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Solid-phase microextraction using poly(pyrrole) film and liquid chromatography with UV detection for analysis of antidepressants in plasma samples

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ABSTRACT

Poly(pyrrole) (PPY) coating was prepared on a stainless-steel (SS) wire for solid-phase microextraction (SPME) by electrochemical deposition (cyclic voltammetric). The PPY was evaluated by analyzing new-generation antidepressants (mirtazapine, citalopram, paroxetine, duloxetine, fluoxetine, and sertraline) in plasma sample by SPME and liquid chromatography with UV detection (LC-UV). The effect of electrolyte solution (lithium perchlorate or tetrabutylammonium perchlorate) and the number of cycles (50, 100 or 200) applied during the polymerization process on the SPME performance was evaluated. Important factors in the optimization of SPME efficiency such as extraction time, temperature, pH, influence of plasma proteins on sorption mechanisms, and desorption conditions are discussed. The SPME–PPY/LC method showed to be linear in concentrations ranging from the limit of quantification (LOQ) to 1200 ng mL^{-1}. The LOQ values range from 16 to 25 ng mL^{-1}. The inter-day precision of the SPME–PPY/LC method presented coefficient of variation (CV) lower than 15%. Based on analytical validation results, the SPME–PPY/LC methodology showed to be adequate for antidepressant analysis, from therapeutic to toxic levels. In order to evaluate the proposed method for clinical use, the SPME–PPY/LC method was applied to the analysis of plasma samples from elderly depressed patients.

1. Introduction

The treatment of depressive disorders is usually accomplished by the use of different compounds known as antidepressants (Fig. 1) [1–3]. These compounds show considerable adverse drug reactions (drug–drug interactions) side effects, and they can have a delayed therapeutic effect, which could result in poor patient compliance.

Therapeutic drug monitoring (TDM) is under-utilized in the field of psychiatry because the therapeutic ranges of antidepressants seem quite broad, leading to the generally accepted notion of low toxicity. On the other hand, the relationship between blood concentration and therapeutic effects is not always fully understood. TDM, though, could be of interest for monitoring patient compliance. In other situations, such as liver and kidney impairment, poor metabolism by CYP450 isoenzymes and comedication with inhibitors and inducers of those enzymes, and in the elderly population, TDM could provide valuable information for a cost-effective and more rational use of psychiatric drugs [3–5].

Liquid–liquid extraction (LLE) [6–9] and solid-phase extraction (SPE) [1,2,10–15] have been the most frequently employed techniques for drug extraction from biological fluids. These classic methods usually consume organic solvents, not to mention that they are laborious and time consuming. However, modern trends in analytical chemistry are moving toward methods that lead to simplification, miniaturization of sample preparation, and minimization of organic solvent and sample volumes.

In the last decade, Arthur and Pawliszyn [16] introduced solid-phase microextraction (SPME). This solventless technique combines extraction and concentration of analyte in a single step, thereby reducing the time required for sample preparation. In SPME, the volume of the extraction phase is very small in relation to the volume of the sample, and extraction of analyte is not exhaustive. The extraction efficiency is determined by the partitioning of the analyte between the sample matrix and the extraction phase [17,18].

SPME has been successfully applied to the extraction of volatile and semi-volatile drugs from biological samples mainly by coupling with gas chromatography [19–21]. However, SPME applications to ionizable compounds species have been limited because of the neutral charge of commercial SPME coatings, which results in low coating/sample partition coefficient and poor analyte recoveries. To overcome this difficulty, chemical modifications have been made to the sample in order to increase the extraction efficiency [18,22–25].

