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Stir bar sorptive extraction and liquid chromatography with UV detection for determination of antidepressants in plasma samples

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Abstract

A sensitive and reproducible stir bar sorptive extraction and liquid chromatography (SBSE/LC–UV) method is described for the determination of sertraline, mirtazapine, fluoxetine, citalopram, paroxetine, imipramine, nortriptyline, amitriptyne, and desipramine in plasma samples. Important factors in the optimization of SBSE efficiency are discussed, such as extraction time, pH, ionic strength, influence of plasma proteins, and desorption conditions: solvents, modes (magnetic stir, ultrasonic), time, and number of desorption steps. The SBSE/LC–UV method showed to be linear in a concentration ranging from the limit of quantification (LOQ) to 1000.0 ng mL^{-1}. The LOQ values ranged from 10.0 ng mL^{-1} to 40.0 ng mL^{-1}. The inter-day precision of the SBSE/LC–UV method presented coefficient of the variation lower than 15%. Based on figures of the merit results, the SBSE/LC–UV methodology showed to be adequate to the antidepressants analyses from therapeutic to toxic therapeutic levels. In order to evaluate the proposed method for clinical use, the SBSE/LC–UV method was applied to the analysis of plasma samples from elderly depressed patients.

Keywords: Stir bar sorptive extraction; Liquid chromatography; Antidepressants; Plasma samples

1. Introduction

The selective serotonin reuptake inhibitors (paroxetine, fluoxetine citalopram and sertraline), and an antagonist of central α_2-adrenergic autoreceptors (mirtazapine) are important classes of antidepressants usually used in psychiatry. They exhibit clinical efficacy comparable with classical tricyclic antidepressants but are devoid of some of the adverse anticholinergic and cardiovascular effects commonly associated with these drugs [1,2]. The structures of these antidepressants are shown in Fig. 1.

Depression is one of the most frequent of all major psychiatric illnesses. Clinically significant depressive symptoms are detectable in approximately 12–36% of geriatric patients with another nonpsychiatric general medical condition. The prevalence of major depression ranges from 10 to 27% in stroke patients, from 40 to 65% in victims of myocardial infarction, from 30 to 40% in patients with Alzheimer's disease, and from 20 to 25% in cancer patients. Because of the aging-related pharmacokinetic and pharmacodynamic changes, it is not possible to automatically extrapolate findings on the efficacy or tolerability of antidepressants from younger to older populations. In older patients, noncompliance and medication errors are disturbingly common. In clinical practice, the effort to determine an individual dose optimum for an antidepressant drug is often guided by a trial-and-error dose titration strategy. However, with the antidepressant drugs used in psychiatry, therapeutic drug monitoring is a long-established tool for finding the individual dose optimum and always increases efficacy and safety [3–7].

The analytical methods described in the literature to analyze antidepressants in biological fluids usually use conventional sample pretreatment techniques that is laborious, time-consuming, and require large amounts of organic solvents [6,7].

Solid-phase microextraction (SPME) has been successfully applied to analyze drugs in biological fluids by chromatography techniques, mainly by coupling to gas chromatography [8–13]. Most of the described methods showed low recoveries [14] that

