BIOMEDICAL CHROMATOGRAPHY
Biomed. Chromatogr. **19**: 343–349 (2005)
Published online in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/bmc.455

ORIGINAL RESEARCH

Therapeutic monitoring of imipramine and desipramine by micellar liquid chromatography with direct injection and electrochemical detection

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Received 23 July 2004; revised 3 September 2004; accepted 20 September 2004

ABSTRACT: A micellar liquid chromatographic (MLC) procedure was developed for the clinical monitoring of imipramine and its active metabolite, desipramine. The determination of these highly hydrophobic substances was carried out after direct injection of the serum samples using a mobile phase composed of 0.15 M SDS–6% (v/v) pentanol buffered at pH 7, pumped at 1.5 mL/min into a C_{18} column (250 × 4.6 mm), and electrochemical detection at 650 mV. Using this MLC method, calibration was linear (r > 0.995) and the limits of detection (ng/mL) were 0.34 and 0.24 for imipramine and desipramine, respectively. Repeatabilities and intermediate precision were tested at three different concentrations in the calibration range and a CV (%) below 2.2 was obtained. In this MLC procedure, the serum is determined without treatment, thus allowing repeated serial injections without changes in retention factors, and reducing the time and consumables required to carry out the pretreatment process. The assay method can be applied to the routine determination of serum imipramine and its metabolite in therapeutic drug monitoring.

KEYWORDS: micellar liquid chromatography (MLC); imipramine; desipramine; serum; monitoring

INTRODUCTION

Imipramine and desipramine are tricyclic antidepressants and belong to the group of dibenzazepine derivatives, consisting of two aromatic rings connected by a seven-member ring which has a propylamino side chain joined to it (Fig. 1). Imipramine and its active metabolite, desipramine, are used in the treatment of depressive affective disorders, principally major depression. The drugs are used to treat the depressive phase of bipolar disorder, but they do not prevent and indeed may precipitate hypomanic or manic attacks in patients with this disorder. These drugs may be beneficial in the treatment of the depressive stages of schizophrenia or in the treatment of depression with psychotic features

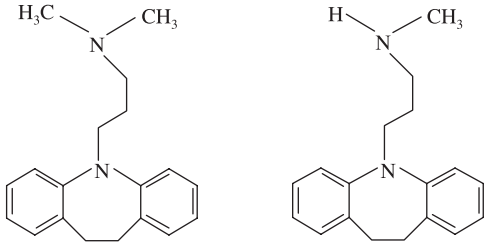

Figure 1. Structures of desipramine and imipramine.

(AHFS, 1998). The therapeutic ranges of desipramine and imipramine are 115–250 and 180–350 ng/mL, respectively, and both become toxic at concentrations higher than 500 ng/mL (Linder and Keck, 1998). Pharmacokinetics of these drugs show that plasma concentrations occur within 1–2 h after oral administration for imipramine, and 4–6 h for desipramine. Imipramine is metabolized to desipramine, its pharmacologically active metabolite. The plasma half-life is 12–28 and 6–28 h for desipramine and imipramine, respectively.

In the last 10 years, only a few procedures have been developed to determine imipramine and desipramine using HPLC with UV (Hilberg *et al.*, 1999; Theurillat and Thormann, 1998; Tanaka *et al.*, 1997; Yoo *et al.*,

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Abbreviations used: CMC, critical micellar concentration; ED, electrochemical detection; MLC, micellar liquid chromatography; RPLC, reversed-phase liquid chromatography; SDS, sodium dodecyl sulphate.

Contract/grant sponsor: Fundació Caixa de Castelló-Bancaixa; Contract/grant number: P1B2003-07.
Contract/grant sponsor: MCYT-FEDER; Contract/grant number: BQU2001-3770.

