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In-tube solid-phase microextraction coupled to liquid chromatography (in-tube SPME/LC) analysis of nontricyclic antidepressants in human plasma

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Abstract

A sensitive, selective, and reproducible in-tube solid-phase microextraction and liquid chromatographic (in-tube SPME/LC-UV) method for simultaneous determination of mirtazapine, citalopram, paroxetine, duloxetine, fluoxetine, and sertraline in human plasma was developed, validated and further applied to the analysis of plasma samples from elderly patients undergoing therapy with antidepressants. Important factors in the optimization of in-tube SPME efficiency are discussed, including the sample draw/eject volume, draw/eject cycle number, draw/eject flow-rate, sample pH, and influence of plasma proteins. The quantification limits of the in-tube SPME/LC method varied between 20 and 50 ng/mL, with a coefficient of variation lower than 10%. The response of the in-tube SPME/LC method for most of the drugs was linear over a dynamic range from 50 to 500 ng/mL, with correlation coefficients higher than 0.9985. The in-tube SPME/LC can be successfully used to analyze plasma samples from ageing patients undergoing therapy with nontricyclic antidepressants.

Keywords: In-tube solid-phase microextraction; Plasma samples; Nontricyclic antidepressants

1. Introduction

Important groups of new antidepressants include the selective serotonin reuptake inhibitors (SSRIs) (fluoxetine, sertraline, paroxetine, and citalopram), the noradrenergic and specific serotonergic antidepressants (mirtazapine), and the serotonin–noradrenaline reuptake inhibitors (duloxetine). SSRIs block the reuptake of serotonin at central synapses selectively and powerfully. Mirtazapine is the only antidepressant that increases noradrenergic and serotonergic neurotransmission through a blockade of central α2-adrenergic auto- and hetero-receptors. Duloxetine is a dual inhibitor of norepinephrine and serotonin uptake; it is a less potent inhibitor of dopamine reuptake [1,2]. The chemical structures of these antidepressants are shown in Fig. 1.

Depression is diagnosed on the basis of abnormal positive effects (anhedonia) and negative effects (low mood, helplessness, coping deficit, fatigue), and associated physiological abnormalities include hyperactivity of the hypothalamic–pituitary–adrenal (HPA) endocrine system and autonomic nervous system. Because of the age-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 such cases, the risk of overdose and adverse effects should be considered, and a laboratory measurement of plasma levels becomes mandatory.

In-tube solid-phase microextraction (in-tube SPME), an effective sample preparation technique, has been successfully applied to the analysis of drugs in biological fluids. In-tube SPME uses an open tubular fused-silica capillary column as an extraction device. Organic compounds in aqueous samples are directly extracted and concentrated into the stationary phase of the capillary column by repeated draw/eject cycles of the sample solution, and they can be directly transferred

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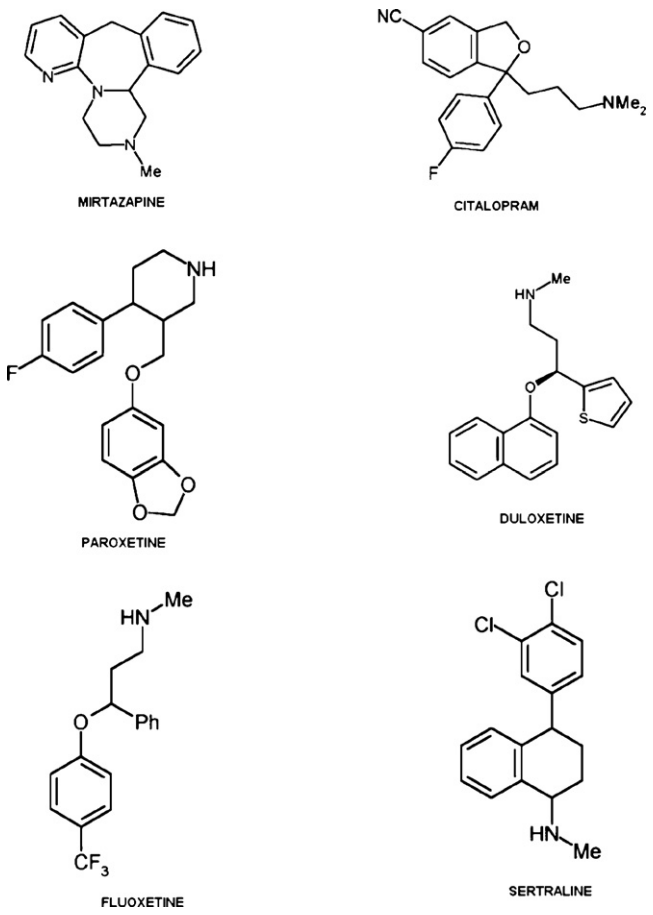

Fig. 1. Chemical structure of selected antidepressants.

to the liquid chromatographic column. In-tube SPME is an ideal sample preparation technique because it is fast to operate, easy to automate, solvent-free, and inexpensive. In-tube SPME performs continuous extraction, concentration, desorption, and injection using an autosampler, which is usually employed in combination with high performance liquid chromatography and liquid chromatography–mass spectrometry [3].