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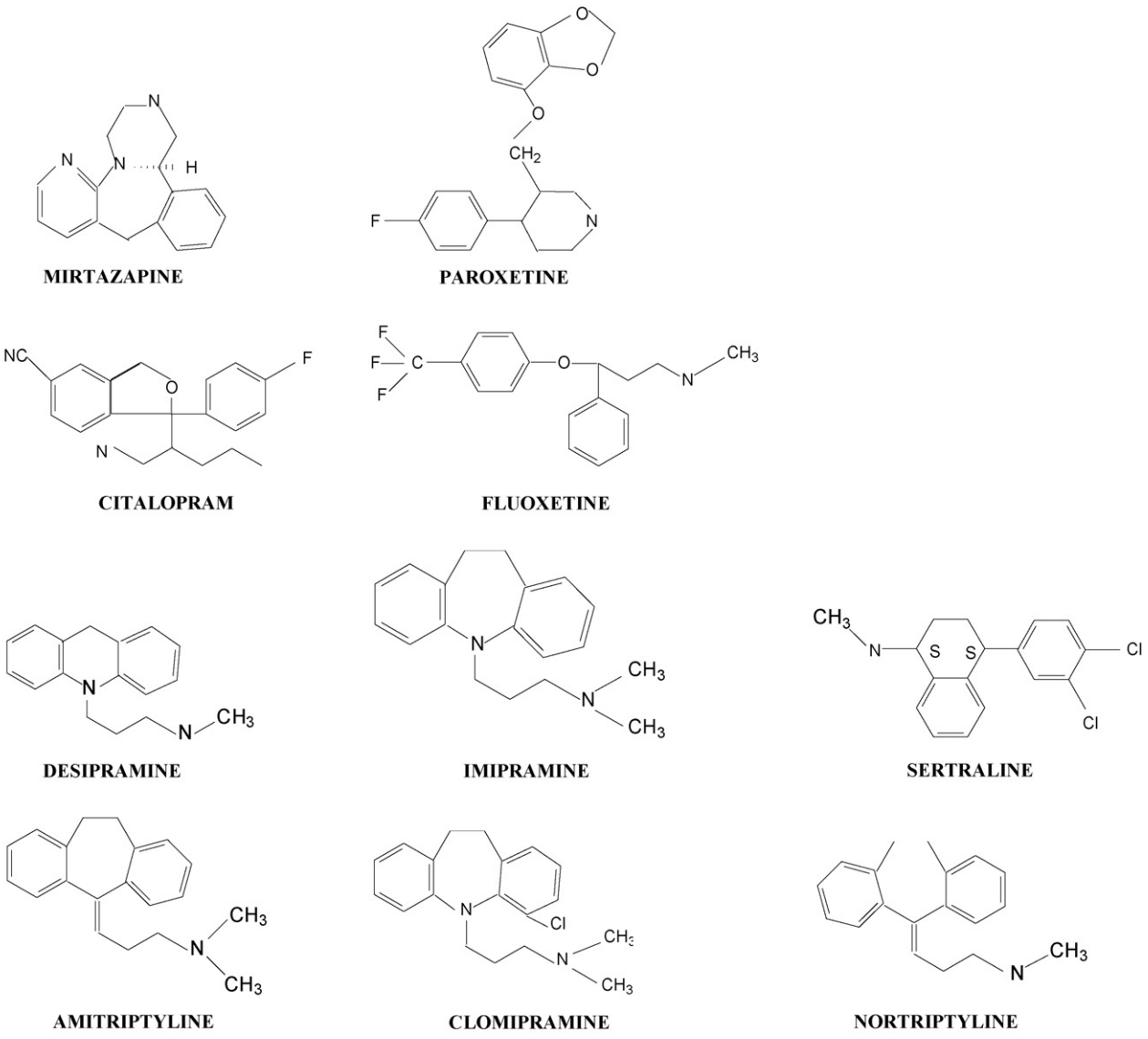

Fig. 1. Structure of selected antidepressants.

became laborious to develop methods to evaluate drugs in very low plasma or serum levels for therapeutic drug monitoring.

More recently, stir bar-sorptive extraction (SBSE) [15], a sample-preparation technique based on the same principles as SPME, partitioning coefficient of the solutes between the silicone phase and the aqueous phase, has been evaluated for the enrichment of organic solutes from biological fluids [16–25]. In SBSE, a stir bar coated with a polydimethylsiloxane (PDMS) layer is stirred for a given time in the sample solution. After this concentration step, analytes are thermally desorbed from the stir bar on-line with gas chromatography and provide a simple and very sensitive tool for analysis of the volatile and semi-volatile analytes. For polar analytes, the in situ derivatization can enhance recoveries into the PDMS layer and chromatographic analysis [17,18,20,22–25]. As an alternative, analysts can also use liquid desorption and liquid chromatographic analysis for high molecular mass drugs or thermolabile solutes. This procedure has not yet been much studied. In SBSE the amount of polydimethylsiloxane (PDMS) typically coated, 24–126 μL is substantially higher than on an SPME fiber, for which the maximum volume is usually 0.5 μL (100 μm film thickness). Consequently the sensitivity is increased by a factor of 50 and 250, reducing detection limits to sub-ng L^{-1} levels [14].

The aim of this study was to evaluate SBSE, followed by liquid desorption and LC–UV analysis, for the determination of mirtazapine, citalopram, paroxetine, desipramine, nortriptyline, amitriptyline, imipramine, fluoxetine and sertraline in plasma samples.

2. Experimental

2.1. Reagents and analytical standards

Fluoxetine (FLU) and paroxetine (PAR) analytical standards were kindly donated by Lilly (São Paulo, Brasil) e Libbs (São Paulo, Brasil), respectively. Citalopram (CIT), mirtazapine (MIR) and sertraline (SER) were donated by Roche (São Paulo, Brasil); nortriptyline (NOR), amitriptyline (AMI), desipramine

(DES) and imipramine (IMI) by Sandoz (São Paulo, Brasil), and clomipramine (CLO) (internal standard) by Pfizer (São Paulo, Brasil).