Published online 7 December 2004

1995; Goldnik *et al.*, 1991; Segatti *et al.*, 1991) or electrochemical (Ivandini *et al.*, 2002; Chen *et al.*, 1997; Koyama *et al.*, 1993; Bouquet *et al.*, 1992; Foglia *et al.*, 1991) detection. In all cases, the procedure requires prior extraction and in most of them an internal standard is used (Theurillat and Thormann, 1998; Yoo *et al.*, 1995; Goldnik *et al.*, 1991; Segatti *et al.*, 1991; Chen *et al.*, 1997; Koyama *et al.*, 1993; Bouquet *et al.*, 1992; Foglia *et al.*, 1991). The columns used were C_{18} (Theurillat and Thormann, 1998; Yoo *et al.*, 1995; Chen *et al.*, 1997; Koyama *et al.*, 1993; Foglia *et al.*, 1991), C_8 (Tanaka *et al.*, 1997; Segatti *et al.*, 1991) and cyano (Hilberg*et al.*, 1999; Foglia *et al.*, 1991). Moreover, the mobile phases employed in conventional HPLC usually require a high percentage of organic solvent (30–60%) to elute these compounds. Acetonitrile or methanol with phosphate buffer are the most commonly used mobile phases. Ternary mobile phase with methanol–acetonitrile–phosphate is also used to determine the two compounds. Sometimes the addition of anamine is useful to increase the efficiencies (Theurillat and Thormann, 1998; Yoo *et al.*, 1995).

The determination of drugs in biological fluids by HPLC has one important drawback due to the presence of proteins and endogenous compounds. These compounds can precipitate inside the chromatographic system, causing a rise in the pressure and occasionally the blockage of the column. This situation becomes a major problem in conventional chromatography and so an extraction step is required. Extraction can be a tedious job and frequently leads to low and variable recoveries.

Micellar liquid chromatography (MLC) is a mode of reversed-phase liquid chromatography (RPLC) in which the mobile phases are aqueous solutions of a surfactant at a concentration above the critical micellar concentration (cmc), that is, in a medium where micelles are present (Knox and Laird, 1976; Armstrong and Nome, 1981). One of the major advantages of MLC is in fact the possibility it offers to determine drugs in physiological fluids without the need for previous separation of the proteins present in the samples. Micelles tend to bind proteins competitively, thereby releasing protein-bound drugs. MLC has proven to be a useful technique in the analysis of several drugs in body fluids, like serum (Martinavarro-Dominguez *et al.*, 2002; Capella-Peiro *et al.*, 2002a,b; Habel *et al.*, 1997) or urine (Gil-Agusti *et al.*, 2003; Ruiz-Angel *et al.*, 2002; Torres-Cartas *et al.*, 2001; Carda-Broch *et al.*, 1999; Martin-Biosca *et al.*, 1999; Chen *et al.*, 1997; Chen and Wang, 1997), using direct injection. These are therefore free to partition into the stationary phase whereas the proteins, rather than precipitating in the column, are solubilized and swept away harmlessly, eluting with or shortly after the solvent front. Another advantage is that the surfactants are non-toxic, non-flammable and relatively inexpensive in comparison to aqueous–organic solvents.

This work shows the development of a fast simple MLC procedure for the determination of imipramine and its main metabolite, desipramine, in serum samples with a mobile phase of sodium dodecyl sulphate (SDS) and a small amount of organic modifier, pentanol and electrochemical detection.

EXPERIMENTAL

Reagents. The analytes studied, imipramine and desipramine, were purchased from Sigma (St Louis, MO, USA). Stock solutions containing 10 μg/mL of the compounds, weighed on a Mettler-Toledo AX105 Delta-Range balance (Greifensee, Switzerland), were dissolved in a micellar solution [0.15 M SDS–6% (v/v)-pH 7] and then suitably diluted before analysis.

Micellar mobile phases were prepared using SDS (99% purity) from Merck (Darmstadt, Germany), the buffer salt sodium dihydrogen phosphate, and the modifiers propanol, butanol and pentanol (Merck). Potassium chloride (Merck) was added as an electrolyte for electrochemical detection. All the solutions were filtered through 0.45 μm Nylon membranes (Micron Separations, Westboro, MA, USA) and stored at 4°C. The vortex shaker and sonification unit were from Selecta (Barcelona).