Poly(pyrrole) (PPY) is a promising alternative as extraction phase for ionizable compounds analysis due to its permeability (porous structure) and multifunctional properties, which result in intermolecular interactions like acid–base, π–π, dipole–dipole, hydrophobic, hydrogen bonding and exchange between the

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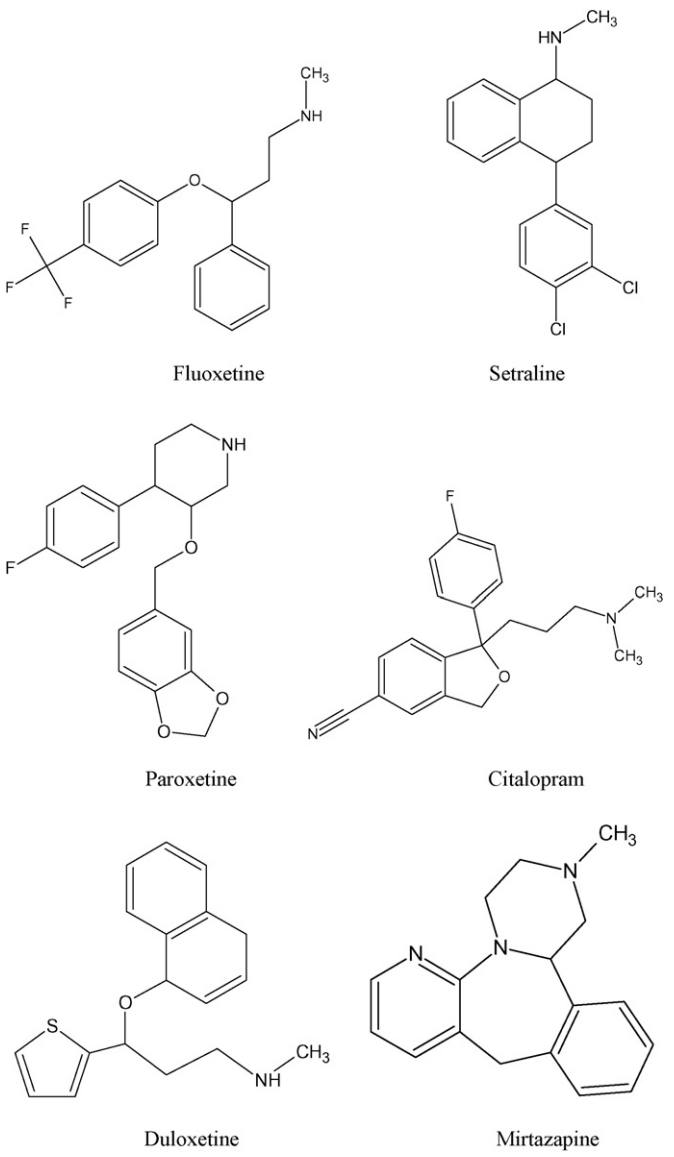

Fig. 1. Representative structure of the antidepressants.

polymer and analytes [26–28]. PPY has been successfully applied as coating for SPME and in-tube SPME of β-blockers, stimulants and polycyclic aromatic compounds from biological samples [29–34].

In this study, the PPY coating was prepared on a stainless-steel (SS) wire for SPME by electrochemical deposition (cyclic voltammetric). The PPY was evaluated by analyzing new-generation antidepressants (mirtazapine, citalopram, paroxetine, duloxetine, fluoxetine and sertraline) in plasma sample by SPME and liquid chromatography with UV detection for TDM.

2. Experimental

2.1. Reagents and analytical standards

The fluoxetine and duloxetine analytical standards were donated by Lilly (São Paulo, Brazil), paroxetine by Libbs (São Paulo, Brazil), and citalopram, mirtazapine and sertraline, by Roche (São Paulo, Brazil).

The working standard drug solutions were prepared by diluting the stock solutions of these drugs (1 mg mL^{-1} in methanol) to a proper methanol volume, based on their therapeutic intervals. These solutions were stable 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). Lithium perchlorate and tetrabutylammonium perchlorate (Sigma–Aldrich, Steinheim, Germany); methanol and acetonitrile HPLC grade were purchased from J.T. Backer (Phillipsburg, USA), monobasic and dibasic phosphates were purchased from Merck (Darmstadt, Germany). Pyrrole (99.8% Sigma–Aldrich, Steinheim, Germany) was distillated before use.Drug-free plasma samplesPlasma from patients not exposed to any drug for at least 72 h (blank plasma) was kindly supplied by *Hospital das Clínicas de Ribeirão Preto*, University of São Paulo, Brazil. These plasma samples spiked with analytes were used for optimization of the SPME process and analytical method validation.