The working standard drug solutions, based on therapeutic interval concentrations, were prepared by diluting the stock solutions of these drugs (1 mg mL^{-1} in methanol) to a proper methanol volume. These solutions were stabile for 45 days, when the temperature was kept at −20 °C. The water used to prepare the mobile phase was previously purified in a Milli-Q system (Millipore, São Paulo, Brazil). Sodium chloride (analytical grade, Merck, Darmstadt, Germany) was used after purification by heating at 300 °C overnight. Methanol and acetonitrile in an HPLC grade were purchased from J.T. Backer (Phillipsburg, USA); monobasic and dibasic phosphate, sodium borate and sodium acetate were from Merck (Darmstadt, Germany).

2.2. Plasma samples

Plasma from patients not exposed to any drug for at least 72 h (blank plasma) was kindly supplied by *Hospital das Clínicas de Ribeirão Preto, Universidade de São Paulo*, Brazil. These plasma samples were used for both SBSE optimization, and analytical method validation. The plasma samples were colleted from geriatric patients subjected to therapy with antidepressants for at least 2 weeks. Blood samples were drawn 12 h after the last drug administration.

2.3. SBSE accessories

The commercial stir bar Twister for sorptive extraction was obtained from Gerstel (Gerstel GmbH, Mulheim an der Ruhr, Germany). It consists of a 10 mm length glass-encapsulated magnetic stir bar, externally coated with 22 μg of PDMS. This layer is 0.5 mm thick, which corresponds to a volume of 24 μL of PDMS. Prior to the first use, the stir bars were placed into a vial containing an acetonitrile:methanol solution (80:20, v/v) and conditioned for 24 h, under agitation. Among the successive extractions, the used stir bars were cleaned in methanol for 30 min at 50 °C, under magnetic stirring rate of 1200 rpm, followed by a drying step using a lint-free tissue.

2.4. Instrumentation

The LC system used was a Varian 230 ProStar (Varian, California, USA). Signals were monitored at 230 nm by a UV detector, Varian 310 ProStar. The separation was performed in RP 18 LichroCART® (125 mm × 4 mm × 5 μm particle size-Merck, Darmstadt, Germany) at room temperature (25 °C) with two different mobile phases: acetate buffer solution (0.25 mol L^{-1}, pH 4.5): acetonitrile:methanol (60:37:3, v/v/v) for mirtazapine, citalopram, paroxetine, nortriptyline, imipramine, fluoxetine, sertraline simultaneous analyses and acetate buffer solution (0.1 mol L^{-1}, pH 5.2): acetonitrile (60:40, v/v); for citalopram, desipramine, nortriptyline, imipramine, amitriptyline, sertraline simultaneous analyses in isocratic mode, at a flow-rate of 1.0 mL min^{-1}. The mobile phases were filtered and degassed, prior to use.

2.5. Optimization of the SBSE process

The influence of the pH matrix on antidepressant extractions was the first step evaluated. For that purpose, different pH values from 7.0 to 11.0 (buffer solutions, 0.05 mol L^{-1}) were investigated. In a glass vial (5 mL), sealed with a silicone septum, 50 μL internal standard (10.0 μg mL^{-1}, clomipramine) and 4.0 mL of buffer solution were added to 1.0 mL of the plasma sample spiked with the standard solutions that resulted in therapeutic levels. The vial was heated up to 50 °C on hotplate; the stir bar was then immersed into the sample, and the extraction was performed under magnetic stirring rate of 1200 rpm during 45 min.

The influence of ionic strength of the matrix solution (NaCl addition), extraction time (15, 30, 45 and 60 min) and temperature (38, 50, 60 and 70 °C) in the SBSE process were also investigated.

To evaluate the best desorption conditions: solvents (acetonitrile and mobile phase), modes (magnetic stir, ultrasonic), desorption time (5, 15, 30 and 60 min), number of desorption steps, and the control of the carryover were all individually evaluated. For the desorption, the stir bars were removed with clean tweezers, rinsed slightly with MilliQ water (1.0 mL), dried with lint-free tissue, and placed in a glass vial containing 1.0 mL of the solvent, ensuring the total immersion. Desorption was performed by ultrasonic treatment for 15 min at room temperature (25 °C) or by magnetic agitation for the same period at the same temperature. After the desorption process, the stir bars were removed by means of a magnetic rod and the solvent was evaporated until dryness. The dry residues were re-dissolved in 100 μL of the mobile phase, and 50 μL of this extract was injected in LC–UV system.

3. Results and discussion

3.1. Optimization of the SBSE variables

PDMS, homogeneous polymer coating, extract analytes *via* absorption, where the analytes dissolve in the coating and diffuse into the bulk of it during the extraction process. This process is non-competitive (in comparison to adsorption), and the amount of analyte extracted from a sample is independent of the matrix composition. This interaction is much weaker as adsorption on an active surface and the degradation of unstable analytes is significantly lesser compared to adsorption. Furthermore, the retaining capacity of the PDMS material is not influenced by other analytes because each analyte has its own partition equilibrium in the PDMS phase [26].

