–22]. All these cases employed SDS (sodium dodecyl sulphate) as surfactant. Recently, a procedure to determine non-steroidal antiinflamatory drugs in pharmaceutical preparations which employs cetyltrimethylammonium bromide (CTAB) as surfactant to prepare the micellar mobile phases has been reported [23].

In the present work, the most used tricyclic antidepressants (amitriptyline, amineptine, clomipramine, doxepin, imipramine, melitracen and nortriptyline) in several pharmaceutical formulations (capsules, pills, tablets, injections) have been determined using micellar mobile phases of cetyltrimethylammonium bromide (CTAB) and UV detection. In those pharmaceutical preparations that contain other active components besides the tricyclic antidepressant, such as benzodiazepines (diazepam and medazepam) and butyrophenones (flupenthixol and perphenazine), these compounds are also determined. The proposed methods are rapid, reproducible and sample preparation by dilution in micellar media is easy.

Experimental

Instrumental and Measurement

An Agilent 1100 chromatograph with an isocratic pump and an UV-visible detector was used (Palo Alto, CA, USA). Data acquisition and processing were performed on an HP Vectra XM computer (Amsterdam, The Netherlands) equipped with HP-ChemStation software from Agilent Co., 1996 version, (Waldbronn, Germany).

The solutions were injected into the chromatograph through a Rheodyne valve (Cotati, CA, USA), with a 20 μL loop. A Kromasil octadecyl-silane C_{18} column (5 μm, 150 × 4.6 mm i. d.) (Scharlau, Barcelona, Spain) were used. The mobile phase flow rate was 1 mL min^{-1}. UV detection was performed near the wavelength of the compound's maximum absorption. All the assays were carried out at room temperature.

An Agilent 8452A Spectrophotometer with diode array and equipped with Hewlett-Packard computer, model Vectra ES/12 (Palo Alto, CA, USA) was used.

A micropH 2000 pH-meter (Crison, Barcelona, Spain) was used for pH adjustment and for determining the protonation constants of compounds in a CTAB micellar medium.

Reagents and Standards

Micellar mobile phases were prepared by mixing aqueous solutions of cetyltrimethylammonium bromide (CTAB) (Acros Chimica, Geel, Belgium) and sufficient 1-propanol (reagent grade, Scharlau, Barcelona, Spain) to obtain the working concentration *(v/v)*. The pH of the micellar eluent was adjusted before the addition of the alcohol with: i) 0.05 M phosphate buffer, prepared with sodium dihydrogen phosphate (analytical reagent, Panreac, Barcelona, Spain) and the appropriate amount of 2 M solutions of sodium hydroxide (for analysis, Panreac) or phosphoric acid (for analysis, Panreac), and ii) 0.05 M citric buffer, prepared with trisodium citrate (Guinama, Valencia, Spain) and the appropriate amount of 2 M solutions of sodium hydroxide (for analysis, Panreac) or hydrochloric acid (for analysis, Merck, Darmstadt, Germany).

Barnstead E-pure, deionized water (Sybron, Boston, MA) was used throughout. The mobile phases and the solutions injected into the chromatograph were vacuum-filtered through 0.45 μm nylon membranes (Micron Separations, Westboro, MA, USA).

Stock standard solutions of TCAs were prepared by dissolving the compound in 0.04 M CTAB. Working solutions were prepared by dilution of the stock standard solutions with mobile phase and injected into the chromatograph. The solutions were stored in a refrigerator at 4 °C.

The drugs involved in this work were obtained from several sources: amitriptyline, diazepam and imipramine from Guinama (Valencia, Spain); clomipramine and perphenazine from Sigma (St. Louis, MO, USA). Other drugs were kindly donated by different laboratories: doxepin (Farmasierra, S. Sebastián de los Reyes, Spain); amineptine (Servier, Madrid, Spain); nortriptyline (Lilly, Madrid, Spain); melitracen and flupentixol (Lundbeck Espaa SA, Barcelona, Spain) and Medazepam (Roche, Basel, Switzerland).