The plasma samples were collected from geriatric patients subjected to therapy with antidepressants for at least 2 weeks. Blood samples were withdrawn 12 h after the last drug administration. Collection of these plasma samples was carried out in agreement with the criteria established by the Ethics Committee of the University of São Paulo.

2.3. Electrochemical polymerization

Electrochemical polymerization of pyrrole was carried out using a potenciostat/galvanostat model PAR 273-A, with the M270 software. The electrochemical cell consisted of a three-electrode arrangement: the auxiliary electrode was a platinum sheet, the reference was a double junction saturated calomel electrode, and the working electrode consisted of cylindrical SS (1.25 mm in diameter). The surfaces of the SS was polished with a steel sponge, and washed with methanol/water solution (1:1, v/v) in ultrasonic bath for 15 min, followed by washing with water purified in a Milli-Q system.

The PPY films were electrodeposited on the surface of the working electrode in a 0.1 mol L^{-1} electrolyte solution containing 0.01 mol L^{-1} of pyrrole monomer. The potential range (0–1.2 V) was applied at a scan rate of 50 mV s^{-1}. Before electropolymerization, the solution was deoxygenated by argon purging for 10 min. The effect of electrolyte solution (lithium perchlorate or tetrabutylammonium perchlorate), and the numbers of cycles (50, 100 or 200) employed during the PPY electrodeposition process on SPME performance was evaluated.

2.4. Scanning electron microscopy (SEM) of PPY coated SS surfaces

The surface of the PPY-coated SS wire was cut into a 1 cm—long piece and then analyzed on a Zeiss EVO50 SEM (20 kV accelerating potential).

2.5. Chromatographic conditions

Varian 230 ProStar (Varian, CA, USA) system was employed. Signals were monitored at 230 nm by a UV-diode array detector (DAD), Varian 310/330 ProStar. The separation was performed in a Lichrosphere 60® RP: Select B (250 mm × 4 mm, 5 μm particle size: Merck) column at room temperature (25 °C); the mobile phase consisted of a phosphate buffer solution (0.05 mol L^{-1}, pH 3.8) and acetonitrile (57:43, v/v) in the isocratic mode, and the flow rate was 1.0 mL min^{-1}. The mobile phase was filtered and degassed prior to use.

2.6. Optimization of the SPME process

The influence of the pH of the matrix on SPME performance was the first step to be evaluated. For that purpose, four pH values were investigated: 4.0, 7.0, 9.0, and 10.0 achieved by

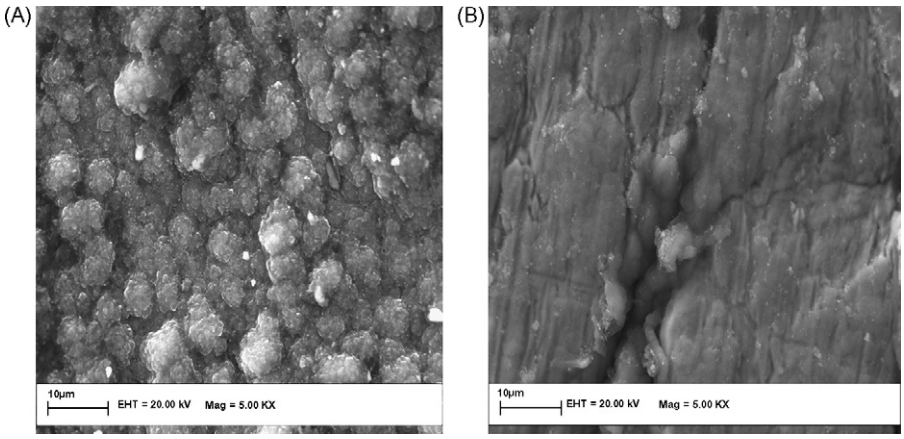

Fig. 2. Scanning electron micrographs of the PPY-coated stainless-steel wires: (A) PPY films polymerized in lithium perchlorate solution and (B) PPY films polymerized in tetrabutylammonium perchlorate solution.

means of different buffer solutions. In a glass vial (5 mL) sealed with a silicone septum, 4 mL of the buffer solution were added to 250 μL of the plasma sample spiked with the drug standard solutions, which resulted in a drug concentration of 500 ng mL^{-1}. The vial was maintained at room temperature (25 °C) and under magnetic stirring. The fiber was then immersed into the sample and the extraction was performed under magnetic stirring at a rate of 1200 rpm, for 40 min.