Instrumentation. The pH of the solutions was measured with a Crison GLP 22 (Barcelona, Spain), fitted with a combined Ag/AgCl/glass electrode. The chromatograph was an Agilent Technologies Series 1100 (Palo Alto, CA, USA) equipped with a quaternary pump (0–5 mL/min), a desgasifier for the mobile phase, an autosampler (5–100 μL injection volume), a temperature control for the columns module (–5–60°C) coupled to a UV–visible detector (190–700 nm range) and an Agilent Technologies electrochemical detector (–400–1400 mV) Series 1049A (Palo Alto, CA, USA). Kromasil 5 C_{18} columns with 5 μm particle size, 250 × 4.6 mm i.d. from Scharlab (Barcelona, Spain) were also used. Chromatographic signals were acquired and treated with the Agilent program (Revision A.09) used in the integration of the peaks. Excel was used in other calculations.

Recommended method. The optimum mobile phase used for the separation of desipramine and imipramine was 0.15 M SDS–6% (v/v) pentanol–0.001 M NaCl–0.01 M NaH_2PO_4 at pH 7. The pH was adjusted before the addition of the pentanol to the micellar solution. The flow rate, injection volume, temperature of the column and voltage applied were 1.5 mL/min, 20 μL, 25 ± 0.2°C and 0.650 V, respectively.

Sample preparation. The analyses of real samples were performed with a few volumes of serum that were injected directly into the chromatographic system without any treatment other than filtration, which was carried out in the autosampler vials through 0.45 μm nylon membranes. Aqueous drug solutions were spiked in the diluted serum to obtain the chromatographic parameters of the individual compounds.

RESULTS AND DISCUSSION

Selection of pH and modifier

In the optimization procedure, the pH was the first parameter to be studied. The two antidepressants have a basic behaviour (Fig. 1), and the first protonation constant for imipramine and desipramine is 9.5 and 10.2, respectively (AHFS, 1998). Log *K* are expected to increase in the presence of the anionic SDS micelles, owing to stabilization of the positive charge of the protonated drugs. Following this point of view, these two analytes will be protonated in the 3–7 range, whereas its chromatographic behaviour is not expected to change within this pH range. When the injection of the two substances was performed in mobile phases containing SDS 0.1 M, SDS 0.1 M–6% (v/v) propanol, SDS 0.1 M–5% (v/v) butanol and SDS 0.1 M–4% (v/v) pentanol, buffered at pH 3, 5 and 7, no change in the chromatographic behaviour was observed due to the factor pH, in agreement with what was previously predicted. pH 7 was selected because is the most suitable value for extending the life of the column.

Additionally, Fig. 1 shows the high values of log $P_{o/w}$, indicating that these substances are highly hydrophobic. In MLC, these kinds of compounds are not eluted in feasible amounts of time if pure micellar mobile phases are used (containing only surfactant), and generally they need the addition of an organic modifier in order to elute them in an adequate analysis time. Alcohols such as propanol, butanol or pentanol could be suitable due to their higher elution strength, which increases according to the length of the carbon chain. To select the appropriate organic modifier, three mobile phases were prepared containing the maximum concentrations of SDS and alcohol, that is, 0.15 M SDS–12% (v/v) propanol, 0.15 M SDS–7% (v/v) butanol and 0.15 M SDS–6% (v/v) pentanol. When the two antidepressants were tested, results showed that the analysis times were too high in the mobile phases containing propanol (>40 min) and butanol (>30 min). Pentanol, which reduces the analysis time to less than 20 min, was thus selected because it has the highest elution strength, the two substances are highly hydrophobic, and on the basis of the results obtained by our group in other studies involving substances like antiepileptics (log $P_{o/w}$ 1.5–2.5; Martinavarro-Dominguez *et al.*, 2002) and barbiturates (log $P_{o/w}$ 0.7–2.1; Capella-Peiro *et al.*, 2002a,b).