The SBSE variables, such as time, temperature, pH matrix, ionic strength, and desorption conditions, were optimized to reach drugs partition equilibrium in shorter analyses time, and to obtain adequate sensitivity to work in therapeutic interval. The sample volume, stirring speed, and stir bar dimensions were maintained constant during the optimization.

The sensitivity of the SBSE/LC–UV method was improved by diluting the samples with the borate buffer solution, to pH 9.0, in which the drugs (pK_a values from 8.7 to 10.2) were partially

or totally in the nonionic form that enabled them to be extracted by the PDMS phase (Fig. 2). The sample dilution favors the stirring SBSE process.

The addition of NaCl, increasing the ionic strength, reduced the amount extracted for some analytes; however for others, it did not alter the efficiency of the SBSE process. Probably the salt itself interacted with the drugs in solution through electrostatic, or pair ion-pairing interactions, thus reducing the ability of the drugs to move to the fiber coating (data not shown).

Fig. 3 shows representative time extraction profiles (15–60 min) in different temperature values (38–70 °C). We observed that an increase in extraction temperature from 38 to 50 °C results in an increased amount of the extracted drugs. This occurs because at lower temperature, extraction is further from equilibrium, and therefore, a low level of analyte is extracted. At higher temperature under the same extraction time, however, the absorption-time profile will be closer to equilibrium, and therefore, the amount extracted is generally greater. The results obtained at 50 °C and 60 °C were very similar for some drugs, and then a lowering of extraction level at 70 °C. As a result, the SBSE conditions: temperature at 50 °C and time extraction for 45 min were selected, although in this time the sorption equilibrium was not reached for few analytes.

Rinsing the stir bar slightly with 1.0 mL of the Milli-Q grade water to remove adsorbed proteins did not cause drugs loss because the sorbed drugs are present in the PDMS phase.

The conditions of the desorption were tested to ensure effective removal of the extracted analytes from the SBSE device. Acetonitrile showed the best results of the desorption solvent investigated (acetonitrile and the mobile phase). The time of the

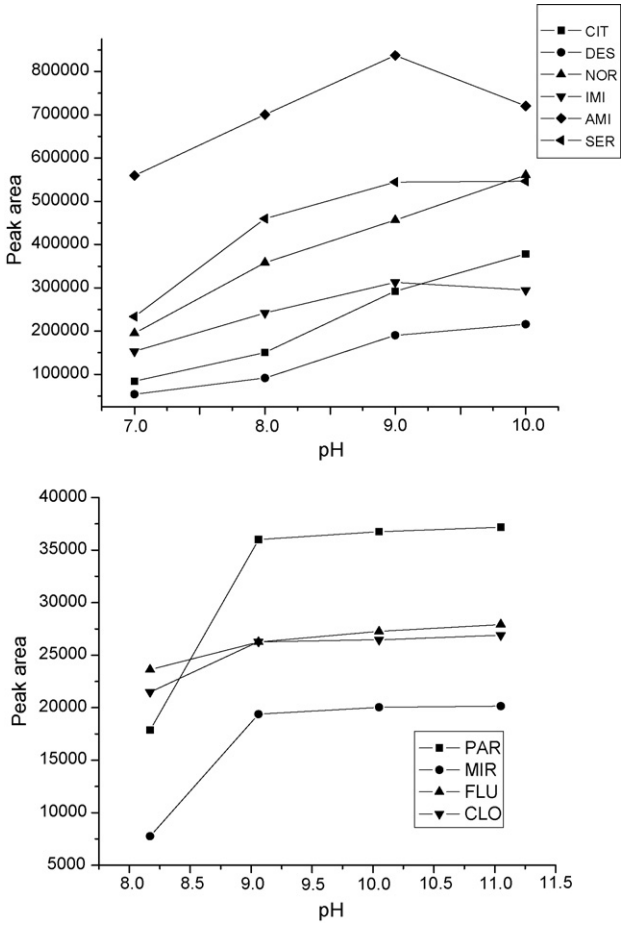

Fig. 2. Effect of the matrix pH on the SBSE efficiency of antidepressants in a plasma sample.