Commercial GC columns such as Omegawax 250 [4], HM/DB-5 (zylon fiber packed in a DB-5 capillary, 5% phenyl-polydimethyl siloxane) [5], polypyrrole [6], poly (methacrylic acid-ethylene glycol dimethacrylate) monolithic [7], and β-cyclodextrin capillaries [8] have been applied to the in-tube SPME determination of drugs in biological fluids.

In this study, the in-tube SPME/LC method was developed and validated for the simultaneous analysis of mirtazapine, citalopram, paroxetine, duloxetine, fluoxetine, and sertraline in human plasma. The in-tube SPME/LC technique was used in the analysis of plasma samples from ageing patients undergoing therapy with nontricyclic antidepressants.

2. Experimental

2.1. Chemical and materials

Fluoxetine and duloxetine analytical standards were donated by Lilly (São Paulo, Brazil); paroxetine by Libbs (São Paulo, Brazil); clomipramine (internal standard) by Pfizer (São Paulo, Brazil); citalopram, mirtazapine, and sertraline by Roche (São Paulo, Brazil).

The working standard drug solutions, based on interval concentrations, were prepared by diluting the stock solutions of these drugs (1 mg/mL in methanol) to a proper methanol volume. These solutions were stable for 45 days, and the temperature was kept at −20 °C during this period. The water used to prepare the mobile phase was previously purified in a Milli-Q system (18 MΩ) (Millipore, São Paulo, Brazil). Methanol and acetonitrile, both HPLC grade, hydrochloric acid and acetic acid were purchased from J.T. Baker (Phillipsburg, USA); monobasic and dibasic phosphate, sodium borate, and sodium acetate were purchased from Merck (Darmstadt, Germany).

2.2. Instrument and analytical conditions

The in-tube SPME/LC-UV system consisted of a pre-extraction segment, and LC-UV analyses, which included a Pro Star Varian (model 230, CA, USA) liquid chromatograph with a Varian autosampler (model 430, CA, USA). Signals were monitored by a diode-array detector (Varian, CA, USA, model 330) and UV detector (Varian, CA, USA, model 310) set at 230 nm (the majority of antidepressants presented the absorbance maximum at this value).

The chromatographic separations were performed using a LiChrospher®60 RP-select B (C_{18}) column (5 μm, 250 mm × 4 mm, Merck) at room temperature (25 °C) with a mobile phase consisting of phosphate buffer solution (0.05 mol/L, pH 3.8)/acetonitrile 53:47 (v/v) in the isocratic mode, at a flow-rate of 1.0 mL/min. The mobile phase was filtered and degassed prior to use.

2.3. Plasma sample

Plasma from healthy volunteers not subjected to pharmacological treatment for at least 72 h (blank plasma) was supplied by the *Hospital das Clínicas de Ribeirão Preto*, University of São Paulo, Brazil. This plasma was used for the in-tube SPME/LC method validation. The principles embodied in the Helsinki Declaration were observed, and the study was approved by the Ethics Committee of the University of São Paulo in Ribeirão Preto, Brazil. The plasma samples were collected from elderly patients subjected to therapy with nontricyclic antidepressants for at least 2 weeks. Blood samples were drawn 12 h after the last drug administration.

2.4. Capillary extraction column preparation

Fused-silica capillary tubing used to prepare extraction columns were purchased from Polymicro Technologies (Phoenix, USA). The capillary (80 cm × 250 μm I.D.) were first flushed with high-purity nitrogen, followed by a washing step with 10 mL of dichloromethane under N_2 pressure (50 psi). The capillary was purged with dry nitrogen for 10 min at a slow flow-rate and coated with the OV-1701 (14% cyanopropylphenyl methylpolysiloxane) solution using the static coating method.

2.5. In-tube SPME optimization

The fused-silica capillary (80 cm × 250 μm I.D.) with a surface area of 6.3 cm^2 and coated with the OV-1701 phase was fixed in the injection loop LC autosampler place. The capillary connections were facilitated by a MicroTight sleeves at each end of the capillary.