Sample Preparation

For the analysis of tablets and pills, five units were weighed, ground in a mortar and finally, some of the solid was taken and dissolved in 0.04M CTAB buffered solution by ultrasonic bath (30 min). If pharmaceuticals were presented as capsules, two were taken, dissolved in CTAB solution by magnetic stirrer (30 min) and then 15 minutes in an ultrasonic bath, allowing a total solution of all components of the capsule including the cover. In the case of injection solutions, an aliquot was taken and diluted in CTAB solution. After appropriate dilution with mobile phase, working solutions were injected into the chromatographic system through a 0.45 μm nylon membrane. For each pharmaceutical, three independent sample solutions were prepared and for each one triplicate determinations were performed.

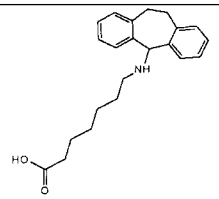
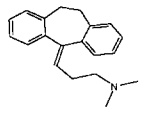
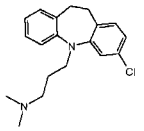
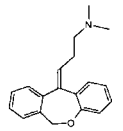
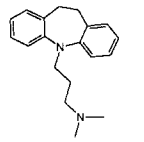
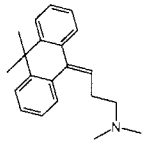
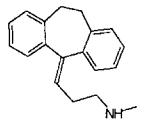
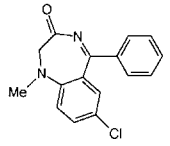
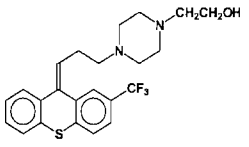
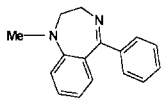
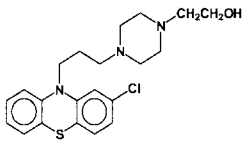
Results and Discussion

In order to determine the detection wavelength, the absorption spectra of compounds in CTAB micellar medium were obtained (Table I). The absorption spectra of all compounds showed absorption bands in the UV region with maximum absorption wavelengths ranged between 226 and 258 nm. A second maximum absorption wavelength at 310 nm for diazepam and perphenazine and at 450 nm for medazepam were also observed. Detection was carried out at 230 nm for amineptine, 254 nm for clomipramine and imipramine and 240 nm for the rest of tricyclic antidepressants and other CNS drugs included in this study.

Retention Behavior of Compounds

Table I shows the structure, molecular mass, log *P*, log *K* (in aqueous medium) [24–25] and maximum and detection wavelengths for the tricyclic antidepressants

Table I. Structure, PM, log*P*, and maximum and detection wavelengths of the coumpounds studied.

Compound	Structure	PM (g mol^{-1})	log $K^{34\text{-}35}$	log $P^{34\text{-}35}$	λmax/λdetection (nm)
Amineptine		337.5	5.24 7.69	2.44	226/230
Amitriptyline		277.4	9.42	4.64	240/240
Clomipramine		314.9	9.38	5.19	256/254
Doxepine		279.4	9.0	3.88	228/240
Imipramine		280.4	9.5	4.53	254/254
Melitracen		291.4	7.38	5.12	230/240
Nortriptyline		263.4	9.7	4.32	240/240
Diazepam		284.7	3.3	3.18	232,312 /240
Flupentixol		434.5	7.8	5.90	232,264 /240
Medazepam		270.8	4.4	4.47	252,360, 454/240
Perphenazine		404.0	3.7 7.8	5.57	258,314 /240