The influence of the extraction time (30, 40, 50, and 60 min) and temperature (20, 40, 50, and 60 °C) on the SPME process was also investigated.

To evaluate the best desorption conditions, different solvents (acetonitrile and mobile phase), desorption times (5, 10, 20, and 30 min), and numbers of desorption steps, were evaluated. To this end, the PPY fiber was placed in a glass vial containing 250 μL of the desorption solvent, which ensured its total immersion. Desorption was performed in the off-line mode for 20 min at room temperature (25 °C), and 50 μL of this extract were injected in to the LC-UV system. After the desorption process, the PPY fiber was washed with methanol/water (1:1, v/v) solution.

2.7. Analytical validation

The analytical validation of the SPME–PPY/LC method was carried out using blank samples spiked with drug standard solutions at concentrations that included the plasma levels. Linearity was evaluated by calibration curves constructed using linear regression of the drug standard peak area (y) versus the drug nominal plasma concentration (x, ng mL^{-1}). The concentration of these samples ranged from limit of quantification (LOQ) to 1200 ng mL^{-1}.

Accuracy and inter-day precision values were determined by calibration curves using quintuplicate SPME–PPY/LC assays of the blank plasma samples spiked with analytes at LOQ, 50, 100, 200, 400, and 1200 ng mL^{-1}.

Recovery values were calculated by comparison of the peak areas of the drugs extracted from the plasma with those of the drugs at the same concentration in standard solutions. Selectivity of the method was also investigated by comparing the retention times of the analytes (antidepressants) with the retention times of other drugs and endogenous compounds.

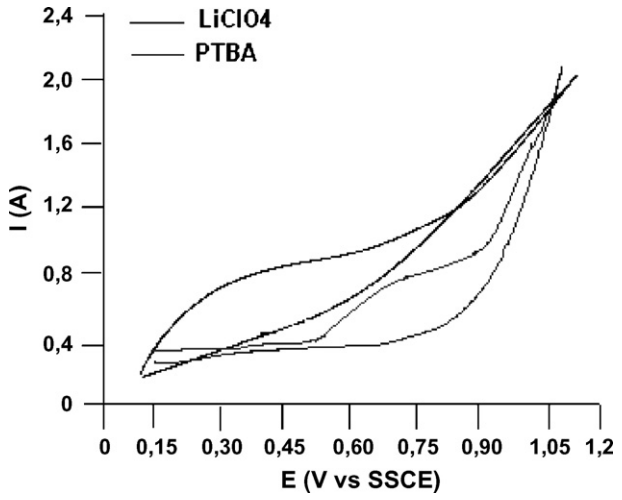

Fig. 3. Cyclic voltammograms of PPY films in lithium perchlorate solution ($LiClO_4$), and tetrabutylammonium perchlorate (PTBA) solution in acetonitrile, both with 100 cycles.

3. Results and discussion

3.1. Electrochemical polymerization process

The PPY film polymerized in lithium perchlorate electrolyte solution led to a more efficient SPME than the film polymerized in tetrabutylammonium perchlorate solution. According to Wu and Pawliszyn [35] the counterions do not play an important role in PPY synthesis; however, they allow manipulation of the functionality of the resulting polymeric chain.

The characteristics of the surface of the polymeric films were investigated by SEM. Fig. 2 depicts the micrographs of PPY films and shows that PPY coating prepared in lithium perchlorate solution has a more porous structure, and the surface of the film is well distributed. The porous structures should significantly increase the effective surface areas of the films, therefore leading to higher extraction efficiency compared with non-porous films [33,36]. The porosity of the structure is very important for the adsorption process, extractions occur on the active sites present on the surface.