In a glass vial (1.5 mL, Sun Sri, USA) sealed with a screw cap containing a silicone septum, 50 μL internal standard (10.0 μg/mL clomipramine) and 0.5 mL buffer solution were added to 0.5 mL of the aqueous sample, which was spiked with standard solutions of the drugs, resulting in a concentration level of 500 ng/mL. Prior to the extractions, the OV-1701 capillary was washed with methanol/water solution (50:50 v/v). The samples were vortexed for 10 s before extraction. The vials were then set on the autosampler to optimize the following in-tube SPME conditions: draw/eject volume, from 40 μL (capillary volume) to 250 μL (syringe capacity); pH of the buffer solutions (4.5, 7.0, 9.0, and 10.0); flow-rate draw/eject (125, 315, and 625 μL/min), draw/eject cycles (1, 5, 10, 15, 20, 25, 30, and 35), waiting time between draw and eject procedures (30, 60, and 120 s). The optimization process was carried out in aqueous solution, in order to prevent protein plasma irreversible adsorption on the capillary.

Extraction of each sample was possible by repeatedly aspirating (draw) and dispensing (eject) the sample through the capillary. After the draw/eject cycles, the capillary was washed with water, to prevent the capillary contamination with serum proteins.

Desorption of the extracted analyte was then possible by redirecting the appropriate mobile phase through the OV-1701 capillary, switching the six port injection valve from the load to the inject position for transport to the analytical column.

2.6. Preparation of plasma samples

The proteins of the plasma samples were precipitated before the in-tube SPME, to prevent clogging of the capillary column and flow lines during extraction. Two solvents were evaluated: acetonitrile (plasma:acetonitrile 1:2 v/v), and acetic acid 1% (plasma:acetic acid 1:4 v/v). The solvent was added to 500 μL of plasma in a 1.5 mL test tube. The samples were immediately vortexed for 3 min and centrifuged at 3000 rpm for 20 min. The supernatant layer was treated before the in-tube SPME analysis. The pH of the acetic acid supernatant was adjusted for neutrality with basic solution, and the acetonitrile supernatant dry residue was re-dissolved in buffer solution. The extracts were used for in-tube SPME analysis, following the procedure described for aqueous samples.

2.7. Analytical validation

The analytical validation of the in-tube SPME/LC method was carried out with blank plasma samples spiked with drugs standard solutions with concentrations that include the therapeutic plasma levels. The linearity was evaluated by calibration curves constructed using linear regression of the drug/internal standard peak area ratio (Y) versus drug nominal plasma concentration (X, ng/mL). These sample concentrations ranged from LOQ to 500 ng/mL.

Accuracy, intra-day, and inter-day precision values were determined by calibration curves by quintuplicate in-tube SPME/LC assays of the blank plasma samples spiked with analytes at LOQ, 200, 300, and 500 ng/mL.

Recovery values were calculated by comparison of the peak areas of the drugs extracted from the plasma with that of the same concentration of the drugs in standard solutions. Furthermore, co-elution was also investigated by comparison with other drugs, and endogenous compounds retention times were compared with those of the analytes (antidepressants).

3. Results and discussion

3.1. Optimization of in-tube SPME process

The sample draw/eject volume was evaluated from values ranging between the capillary volume (~40 μL) and the autosampler injector syringe volume (250 μL). Volumes lower than the capacity and higher than the maximum volume of the syringe were impracticable. In general, the extraction efficiency increased with higher volumes (Fig. 2), so 100 μL was selected as the optimum draw/eject volume, where the equilibrium partition was achieved for the majority of the analytes. Sample volumes higher than this value, did not intervene with the extraction efficiency.

Adjustment of the sample pH can improve the sensitivity of the method for basic and acidic analytes, since the OV-1701 capillary can extract only nonionic species from plasma samples [9,10]. Moreover, the interference of the protein plasma in SPME process was minimized by adopting the buffer solution dilution procedure. This procedure decreased the matrix viscosity and increased the diffusion coefficients. Thus, the sensitivity

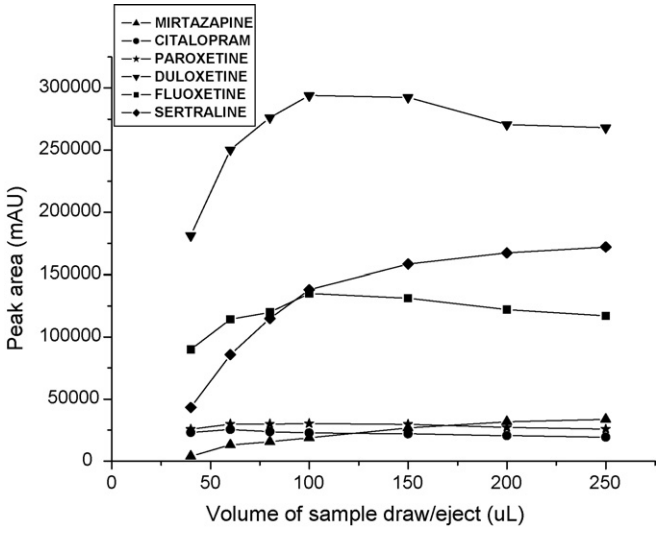

Fig. 2. Evaluation of the draw/eject volume in-tube SPME efficiency. In-tube SPME conditions: aqueous sample (0.5 mL) spiked with antidepressants (500 ng/mL), diluted with 0.5 mL of borate buffer solution (0.05 mol/L, pH 9.0), with 25 draw/eject cycles, and flow-rate of 315 μL/min.