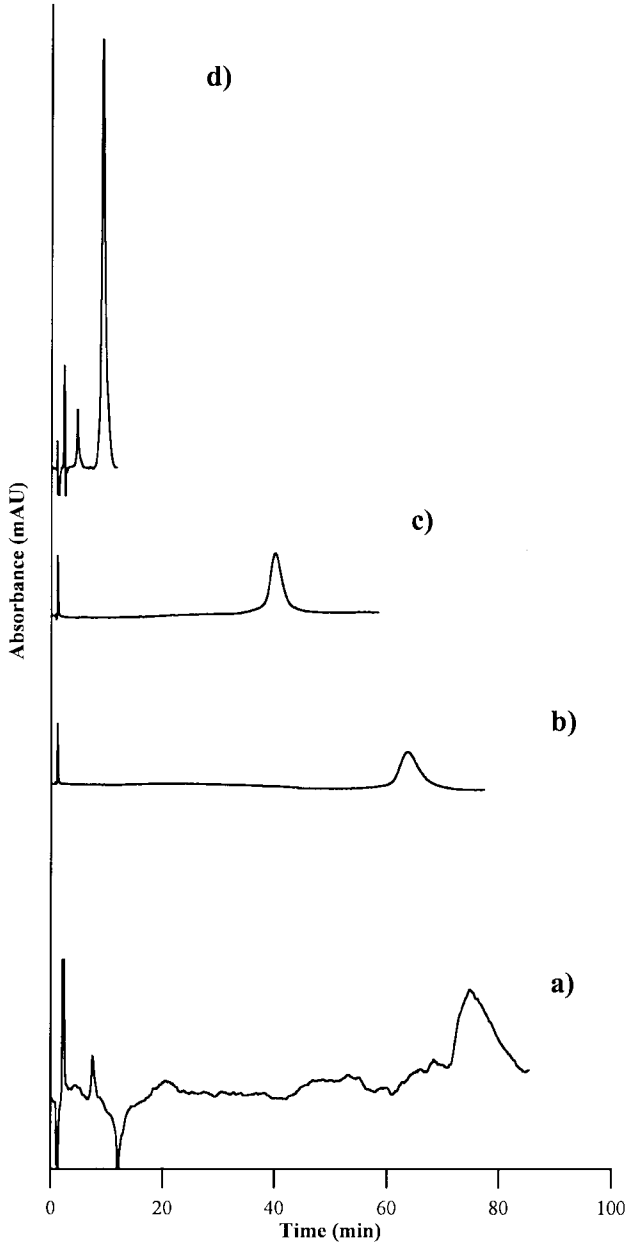


Figure 1. Influence of the mobile phase composition on clomipramine retention: **a)** 0.04M CTAB, pH = 6.5, **b)** 0.06 CTAB M, pH = 6.5, **c)** 0.04M CTAB, pH = 7, 5% 1-propanol and **d)** 0.04M CTAB, pH = 3, 5% 1-propanol.

Table II. Retention data of compounds in different CTAB mobiles phases.

CTAB, M	0.04	0.06	0.04	0.04	0.04	0.04	0.04	0.04
pH	6.5[a]	6.5[a]	7[a]	3[a]	3.5[a]	3.5[b]	4[b]	4.3[b]
% 1-Propanol	–	–	5	5	10	10	10	10
Amineptine	83	50	–	5.6	–	–	–	–
Amitriptyline	67	44	62	7.1	5.1	6.8	7.4	8.3
Clomipramine	86	54	83	8.9	–	–	–	–
Doxepin	43	28	36	4.4	–	–	–	–
Imipramine	52	35	51	5.1	–	–	–	–
Melitracen	96	59	–	12.7	8.3	9.8	–	13.1
Nortriptyline	24	14	21	9.1	–	–	–	10.6
Diazepam	–	–	–	37.0	–	–	–	26.6
Flupentixol	–	–	–	11.6	–	–	–	13.7
Medazepam	–	–	–	6.4	2.7	6.3	11.4	19.8
Perphenazine	–	–	–	7.2	5.6	7.5	8.6	9.6

[a] Phosphate buffer 0.05 M, I = 0.05; [b] Citrate buffer 0.05 M, I = 0.15

and other CNS drugs that are also present in the pharmaceutical preparations, the anxiolytics, diazepam and medazepam and the antipsychotics, flupentixol and perphenazine.