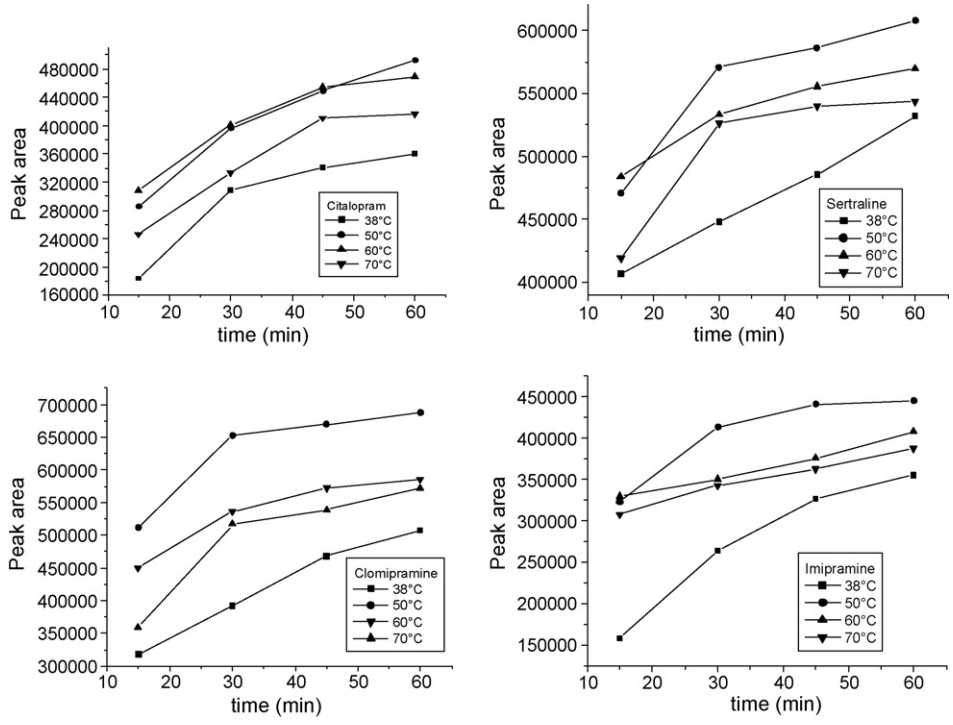

Fig. 3. SBSE time extraction profiles of antidepressants at different temperatures values.

Table 1
Linearity and limit of quantification (LOQ) of the proposed SBSE/LC method

Drugs	Linear regression (LOQ-1000 ng mL^{-1})	r^2	LOQ (ng mL^{-1})
Paroxetine	$y = -16693.58 + 1186.827 \times$	0.9973	40.0
Citalopram	$y = 42655.79 + 1390.24 \times$	0.9987	10.0
Mirtazapine	$y = 15432.48 + 1194.314 \times$	0.9953	40.0
Fluoxetine	$y = 6343.09 + 1091.106 \times$	0.9986	25.0
Sertraline	$y = 55986.42 + 411.72 \times$	0.9960	35.0
Imipramine	$y = 39466.7 + 792.67 \times$	0.9958	35.0
Amitriptyline	$y = 2089.3 + 8.9 \times$	0.9975	15.0
Nortriptyline	$y = 1958.92 + 8.2 \times$	0.9996	15.0
Desipramine	$y = 4886.56 + 7.69 \times$	0.9965	35.0

desorption was varied from 5 to 60 min (Fig. 4). It was found that the peak areas increased from 5 to 15 min desorption time, but remained nearly constant for desorption time of 15–60 min that corresponds to the complete desorption drugs from the SBSE bar (magnetic stirring), as no detectable carryover was observed. The magnetic stirring desorption ($T = 50\,^{\circ}C$), using acetonitrile was more effective than sonication performed in the same period (15 min, ambient temperature, $T = 25\,^{\circ}C$). Probably, the desorption process was favored at higher temperature. Therefore, thermal magnetic stirring was selected for desorption process.

Table 2
Inter-day precision (CV: coefficient of the variation) and recovery of the SBSE method

Drugs	Added concentration (ng mL^{-1})	CV (%) $n = 5$	Recovery (%) ($n = 5$)
Paroxetine	50.00	14.2	52
	300.00	12.1	60
	500.00	2.6	69
Citalopram	50.00	13.0	92
	200.00	3.5	84
	400.00	4.3	97
Mirtazapine	50.00	8.4	57
	300.00	4.9	86
	500.00	7.4	97
Fluoxetine	50.00	6.5	77
	300.00	13.2	79
	500.00	2.7	90
Sertraline	50.00	9.1	95
	200.00	4.9	103
	400.00	3.5	100
Imipramine	50.00	12.1	98
	200.00	7.3	105
	400.00	7.8	100
Amitriptiline	50.00	8.85	91
	200.00	6.13	100
	400.00	9.43	110
Notripiline	50.00	7.91	107
	200.00	7.30	98
	400.00	3.14	107
Desipramine	50.00	9.38	100
	200.00	4.70	83
	400.00	3.02	100