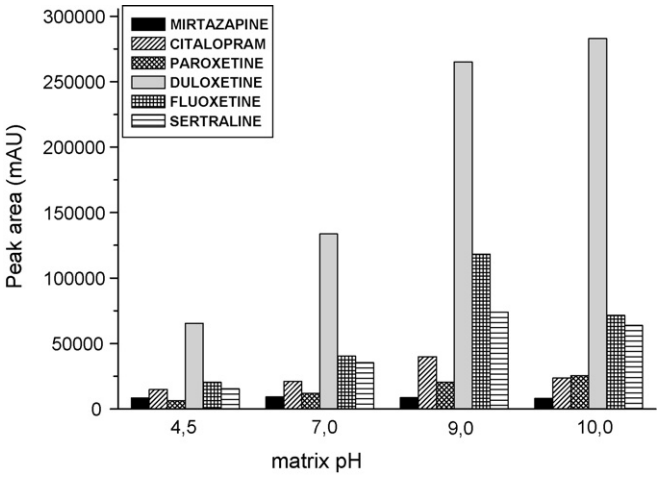

Fig. 3. Effect of the pH on in-tube SPME efficiency. In-tube SPME conditions: aqueous sample (0.5 mL) spiked with antidepressants (500 ng/mL), diluted with 0.5 mL of buffer solution. Draw/eject volume: 100 μL, draw/eject cycles: 25, and draw/eject flow-rate: 315 μL/min.

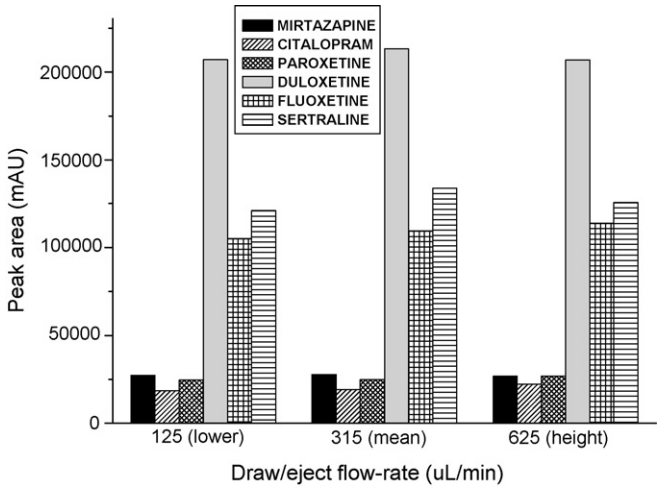

Fig. 5. Effect of the flow-rate draw/eject on the in-tube SPME efficiency. In-tube SPME conditions: aqueous sample (0.5 mL) spiked with antidepressants (500 ng/mL), diluted with 0.5 mL of borate buffer solution (0.05 mol/L, pH 9.0). Draw/eject volume: 100 μL and draw/eject cycles: 15.

of the in-tube SPME/LC method was significantly improved by diluting the samples with the borate buffer solution pH 9.0, in which drugs (pK_a values from 7.1 to 9.9) were partially, or totally, in the nonionic form (Fig. 3).

Organic compounds in aqueous samples are directly extracted and concentrated into the stationary phase of the capillary columns by repeated draw/eject cycles of the sample solution. However, increments in draw/eject cycles should result in partial analyte desorption [4] during the eject step. The partition equilibrium was reached after 15 draw/eject cycles (Fig. 4). After this value, the increments in the draw/eject cycles did not significantly increase the sensitivity of the method.

Fan et al. [7,8] observed increment in the in-tube SPME analytical sensitivity at low flow-rates (≤100 μL/min). On the other hand, Kataoka [3] described that the draw/eject flow-rate corresponds to the agitation speed of the SPME fiber, and the extraction efficiency increases with speed. In accordance with Fig. 5, no significant variations in the method sensitivity were observed among the evaluated flow-rates. The partition equilibrium was probably reached at 125 μL/min (low autosampler injector flow-rate). The selected flow-rate draw/eject was 315 μL/min. Below this value, extraction required an inconvenient long time and, above this level, bubbles formed inside the capillary.

The waiting time (30, 60, and 120 s) between the draw and eject procedures, was evaluated in order to increase in-tube SPME sensitivity and decrease the number of draw/eject cycles. However, even when the sample was kept in contact with the OV-1701 extraction phase for a longer time, enhancement in method sensitivity was not observed (Fig. 6).