Selection of the mobile phase

To select the optimum mobile phase, under the criteria of minimum analysis time, maximum resolution and best chromatographic parameters, we injected the stock solutions (10 μg/mL) of desipramine and imipramine in five mobile phases containing SDS (M)–pentanol (%, v/v): 0.05–2, 0.05–6, 0.15–2, 0.15–6 and 0.10–4, buffered with 0.01 M NaH_2PO_4 at pH 7 and containing NaCl 0.001 M. Later, measurements of the chromatographic parameters, retention factors (*k*), efficiencies (*N*) and asymmetry factors (*B/A*) were performed. In all chromatograms, the dead volume was determined as the mean value of the first significant deviation from the base-line in the chromatograms.

Table 1. Capacity factors (*k*) for desipramine and its metabolite in the five mobile phases studied for the method optimization using SDS (M)–pentanol (% v/v), pH 7 at 1 mL/min

Compound	0.05–2	0.05–6	0.1–4	0.15–2	0.15–6
Imipramine	55	16	21	29	11
Desipramine	68	18	26	38	13

Table 1 shows the values of the retention factors for the two antidepressants in the five micellar mobile phases at 1 mL/min. The behavior of the drugs was as expected in MLC: retention decreased as the concentration of surfactant and/or organic modifier increased. The elution strengths of pentanol and SDS were similar, and when the concentration of SDS or pentanol was fixed, at 0.05 or 0.15 M and 2 or 6%, the retention time decreased by 20–30 min. The biggest change (50 min) was observed in the retention of desipramine when the concentration of SDS was 0.05 M and the amount of pentanol was increased to 6%. On the other hand, the efficiencies (*N*) obtained in the SDS–pentanol hybrid mobile phases, for the compounds studied, were in all cases around 3000. These values of efficiency for MLC were in accordance with the normal values reported by Berthod (1997). Finally, asymmetry factors were around 1.

As a consequence of these results, the mobile phase selected was 0.15 M SDS–6% (v/v) pentanol–0.001 M NaCl–phosphate buffer at pH 7. It should be noted that the selected mobile phase is in the corner of highest concentrations of the optimization space. Higher concentrations of SDS or pentanol increase the pressure of the chromatographic system and lead to microemulsion media.

Influence of the flow rate

As the analysis time in the optimum mobile phase was 20 min, we checked the possibility of decreasing this time by raising the flow-rate of the pump. Flows above 1.5 mL/min combined with the use of the recommended mobile phase produced high pressure inside the chromatographic system. When the flow was increased within the 1–1.5 mL/min range, the analysis time decreased linearly until 16 min, thus reducing the analysis time by 4 min. On the other hand, the other chromatographic parameters (*N* and *B/A*), as well as

the resolution, were almost similar. A flow-rate of 1.5 mL/min was finally selected as a compromise between minimum analysis time, maximum resolution and best chromatographic parameters.

Electrochemical detection

Optimization studies were performed using UV detection at 240 nm, but the two antidepressants, desipramine and imipramine, are electrochemically detectable, and this fact can increase the peak area, thus decreasing the limits of detection. In order to establish the optimum oxidation potential for the detection of the substances studied, the two antidepressants were chromatographed in SDS 0.15 M–6% (v/v) pentanol, pH 7 at a flow of 1.5 mL/min, and the potential applied was varied within the 100–1000 mV range, in steps of 50 mV. At each voltage, 10 injections of each substance were made and the peak area was measured. Potentials above 700 mV could not be used because a lack of reproducibility was observed. Yet, within the 100–700 range, the maximum area, with no changes in the other chromatographic parameters (t_R, N, B/A or R), occurred at 650 mV. This value was therefore the optimum oxidation potential.

Influence of the ED on the chromatographic parameters

Chromatographic parameters of desipramine and imipramine were obtained by employing the two detection systems: UV at 240 nm and ED at 650 mV, in the selected mobile phase of 0.15 M SDS–6% (v/v) pentanol, and 1.5 mL/min for the flow-rate. Retention time was unchanging, and N and B/A were 2400–1.12 and 2520–1.09 for imipramine, and 2200–1.21 and 2760–1.03 for desipramine, using UV and ED, respectively. According to these results, ED and UV can be used for monitorization of desipramine and imipramine in serum samples, but the higher sensitivity of ED makes it more suitable for physiological studies.