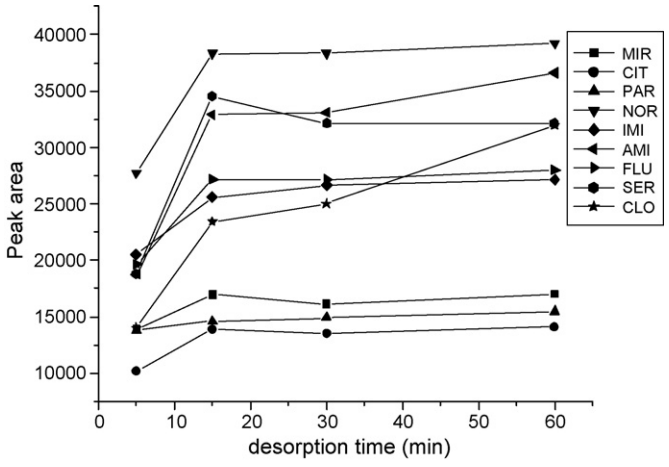

Fig. 4. SBSE desorption time profile of antidepressants in plasma samples.

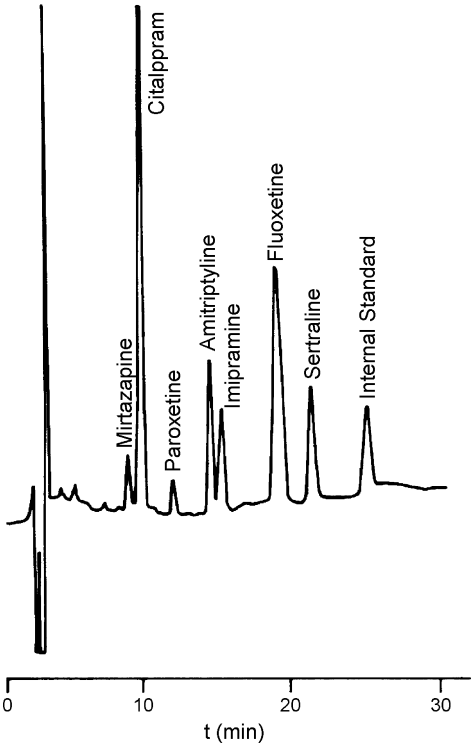

Fig. 5. SBSE-LC chromatograms using acetate buffer solution (0.25 mol L^{-1}, pH 4.5): acetonitrile:methanol (60:37:3, v/v/v) as mobile phase. Blank plasma sample spiked with antidepressants at 500.0 ng mL^{-1}.

The efficiency of desorption process (magnetic stirring) was also confirmed by performance of two consecutive acetonitrile desorptions, in which a unique step provides the maximum yield. Furthermore, no evidence of interference was found during blank assays, and the PDMS phase of the stir bars was very highly stable, with no evidence of deterioration. Lambert et al. [21] observed some degradation of the restricted access material stir bar coating, after 30 desorption cycles, using sonication process [21].

Although it is possible to re-use stir bars without additional clean up, a cleaning procedure was carried out using methanol for 30 min at 50 °C, under magnetic stirring rate of 1200 rpm, between extractions, to assure efficient protein removal. The robustness of the stir bar was confirmed with over 50 extractions with a minimum loss of extraction efficiency.

Based upon this data, we concluded that the best SBSE experimental conditions, among those investigated for the antidepressants assays (Figs. 2–4), were as follows: 1.0 mL of plasma sample modified with 4 mL borate buffer (pH 9.0), extraction temperature at 50 °C, under magnetic stirring during 45 min, followed by the drugs off-line liquid desorption by immersion of the PDMS bar on acetonitrile at 50 °C, under magnetic stirring during 15 min.