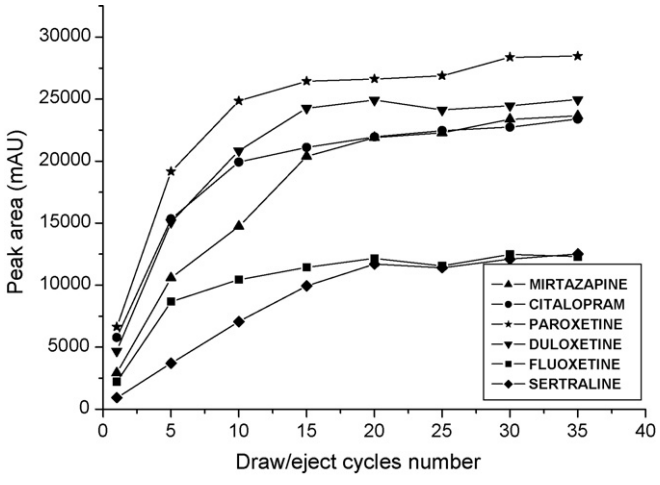

Fig. 4. Effect of the draw/eject cycles on in-tube SPME efficiency. In-tube SPME conditions: aqueous sample (0.5 mL) spiked with antidepressants (500 ng/mL), diluted with 0.5 mL of borate buffer solution (0.05 mol/L, pH 9.0). Draw/eject volume: 100 μL and draw/eject flow-rate: 315 μL/min.

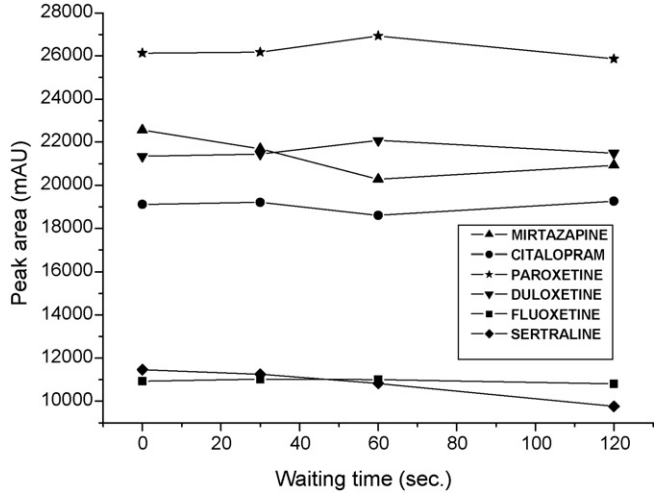

Fig. 6. Effect of the waiting time on the in-tube SPME efficiency. In-tube SPME conditions: aqueous sample (0.5 mL) spiked with antidepressants (500 ng/mL), diluted with 0.5 mL of borate buffer solution (0.05 mol/L, pH 9.0). Draw/eject volume: 100 μL, draw/eject cycles: 15, and draw/eject flow-rate: 315 μL/min.

3.2. Plasma sample preparation

Analysis of blank plasma samples by in-tube SPME/LC was carried out to evaluate the efficiency of protein precipitation for both acetic acid 1% and acetonitrile procedures. The acetonitrile procedure was more efficient than the acetic acid 1% procedure; endogenous compound peaks were less intense, and they were not co-eluted with drugs. Therefore, protein precipitation with acetonitrile was selected for subsequent analyses. During the precipitation process, the analytes did not precipitate together with the endogenous compounds.

3.3. Analytical validation

The use of internal standards for quantification is done routinely in the case of many methods, and this can give satisfactory results for microextraction as well. Clomipramine, the selected internal standard, is closely related to the analytes of interest, particularly in terms of partition coefficient for the extraction phase. If the internal standard is extracted with extent significantly different from that of the analyte, analysis error will be either under- or over-stated [4].Based on previously published methods [11,12], the analytes in the plasma samples were stable in the conditions that they were determined.

The specificity (selectivity) of the in-tube SPME/LC method is demonstrated by representative chromatograms of a drug-free plasma sample (blank plasma) (Fig. 7), blank plasma sample spiked with antidepressants, resulting in 500 ng/mL (Fig. 8), and plasma samples obtained from elderly patients subjected to therapy with nontricyclic antidepressants (Fig. 9). Additional drug-free human plasma from several individuals were tested and showed no significant interference with the analyte retention times. This gives evidence of the ability of the method to unequivocally measure the drugs in the presence of endogenous plasma components. Antidepressants may be prescribed in combination with different psychotropic agents and other drugs [13], so it is important to assess probable interferences from potentially co-administered compounds (Table 1). No drugs co-elution with the retention times of the analytes was observed.