Method validation

Calibration curves. Calibration curves for desipramine and imipramine were constructed after triplicate injection of seven solutions of the drugs dissolved in SDS 0.15 M–6% (v/v) pentanol, pH 7 and in spiked serum samples. Concentrations varied between 50 and 1000 ng/mL. In both media, after chromatography, the peak area was measured and the regression parameters vs. concentration were calculated. The calibration parameters were statistically equal using the two matrices, i.e. micellar and serum solution, meaning that the serum does not cause a matrix effect or any kind of interference in the method. Table 2 shows the slopes and intercepts and, in addition, linear regression coefficients were always better than 0.995.

Limits of detection and quantification. Both values were obtained based on the standard deviation of the response and the slope, according to the *3 s* and *10 s criteria*. The limits of detection were calculated for both detection modes. As can be seen in Table 2, ED is five times more sensitive than UV detection. Furthermore, the limits of quantification (Table 2) results were suitable for the monitorization of imipramine and desipramine in serum samples. Finally, it can be noted that the limits of detection and quantitation obtained using UV were very low and below those reported in the literature using conventional HPLC with extraction steps.

Precision. Intra-day precision (the average of 10 determinations covering the specified range for the procedure made on the same day) and the intermediate precision (the average of intra-day values taken for 10 days over a 2 month period) were determined at three different drug concentrations within the therapeutic ranges of each drug (115–250 ng/mL for desipramine and 180–350 for imipramine). The relative standard deviations (RSD) were always below 2.2% (Table 3).

Analysis of imipramine and desipramine in real serum samples

Table 4 shows the satisfactory recoveries data (obtained when blank plasma samples, provided by the Analytical Service of the Hospital Verge dels Lliris d'Alcoi, were spiked with known amounts at three different concentrations within the therapeutic range for each antidepressant).

Furthermore, the analytical accuracy of the MLC method was confirmed by comparison with the HPLC

Table 2. Slope, intercept and limits of detection and quantification (ng/mL), for the calibration curves of desipramine and imipramine using the recommended MLC procedure

	Slope		Intercept		LOD		LOQ	
Compound	UV	ED	UV	ED	UV	ED	UV	ED
Imipramine	36.6	174.7	−6.86	1.4	1.6	0.34	14	3
Desipramine	43.3	224.4	−4.01	4.72	1.4	0.24	12	1

Table 3. Intraday and interday precision (RSD, %, $n = 5$) at three different concentrations (ng/mL): $c_1 = 115$, $c_2 = 175$ and $c_3 = 250$ for desipramine and $c_1 = 180$, $c_2 = 250$ and $c_3 = 350$ for imipramine eluted with 150 mM SDS–6% (v/v) pentanol, pH 7, 1.5 mL/min and electrochemical detection

	Intraday			Interday		
Compound	c_1	c_2	c_3	c_1	c_2	c_3
Imipramine	0.52	0.47	0.85	2.2	1.01	1.42
Desipramine	0.41	0.83	0.88	1.6	1.12	0.98

Table 4. Determination of imipramine ($c_1 = 180$, $c_2 = 250$, $c_3 = 350$ ng/mL) and desipramine ($c_1 = 115$, $c_2 = 175$, $c_3 = 250$ ng/mL) in serum samples ($n = 10$)

	Found ± RSD (%)		
Compound	c_1	c_2	c_3
Imipramine	175.1 ± 3.2	244.9 ± 2.6	352.5 ± 1.9
Desipramine	114.8 ± 0.9	174.7 ± 0.4	250.4 ± 0.2

reference method used at the Hospital. Both methods were applied to serum samples from patients treated withdesipramine ($n = 12$) and imipramine ($n = 15$). The correlation between the concentrations of the two antidepressants was good with the MLC vs. HPLC method: MLC = −0.011 + 0.963*HPLC ($r = 0.977$) for desipramine and MLC = 0.028 + 0.951*HPLC ($r = 0.969$) for imipramine. Figure 2 shows some of the chromatograms obtained in the determination of antiepileptics in patient serum samples using the MLC method. The high background signal of the matrix at the beginning of the chromatogram shown in Fig. 3, caused by the proteins and an endogenous compounds band, which can be observed using the MLC method