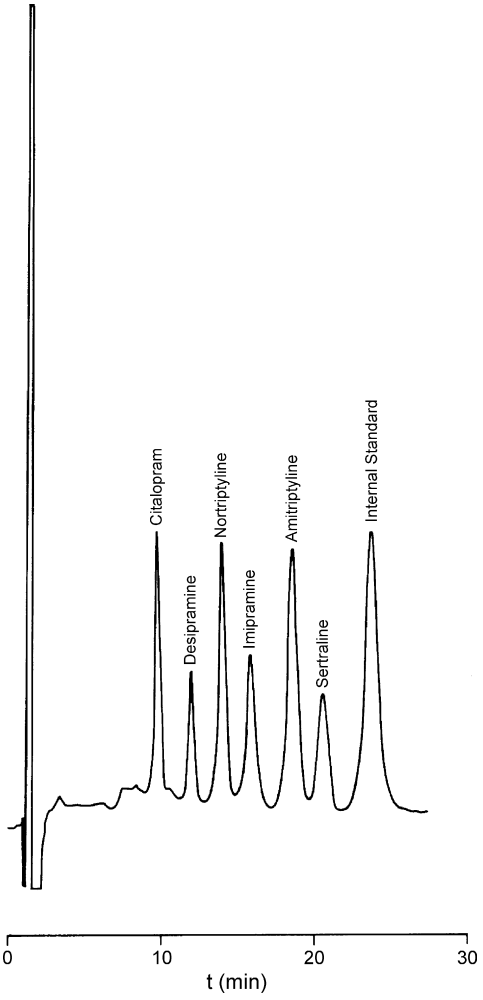

Fig. 6. SBSE-LC chromatograms using acetate buffer solution (0.1 mol L^{-1}, pH 5.2): acetonitrile (60:40, v/v) as mobile phase. Blank plasma sample spiked with antidepressants at 500.0 ng mL^{-1}.

Table 3
Retention time of the drugs studied as possible interferents

Drugs	Retention time (min)
Cafeine	2.93
Metoprolol	4.05
Moclobemide	4.83
Etidocaine	7.78
Carbamazepine	8.41
Sulfamethazaxol	8.43
Propanolol	8.60
Mirtazapine	**8.90**
Lorazepam	9.75
Citalopram	**10.05**
Paroxetine	**12.00**
Despramine	**12.38**
Amitryptiline	**14.38**
Nortryptiline	**15.26**
Imipramine	**15.43**
Diazepam	15.27
Duloxetine	16.21
Diclofenac	17.78
Fluoxetine	**19.02**
Sertraline	**21.50**
Levomepromazine	22.09
Clomipramine	**25.55**
Haloperidol	nd[a]
Indometacin	nd
Metildopa	nd
Amiodarone	nd
Clonazepam	nd
Fenobarbital	nd
Primidone	nd
Indomethacin	nd
Furosemide	nd
Cimetidine	nd
Ranitidine	nd

Mobile phase: acetate buffer solution (0.25 mol L^{-1}, pH 4.5): acetonitrile:methanol (60:37:3, v/v/v).
[a] Not detected in this extraction conditions.

The internal standard selected (clomipramine) is closely related to the analytes of interest (Figs. 2–4). In cases were the internal standard is extracted to a significantly different extent than the analyte, error in the analysis will be either under- or over-stated.

3.2. Figures of merit

The linearity of the SBSE/LC method was determined with plasma samples spiked with analytical standards that result in a concentration ranging from the limit of quantification (LOQ) up to 1000.0 ng mL^{-1}. The regression equations and corresponding correlation coefficients for all drugs are given in Table 1. The LOQ values were determined as the lowest concentration on the calibration curve in which the coefficient of the variation was lower than 15% (Tables 1 and 2) and based on a signal-to-noise ratio about 10.

The average recovery and inter-day precision of the SBSE method were assessed by replicate analysis ($n = 5$) of plasma

samples spiked with standards in three different concentrations (Table 2). The recoveries were calculated by comparing the UV-peak areas of the spiked samples with the direct injection of standard solutions of equal concentrations.

In accordance with the literature [20,24,26], we observed that recovery appeared not to be influenced by the type of liquid sample analyzed, plasma or water, for most of the evaluated drugs.

The specificity (selectivity) of the developed method is demonstrated by representative chromatograms from drug-free human plasma sample spiked with antidepressants in therapeutic interval concentration (Figs. 5 and 6), which showed the ability of the method to measure unequivocally the drugs in the presence of endogenous plasma components. The drug-free human plasma from several individuals were tested and showed no significant interference at the retention times of the analytes.

Antidepressants may be prescribed in combination with different psychotropic agents and other drugs [27], so it was important to assess probable interferences from potentially co-administered compounds (Table 3). Among those drugs tested, desipramine co-eluted with paroxetine, but this type of combination therapy is extremely unlikely to be encountered in clinical practice [27]. Diazepam also co-eluted with imipramine in our chromatographic conditions. Some suitable modifications in the mobile phase can be made to overcome this problem, such as acetonitrile–methanol (52:8, v/v) and 0.25 mol L^{-1} sodium acetate buffer, pH 4.5 (35:65, v/v), at a flow-rate of 1.0 mL/min.

4. Clinical application of the method

In order to evaluate the proposed method for clinical use, the described protocol was applied to the analysis of plasma samples from elderly depressed patients (Fig. 7). Peak shapes and resolution are very similar to those obtained using spiked blank plasma, and no interference is apparent.