All these compounds possess polycyclic structures with molecular mass ranged between 263.4 (nortriptyline) and 434.5 (flupentixol) that give them a high hydrophobicity, their corresponding log P values vary between 2.44 (amineptine) and 5.90 (flupentixol). In addition, they are basic compounds with secondary or tertiary amines in their molecules, except amineptine, which has a basic and an acid group.

It is known that the acid-base equilibrium can be modified by the presence of micelles. In accordance with previous studies of our research group [26] an increase in micellar anionic medium and a decrease in micellar cationic medium of the protonation constants, log K, are usually observed.

The log K values of some of the compounds were potentiometrically estimated in 0.04 M CTAB. The log K values obtained in the presence of CTAB micelles were 1–2 units lower than those corresponding to aqueous medium. For instance, amitriptyline and perphenazine log K values decreased from 9.42 and 7.8 to 7.32 ± 0.05 and 6.50 ± 0.11 (mean value of triplicate estimations), respectively.

In order to select the particular surfactant for preparing micellar mobile phases, some assays were carried out. The retention times of analytes when a non-ionic surfactant such as Brij-35, was used, were very long due to strong hydrophobic interactions between the analytes and the modified stationary phase [27]. The use of an anionic surfactant like SDS, also gave long retention times due to the electrostatic attractions between analytes and surfactant adsorbed on the stationary phase in addition to the high hydrophobicity of compounds. The addition of a large amount of propanol to the mobile phase did not sufficiently decrease the retention. When a 0.15 M SDS/pH = 3 mobile phase containing 10% propanol was used, the retention times varied between 17 minutes for doxepine and 21 minutes for melitracen. In order to reduce retention times, micellar mobile phases containing the cationic surfactant CTAB were assayed.

Table II shows the retention of compounds obtained for different CTAB mobile phases. Two CTAB concentration levels in the mobile phase at pH 6.5 were as-

sayed: 0.04 and 0.06 M, in the absence and in the presence of 10% 1-propanol. The increase of the surfactant concentration in the mobile phase provided a diminution of the retention of about 30–40% in the absence of the alcohol. In the presence of the 1-propanol, the reduction of retention when the surfactant concentration increased was lower. The use of pure micellar mobile phases of CTAB has the drawback that it requires close control of temperature because this surfactant has a high Kraft temperature. The presence of alcohol reduces these problems and besides. generally produces a diminution of the retention times and improves the efficiency of peaks. A 0.04 M CTAB concentration in the micellar mobile phase was selected and different concentrations of 1-propanol (5, 10 and 15%), were assayed. The retention times were reduced when alcohol concentration in the mobile phase increased.

The mobile phase pH is a very important variable affecting the retention of the tricyclic antidepressants as expected from the protonation constant values. Different mobile phase pH values ranged between 3 and 7 were tested. When mobile phase pH decreased, the retention also decreased due to the electrostatic repulsion between the ionic form of the compounds and the surfactant monomers adsorbed on the stationary phase. Figure 1 shows the influence of the mobile phase composition on the chromatographic behavior of clomipramine.

Table II shows that a micellar mobile phase containing 0.04M CTAB, 0.05M phosphate buffer pH 3 and 5% 1-propanol, gives retention times of tricyclic antidepressants appropriate for quantitative purpose, (between 4.5 min for doxepine and 12 min for melitracen). However under these conditions amitriptyline, medazepam and perphenazine, and melitracen and flupentixol overlapped and the retention time of diazepam was too long for quantitative purpose ($t_r \approx 35$ min). In the case of the pharmaceutical preparation containing nortriptyline and diazepam, a mobile phases of 0.04 M CTAB, pH 3 and 15% 1-propanol provided adequate retention times (4.9 and 13 min for nortriptiline and diazepam, respectively).