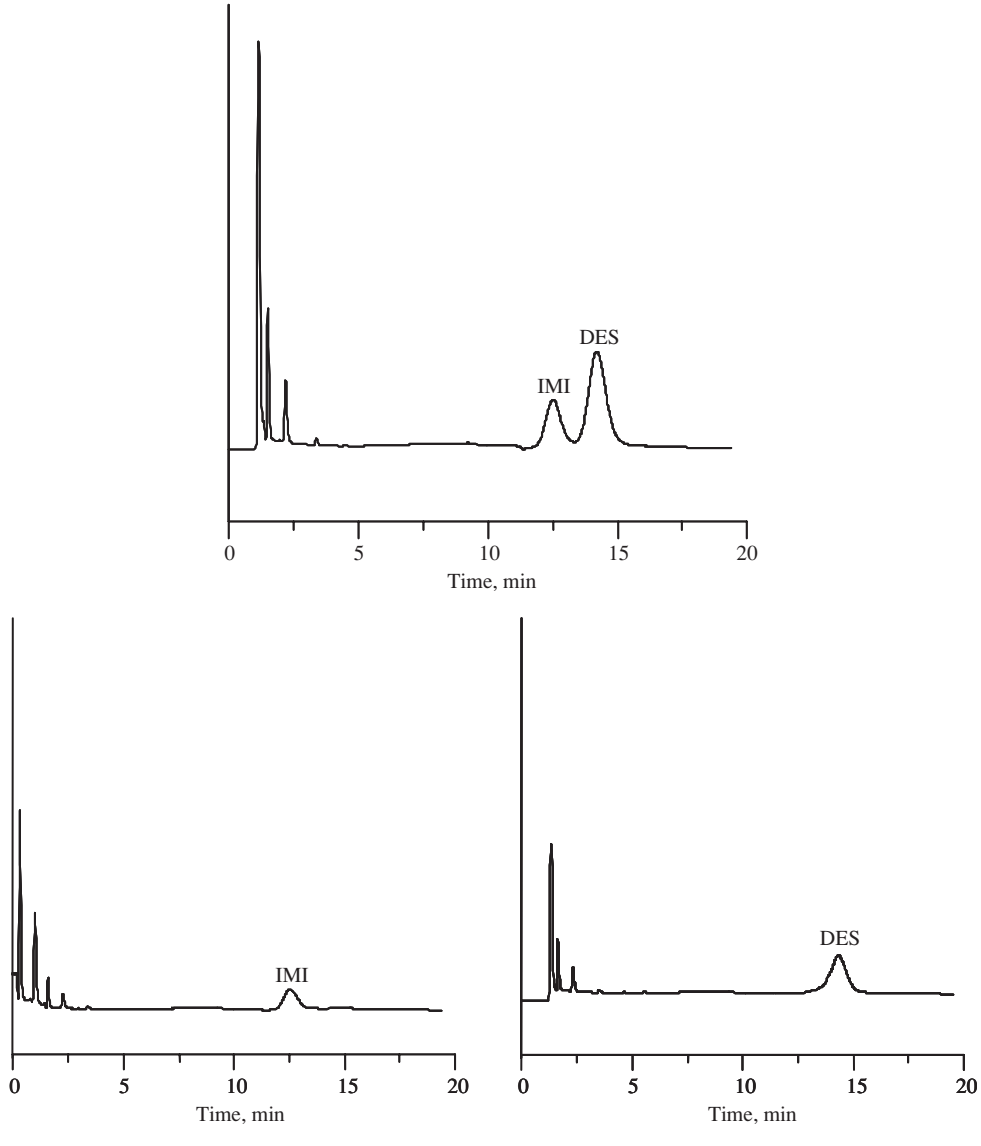

Figure 2. Chromatograms corresponding to real serum samples containing desipramine (A) and imipramine (B).