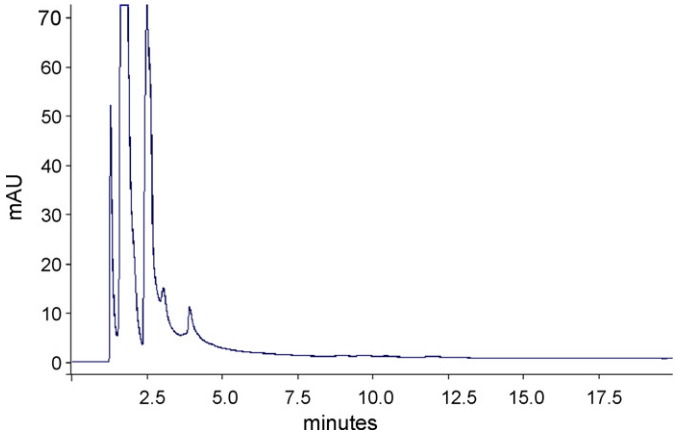

Fig. 7. In-tube SPME/LC chromatogram of a representative blank plasma sample.

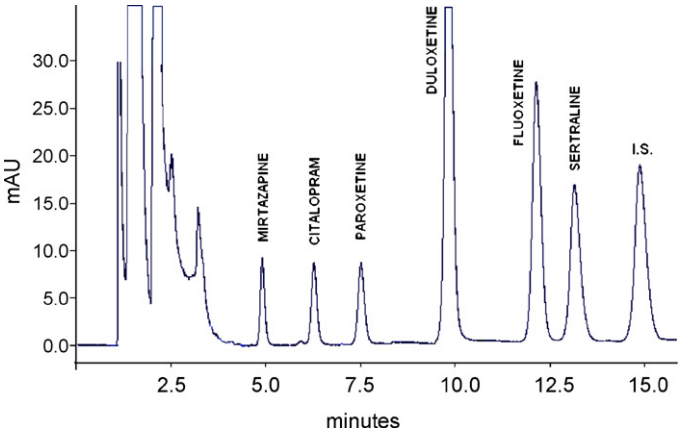

Fig. 8. In-tube SPME/LC chromatogram of a blank plasma sample spiked with antidepressants, resulting in 500 ng/mL (plasma level).

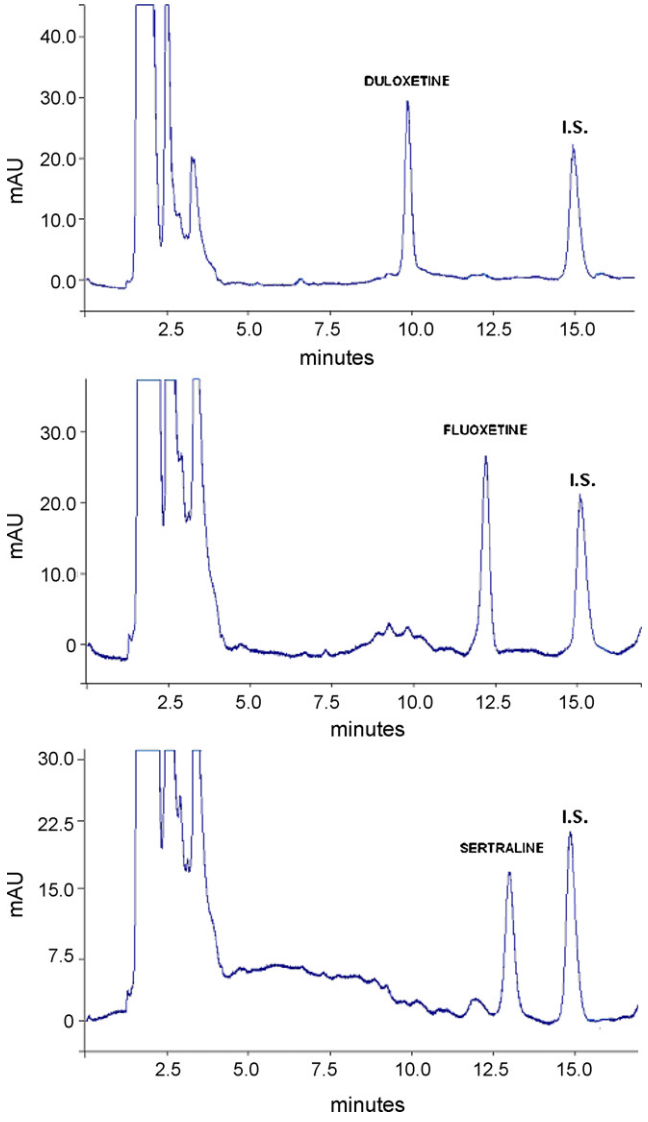

Fig. 9. In-tube SPME/LC analysis of plasma samples from elderly depressed patients receiving therapeutic dosages. Drug concentrations found were: 406 ng/mL of the duloxetine (a), 314 ng/mL of the fluoxetine (b), and 445 ng/mL of the sertraline (c).