Drug concentrations found in these samples were: 191.0 ng mL^{-1} for fluoxetine (Fig. 7a), 43.8 ng mL^{-1} for paroxetine (Fig. 7b) and 225.2 ng mL^{-1} for sertraline (Fig. 7c). The

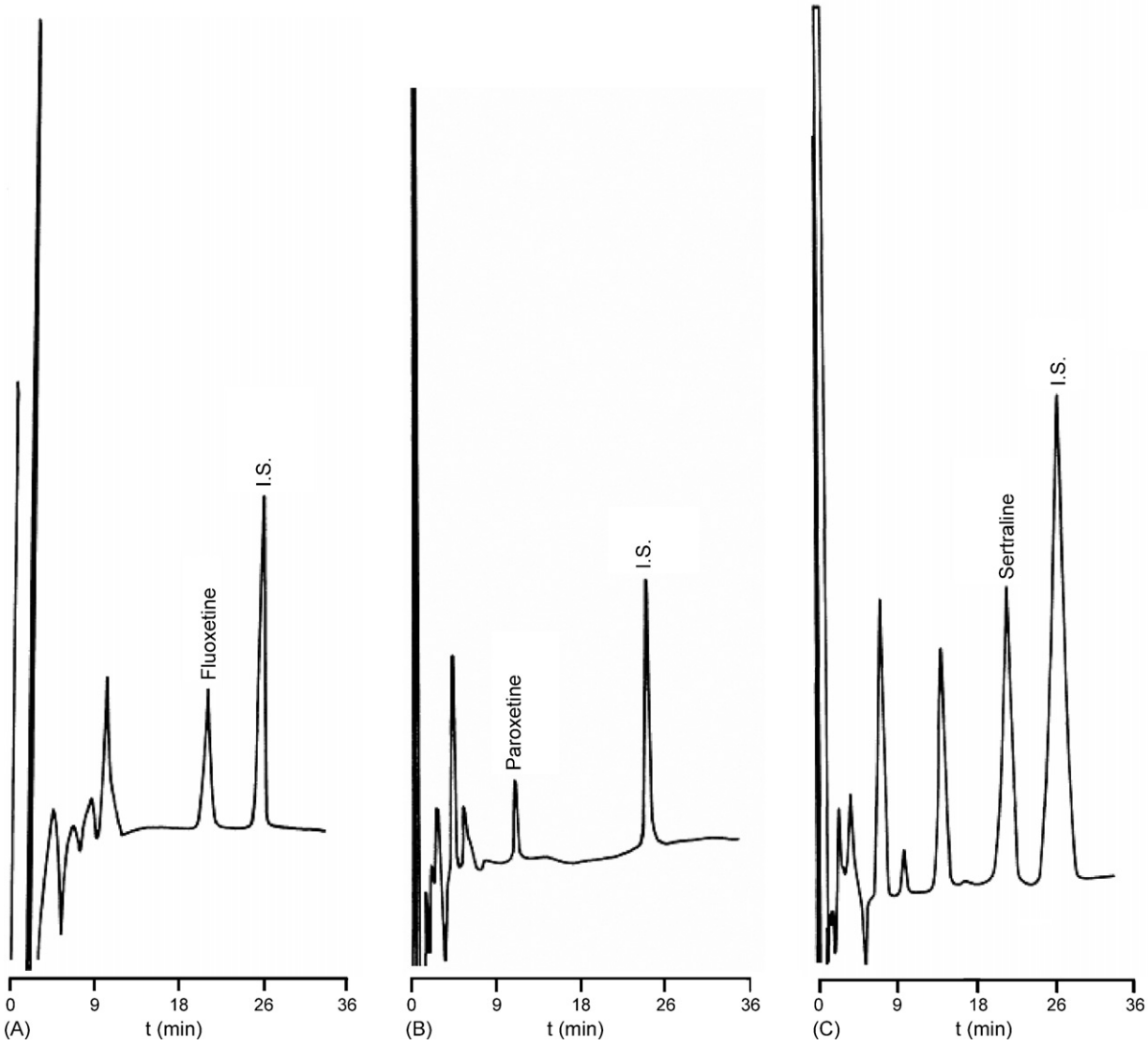

Fig. 7. SBSE/LC analysis of plasma samples from elderly depressed patients receiving therapeutic dosages.

plasma samples were colleted from elderly depressed patients in therapy with Prozac® (40 mg/day), Aropax® (40 mg/day) and Zoloft® (150 mg/day). These patients showed to be inside of therapeutic levels [28].

5. Conclusion

It was demonstrated that a novel SBSE/LC methodology was developed, presenting high sensitivity and enough reproducibility to permit the quantification of tricyclic and nontricyclic antidepressants in human plasma after oral administration of the antidepressants. Thus, the proposed SBSE/LC method can be an useful tool to determine antidepressants in plasma samples from patients receiving therapeutic dosages. The method may be also applied to evaluate plasma levels in urgent toxicological analyses after the accidental or suicidal intake of higher doses.

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