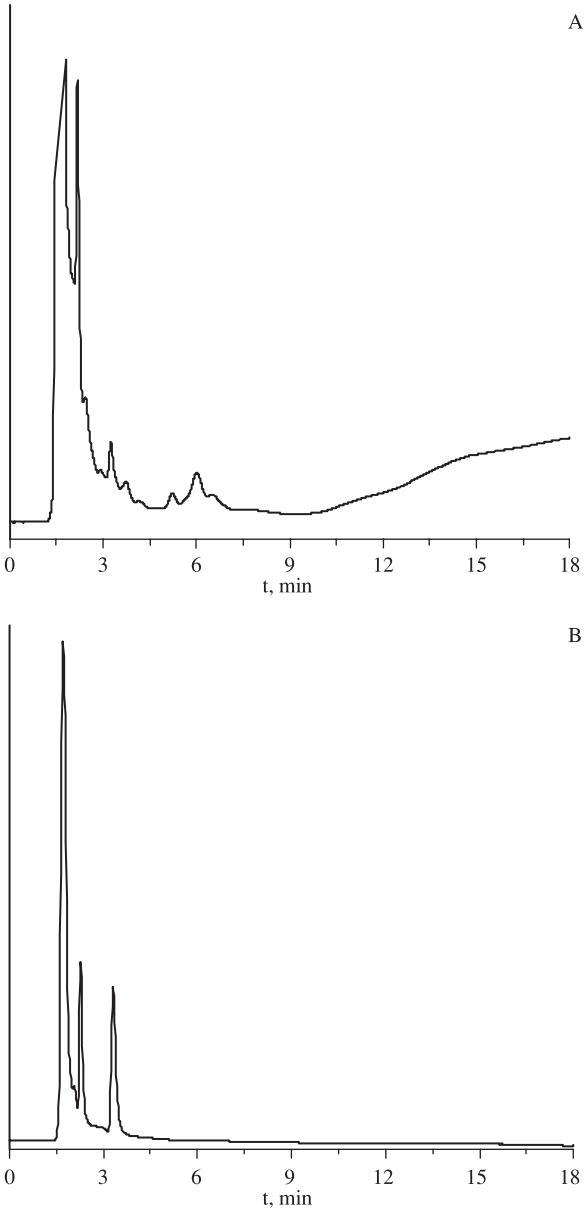

Figure 3. Chromatograms corresponding to the serum blank using UV at 240 nm (A) and ED at 650 mV (B).

with direct injection, is one of the limitations of the procedure for hydrophilic substances that are not retained for a longer time. In our case, this is not a problem because imipramine and desipramine are eluted far from this band. The protein band is less visible using ED than in UV detection.

Finally, the MLC method proposed here was applied to the determination of the biological half-life of the antidepressants in blood, after collecting the blood samples of healthy volunteers ($n = 5$) at various times following a single oral dose (50 mg) of desipramine or imipramine. The half-life obtained using the SDS–pentanol mobile phase was 22.1 ± 3.2 and 19.8 ± 2.9 min, for desipramine and imipramine, respectively.

CONCLUSIONS

Analytical methods for the determination of imipramine and desipramine in serum samples require extraction and preconcentration, which are two time- and reactant consuming steps. Using the MLC-ED method proposed here, the serum samples can be directly injected into the chromatographic system and easily determined, and the detection limits achieved improve those obtained with conventional HPLC reported in the literature. The sensitivity of the method allows the monitorization of antidepressants in serum and can be especially useful for physiological determinations.

Acknowledgements

This work was funded by Projects BQU2001-3770 (MCYT-FEDER) and P1B2003-07 (Fundació Caixa de Castelló-Bancaixa). Thanks are also due to the Conselleria d'Educació i Ciència de la Generalitat Valenciana for the fellowship offered to Dr D. Bose and Dr A. Durgbanshi. We are also grateful to the Hospital Verge dels Lliris d'Alcoi (Spain) for providing the serum samples.

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