Table 1
Retention time of the drugs studied as possible interferents

Drugs	Retention time (min)
Methyldope	1.65
Ranitidine	1.95
Cafeine	2.42
PEMA	2.54
Primidone	2.55
Moclobemide	2.62
Diclofenac	2.68
Diazepam	2.72
Flurazepam	3.42
Propanolol	3.69
Phenobarbital	4.07
Clonazepam	4.16
Carbamazepine	4.20
Phenytoin	4.26
Mirtazapine	**4.88**
Desipramine	5.48
Citalopram	**6.23**
Amitryptiline	7.05
Paroxetine	**7.56**
Duloxetine	**9.86**
Lidocaine	10.33
Fluoxetine	**12.24**
Sertraline	**13.08**
Clomipramine	**14.92**

The bold values represent the retention times of the analytes.

The inter- and intra-assay precision of the in-tube SPME/LC method was determined using blank plasma samples spiked with analytes, which resulted in LOQ, 200, 300, and 500 ng/mL concentrations (Table 2). The coefficients of the variation were lower than 10% for all evaluated concentrations.

The developed method showed adequate accuracy, with values ranging between 94.5% and 102.8% (Table 2).

The absolute recoveries were evaluated in replicates ($n = 5$) from plasma samples spiked with the analytical standard (500 ng/mL) (Table 2). The recovery values were low, but this fact does not necessarily imply in insufficient sensitivity, accuracy or precision of the method [10]. SPME is not an exhaustive process (as well as liquid–liquid extraction); it is based on sorption equilibrium between the matrix and polymeric phases.

Nevertheless, the in-tube SPME/LC method recoveries were significantly higher than those of the traditional SPME/LC method for the same antidepressants in the plasma samples [14]. The sorption (diffusion capability) of antidepressants into the OV-1701 capillary (in-tube SPME) was higher than on the PDMS/DVB fiber surface (SPME), an adsorption process.

The limit of quantification (LOQ) of the analytes in plasma samples varied from 20 to 50 ng/mL (Table 3). These values were determined as the lowest analytes concentration in the analytical curve, with a coefficient of variation (precision) lower than

Table 2
In-tube SPME/LC inter-assay and intra-assay precision, accuracy, and extraction efficiency (recovery)

Antidepressants	Concentration evaluated (ng/mL)	Intra-assay precision ($n = 5$)		Inter-assay precision ($n = 5$)		Recovery (%)	
		Measured concentration (ng/mL)	CV (%) $n = 5$	Measured concentration (ng/mL)	CV (%) $n = 5$	$n = 5$ (500 ng/mL)	Accuracy (%)
Mirtazapine	50 (LOQ)	51.2 ± 3.0	4.71	47.7 ± 5.9	9.40		97.6
	200	202.3 ± 6.2	2.10	194.3 ± 10.3	5.30		98.8
	300	300.6 ± 9.0	1.43	292.4 ± 12.5	4.30	5.3 ± 1.8	99.8
	500	508.6 ± 14.0	1.67	505.1 ± 20.9	4.14		98.3
Citalopram	50 (LOQ)	49.5 ± 2.8	2.82	46.3 ± 3.1	6.67		101.0
	200	194.4 ± 8.6	4.58	188.8 ± 11.0	5.85		102.8
	300	308.0 ± 10.1	2.20	313.4 ± 12.3	3.94	12.6 ± 2.1	97.3
	500	502.4 ± 3.8	1.09	496.1 ± 5.8	1.20		99.5
Paroxetine	50 (LOQ)	49.0 ± 2.6	6.15	51.2 ± 3.9	7.80		102.0
	200	206.0 ± 4.2	2.15	200.4 ± 6.1	3.03		97.0
	300	293.9 ± 2.2	0.99	288.9 ± 3.6	1.25	33.9 ± 2.7	102.0
	500	502.1 ± 7.9	1.57	505.6 ± 12.4	2.47		99.6
Duloxetine	20 (LOQ)	21.1 ± 2.0	2.40	22.3 ± 1.2	4.17		94.5
	200	202.5 ± 4.4	2.84	194.8 ± 8.9	4.60		98.8
	300	293.2 ± 6.0	1.07	284.0 ± 5.2	1.83	42.7 ± 4.3	102.3
	500	507.4 ± 6.3	0.75	511.0 ± 8.3	1.64		98.5
Fluoxetine	40 (LOQ)	41.8 ± 4.5	5.54	39.2 ± 3.9	7.80		95.5
	200	205.4 ± 5.3	2.24	193.4 ± 7.7	3.97		97.3
	300	305.4 ± 7.1	1.89	297.4 ± 12.2	4.12	41.9 ± 1.6	98.2
	500	505.2 ± 9.2	1.23	503.0 ± 14.3	2.85		99.0
Sertraline	40 (LOQ)	41.7 ± 3.1	5.04	43.0 ± 2.8	6.00		95.8
	200	198.1 ± 9.3	2.61	205.8 ± 14.0	6.84		100.9
	300	303.4 ± 8.1	2.09	305.8 ± 9.0	2.96	43.5 ± 1.5	98.9
	500	497.8 ± 13.4	5.41	495.8 ± 23.2	6.70		100.4

LOQ concentration that correspond to the limit of quantification.