In order to analyze pharmaceutical preparations that contain in addition to TCAs, the CNS drugs flupentixol, medazepam, melitracen and perphenazine, other mobile phases with pHs between 3 and 4.5 using citrate buffer and/or the organic modifier concentration were assayed. As can be observed in Table II, for a 0.04M CTAB, pH = 3.5 mobile phase containing 10% 1-propanol, longer retention times were observed when citrate buffer (ionic strength 0.14) was used instead of phosphate buffer (ionic strength 0.05). These differences may be due to an increase of ionic strength producing a diminution of electrostatic repulsions between analytes and surfactant monomers adsorbed on the stationary phase thus increasing the retention of compounds. When the mobile phase pH was changed, the retention time of amitriptiline scarcely changed, whereas the change in the retention of perphenazine was enough to achieve adequate separation from amitriptiline. Large changes in the retention were observed for medazepam (t_r 6.9 to 21.3 min. for pH values 3.5 and 4.3, respectively); even an inversion of the elution order respecting to amitriptiline took place (Table II). Good resolution for the mixtures amitriptiline-medazepam and amitriptiline-perphenazine was obtained using 0.04M CTAB, pH = 4, 10% 1-propanol and 0.04M CTAB, pH = 4.3, 10% 1-propanol, respectively.

Under the experimental conditions used in this work, the resolution of melitracen-flupentixol was not possible. Both compounds have similar log P and pK_a (Table I). Probably, better resolution could be achieved using a mobile phase pH close to their protonation constants, but, under these conditions the retention times are very long (e.g. at pH 7 the retention time of melitracen in 0.04M CTAB, 5% propanol was greater than 180 min).

Table III. Regression statistics of the calibration graphs, coefficients of variation and limits of detection (peak area).

Compounds	m ± ts	n ± ts	R	CV (%)[a]	CV (%)[b]	LOD (ppm)
Amineptine[a]	13.9 ± 0.9	− 20 ± 30	0.9993	9.0	2.0	1.3
Amitriptyline[a]	58 ± 2	0 ± 60	0.9998	1.8	1.5	0.33
Amitriptyline[b]	52 ± 3	10 ± 80	0.9990	2.1	1.7	0.37
Amitriptyline[c]	55 ± 3	30 ± 70	0.9993	2.5	2.2	0.36
Clomipramine[a]	28.8 ± 1.6	40 ± 50	0.9996	3.0	1.8	0.64
Doxepin[a]	43 ± 4	30 ± 130	0.998	1.2	0.6	0.19
Imipramine[a]	34 ± 2	0 ± 70	0.9994	4.0	2.0	0.88
Melitracen[a]	51 ± 5	270 ± 160	0.998	8.0	6.0	2.47
Nortriptyline[a]	63 ± 2	−160 ± 80	0.9998	0.8	0.3	0.15
Nortriptyline[d]	63.7 ± 0.7	10 ± 20	0.9999	0.8	0.3	0.15
Diazepam[d]	107 ± 3	0 ± 30	0.9998	0.6	1.0	0.03
Medazepam[b]	72 ± 3	0 ± 30	0.9997	8.5	2.8	0.45
Perphenazine[c]	44 ± 3	10 ± 40	0.9990	6.5	6.0	1.02

m: slope; n: intercept value; R: regression coefficient; CV: coefficient of variation, LOD: Limit of detection, (see text for details).
[a] 0.04 M CTAB, 0.05 M phosphate buffer pH 3, 5% 1-propanol, [b] 0.04M CTAB, 0.05 M citrate buffer pH 4, 10% 1-propanol, [c] 0.04 M CTAB, 0.05 M citrate buffer pH 4.3, 10% 1-propanol, [d] 0.04 M CTAB, 0.05 M phosphate buffer pH 3, 15% 1-propanol.

Table IV. Regression statistics of the calibration graphs, coefficients of variation and limits of detection (peak height).