Table 3
Regression line, limit of detection (LOD), and limit of quantification (LOQ) for in-tube SPME/LC developed method

Antidepressants	Regression line LOQ[a] (500 ng/mL)			LOD[b] (ng/mL)	LOQ[c] (ng/mL)
	Slope	Intercept	r^2-Value		
Mirtazapine	1.017 ± 0.017	−6.975 ± 5.372	0.9997	20.0	50.0
Citalopram	1.010 ± 0.038	−3.959 ± 11.891	0.9985	20.0	50.0
Paroxetine	1.006 ± 0.026	−2.532 ± 8.154	0.9993	20.0	50.0
Duloxetine	1.014 ± 0.039	−5.622 ± 12.079	0.9985	5.0	20.0
Fluoxetine	1.011 ± 0.012	−4.528 ± 3.876	0.9998	10.0	40.0
Sertraline	0.984 ± 0.013	6.771 ± 4.051	0.9998	20.0	40.0

[a] LOQ: concentration that correspond to the limit of quantification.
[b] LOD: signal/noise = 3.
[c] LOQ: CV < 10%.

10% (Table 2). The limit of detection (LOD) ranged from 5 to 20 ng/mL (Table 3), which were based on a signal-to-noise ratio 3 [15].

The linearity of the in-tube SPME–LC method was determined using blank plasma spiked with antidepressants at concentrations ranging from 20 to 500 ng/mL for duloxetine; 50 to 500 ng/mL for mirtazapine, citalopram, and paroxetine; 40 to 500 ng/mL for fluoxetine and sertraline. These intervals were linear, with correlation coefficients better than 0.9985 (Table 3) and coefficients of the variation were lower than 15% in all cases (Table 2). These results demonstrate that the developed method allows the quantification of antidepressants in the therapeutic interval, although no therapeutic levels have been clearly defined for selective serotonin reuptake inhibitors [16]. The in-tube SPME/LC method presented LOQ values and linearity close to those described in the literature using a UV detector, liquid–liquid extraction (LLE/LC-UV), and solid-phase extraction (SPE/LC-UV) [17–19], and SPME/LC-UV with off-line desorption [14], for the analysis of the same antidepressants in biological samples. However, the developed method presented fluoxetine recovery values higher than those achieved with the SPME/LC-UV method using an interface SPME/LC with heating, as well as lower coefficients of the variation (inter-assay precision) [20,21]. The use of more sensitive detectors, like those of a mass spectrometer and fluorescence, could improve the sensitivity of the method.

The robustness of the OV1701 capillary was confirmed over 100 extractions without extraction efficiency losses (data not shown).

Based on the analytical validation results, it can be expected that the in-tube SPME/LC methodology developed here should be adequate for antidepressants analysis at therapeutic levels.

3.4. Clinical application of the method

The in-tube SPME/LC was used to analyze plasma samples from three elderly patients undergoing therapy with duloxetine (Cymbalta®, 60 mg/day), fluoxetine (Prozac®, 20 mg/day) and sertraline (Zoloft®, 150 mg/day). The effectiveness of the method was proved. The calibration curves for patient's plasma samples analysis were carried out with blank plasma samples spiked with analytical standards of the target drugs at different concentrations.

No interference with the retention times of the drugs was observed (Fig. 9). Drug concentrations found in these samples were: 406 ng/mL duloxetine (Fig. 9a), 314 ng/mL fluoxetine (Fig. 9b), and 445 ng/mL sertraline (Fig. 9c). These measured concentrations fall within the therapeutic levels established for fluoxetine (15–1000 ng/mL) and sertraline (50–500 ng/mL) [22].

4. Conclusion

It was demonstrated that in-tube SPME in combination with LC-UV/DAD, offers high sensitivity, accuracy, and enough reproducibility for the quantification of nontricyclic antidepressants in human plasma after the oral administration of the antidepressant.

The in-tube SPME compared with other extraction techniques (on fibre SPME, LLE, and SPE) allows automation analysis, presents minor exposition of the analyst to the biological samples and organic solvent, and provides short analysis time.

The developed and validated in-tube SPME/LC method was successfully used to analyze plasma samples from elderly patients undergoing therapy with nontricyclic antidepressants.

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