Compounds	m ± ts	n ± ts	R	CV (%)[a]	CV (%)[b]	LOD (ppm)
Amineptine[a]	0.404 ± 0.009	−0.4 ± 0.3	0.9999	2.0	1.5	0.41
Amitriptyline[a]	1.38 ± 0.02	0.3 ± 0.8	0.9999	1.3	0.2	0.25
Amitriptyline[b]	1.21 ± 0.04	0.5 ± 1.2	0.9996	4.8	0.8	0.78
Amitriptyline[c]	1.22 ± 0.04	0.1 ± 1.2	0.9997	5.0	6.9	0.74
Clomipramine[a]	0.59 ± 0.02	−0.1 ± 0.8	0.9997	2.0	0.5	0.42
Doxepin[a]	1.25 ± 0.07	0 ± 2	0.9994	0.9	0.6	0.21
Imipramine[a]	1.01 ± 0.04	0.6 ± 1.3	0.9997	2.0	1.8	0.47
Melitracen[a]	0.74 ± 0.03	1.0 ± 0.8	0.9998	6.0	4.0	1.4
Nortriptyline[a]	1.22 ± 0.04	−2.3 ± 1.2	0.9998	1.1	1.2	0.21
Nortriptyline[d]	2.74 ± 0.11	−1 ± 3	0.9996	1.1	1.2	0.21
Diazepam[d]	1.76 ± 0.03	0.0 ± 0.4	0.9999	2.2	1.5	0.14
Medazepam[b]	1.28 ± 0.02	0.0 ± 0.3	0.9999	8.9	1.5	0.49
Perphenazine[c]	0.75 ± 0.02	−0.1 ± 0.3	0.9998	3.5	2.5	0.49

m: slope; n: intercept value; R: regression coefficient; CV: coefficient of variation, LOD: Limit of detection, (see text for details).
[a] 0.04 M CTAB, 0.05 M phosphate buffer pH 3, 5% 1-propanol, [b] 0.04 M CTAB, 0.05 M citrate buffer pH 4, 10% 1-propanol, [c] 0.04 M CTAB, 0.05 M citrate buffer pH 4.3, 10% 1-propanol, [d] 0.04 M CTAB, 0.05 M phosphate buffer pH 3, 15% 1-propanol.

Table V. Pharmaceutical preparations, composition and recoveries of compounds determined.

Preparation (presentation) source	Composition	Recoveries ± s(%) Peak areas	Recoveries ± s(%) Peak heights
SURVECTOR® (tablets) Danval,SA	Amineptine hydrochlor. (100 mg) Lactose and other excipients	94 ±2	97.9 ± 0.9
NOBRITOL® (capsules) Kern Pharma	Amitriptyline hydrochlor. (12.5 mg) Medazepam (5 mg) Excipients	107 ±5 98 ±9	110 ±7 98 ±6
TRYPTIZOL® (tablets) MSD	Amitriptyline hydrochlor. (10 mg) Excipients	95 ±3	97 ±3
MUTABASE® (pills) Schering plough	Amitriptyline hydrochlor. (10 mg) Perphenazine (2 mg) Lactose, starch, sacarose and other excipients	104.1 ± 1.4 118 ±6	103 ±2 114 ±5
ANAFRANIL® (pills) Novartis farmacéutica	Clomipramine hydrochlor. (25 mg) Glycerine and other excipients	98.7 ± 0.9	102.1 ± 1.8
ANAFRANIL® (injection solution) Novartis farmacéutica	Clomipramine hydrochlor. (25 mg/2 ml) Glycerine and water.	98.6 ± 1.4	97 ±2
ANAFRANIL® (tablets) Novartis farmacéutica	Clomipramine hydrochlor. (75 mg) Ricine oil hydrogenated and other excipients	95.6 ± 0.4	93.6 ± 0.9
SINEQUAN® (capsules) Farmasierra	Doxepin hydrochlor. (25 mg) Excipients.	111 ±7	106 ±9
TOFRANIL® (pills) Novartis farmacéutica	Imipramine hydrochlor. (50 mg) Excipients.	98 ±6	97 ±6
DEANXIT® (pills) Lundbeck España, SA	Melitracen hydrochlor. (10 mg) Flupentixol dichlor. (0.5 mg) Sacarose and other excipients.	98 ±3	108 ±3
TROPARGAL® (capsules) Synthelabo Alonga, SA	Nortriptyline hydrochlor. (12.5 mg) Diazepam (2.5 mg) Excipients	97 ±6 99 ±7	97 ±4 102 ±7

Analytical Data

The calibration curve of each compound studied was obtained by triplicate injections of standard solutions containing analyte concentration in the range 5–50 $\mu g mL^{-1}$ for all compounds studied except for medazepam and diazepam (2–20 $\mu g\ mL^{-1}$). Both peak area and height were used as dependent variables. Tables III and IV show the regression statistics for the calibration curves of each compound. The curves showed adequate regression coefficients ($r^2 \geq 0.998$) over the working range.

The repeatability (expressed as coefficient of variation), was evaluated at two concentration levels 6 and 25 $\mu g\ mL^{-1}$ for the TCAs and perphenazine and 2 $\mu g\ mL^{-1}$ and 10 $\mu g\ mL^{-1}$ for medazepam and diazepam, (n = 5). Tables III and IV show the variation coefficients obtained using peak areas or heights as dependent variables. The coefficients of variation in general ranged between 2% and 6% for the higher and lower concentration levels studied.

The limits of detection (LOD) were calculated according to the 3σ criterion from the standard deviation related to peak area or height obtained by injecting five solutions containing 6 $\mu g\ mL^{-1}$ of each TCA and perphenazine and 2$\mu g\ mL^{-1}$ of medazepam or diazepam. In general, LOD values were lower than 1 $\mu g\ mL^{-1}$ (Tables III and IV).

Analysis of Pharmaceutical Formulations

Table V shows the composition of the pharmaceutical preparations analyzed. The majority of the pharmaceutical preparations contain only one TCA as the active principle. All pharmaceutical preparations commercially available in Spain containing TCAs together with other drugs have been analyzed. The content of each analyte in the pharmaceutical formulations was determined by triplicate injections of three independently prepared solutions. Figure 2 shows the chromatograms obtained from some of the pharmaceutical preparations analyzed. Except for deanxit, the analyte peaks were adequately separated from other compounds present. Table V shows the recoveries and standard deviations obtained; the results were reproducible and the recoveries ranged between 94 and 110% with respect to the manufacurers' declared values.

The pharmaceutical preparation deanxit® that contains melitracen and flupentixol in the concentration ratio 20:1, was also analyzed using a 0.04M CTAB, pH 3, 5% 1-propanol as mobile phase. In spite of overlapping peaks, the recovery of melitracen was 98 ± 3%. This result can be explained taking into account that the concentration of flupentixol in the sample solutions injected (1.2 $\mu g\ mL^{-1}$) is very close to its LOD.

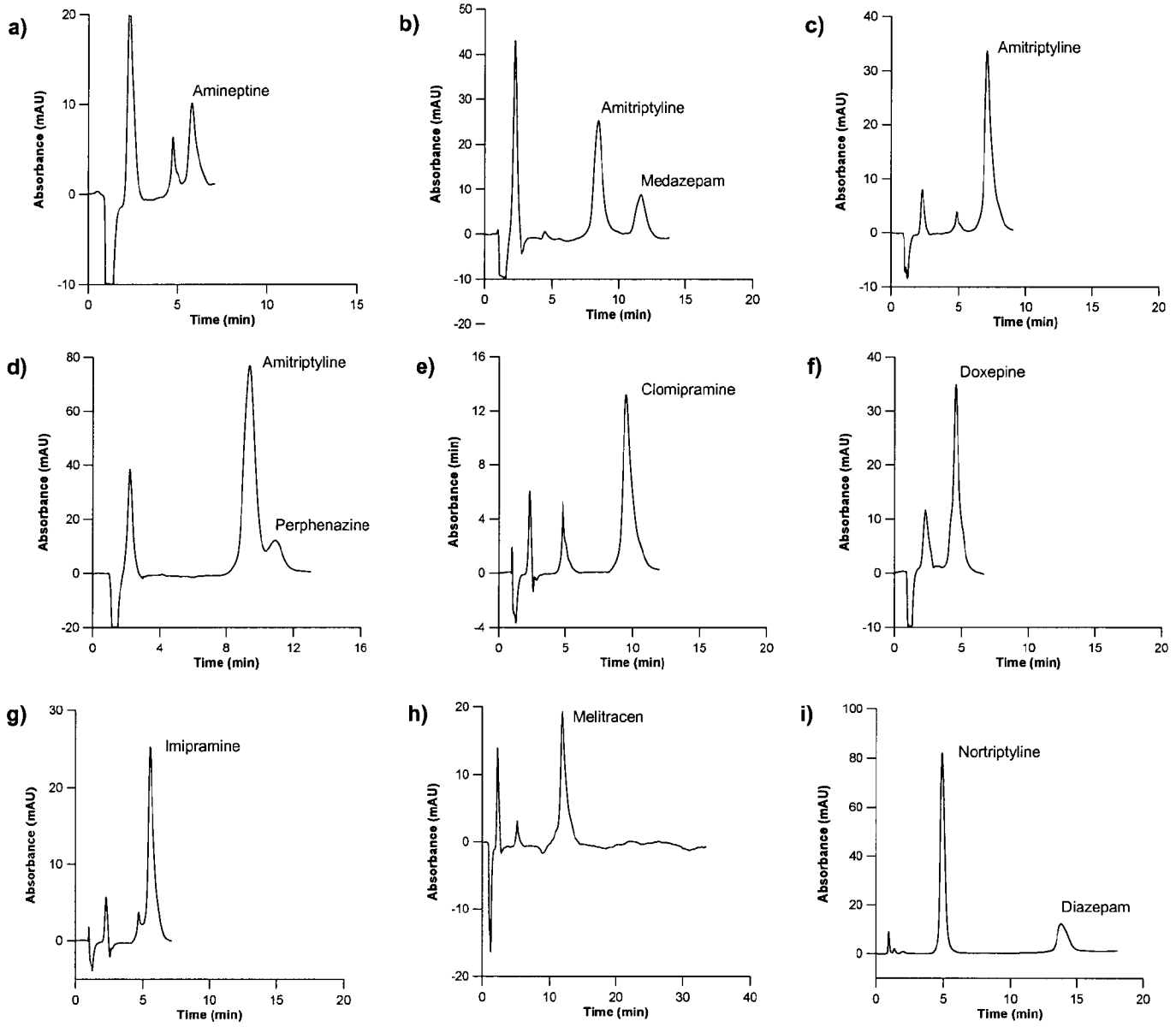


Figure 2. Chromatograms of some of the pharmaceutical preparations analyzed: **a)** Survector®, **b)** Nobritol®, **c)** Tryptizol®, **d)** Mutabase®, **e)** Anafranil® (injection solution), **f)** Sinequan®, **g)** Tofranil, **h)** Deanxit® and **i)** Tropargal®.

Conclusions

The methods described allow a rapid and reproducible determination of tricyclic antidepressants and other CNS drugs present in pharmaceutical formulations. Due to the versatility of interactions in micellar liquid chromatography, it is possible determine highly hydrophobic compounds such as TCAs in a short time at usual flow rates. Pharmaceutical preparations are easily dissolved in micellar media. The CTAB mobile phases used contained low organic solvent concentration and are less polluting than the proposed reference methods. The advantages of the proposed methods over the current RP-HPLC methods make the MLC methods attractive alternatives for the determination of CNS drugs in pharmaceutical preparations.

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