The PPY film prepared in lithium perchlorate solution displayed a more porous surface [37], so it presented higher charges in the cyclic voltammograms, Fig. 3. The specific overall charge was obtained by voltammogram areas integration.

The thickness of the fiber coating can be controlled by tailoring the electrochemical conditions. The effect of the number of cycle (film thickness) during PPY polymerization on SPME efficiency is shown in Fig. 4. For both electrolyte solutions, 100 cycles led to the best results, but lithium perchlorate resulted in better efficiency for most of the antidepressants, Fig. 4A and B.

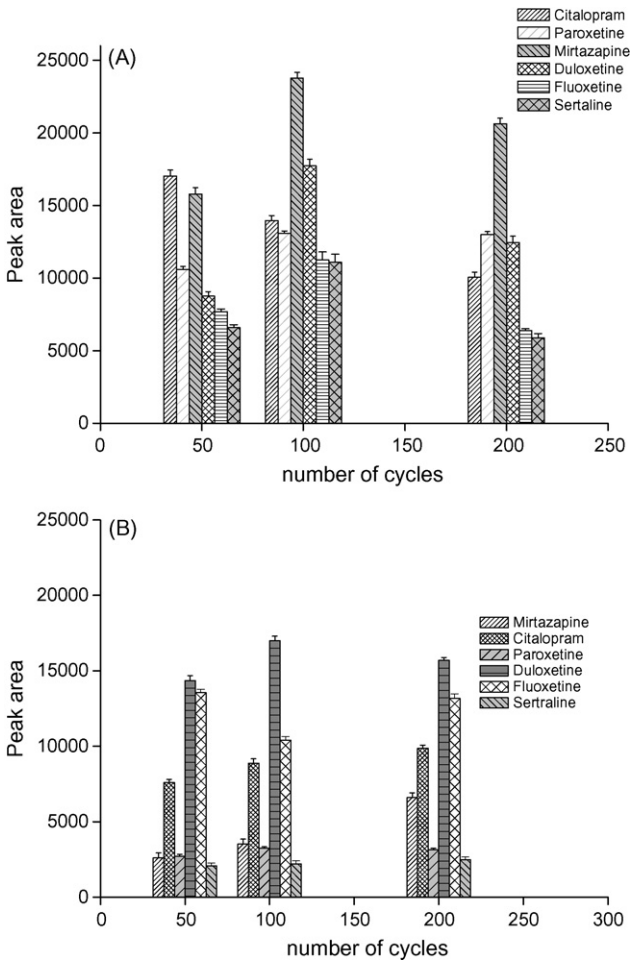

Fig. 4. Effect of the number of cycle during PPY polymerization at 50 mV s^{-1} on the intensity of the antidepressants signals: (A) Lithium perchlorate solution; (B) tetrabutylammonium perchlorate solution.

The shape and the peak positions in the cyclic voltammograms can reflect properties of the polymer structure [38]. The cyclic voltammograms were monitored so as to keep the properties of the PPY films constant.

The PPY coating thickness (d) was estimated to be 20 μm, in agreement with the Faraday law, Eq. (1):

$$d = \frac{QM}{2F\rho} \quad (1)$$

where ρ is the PPY density, M is the pyrrole molar mass, Q specific overall charge for electropolymerization, and F is Faraday's constant [37,38]. According to the SS wire geometry and the voltammograms area integration; the film developed here presented an average volume of 19.6 μL.

3.2. Optimization of SPME–PPY variables

SPME variables such as time, temperature, pH of the matrix, ionic strength, and desorption conditions were optimized. The SPME variables were investigated in triplicate assays. The sample volume, stirring speed, and PPY films dimension were kept constant during the optimization.

The SPME efficiency was improved by diluting the plasma samples with phosphate buffer solution to pH 7.0, in which the drugs (pK_a values from 8.7 to 10.2) were totally or partially in the ionic form (Fig. 5). According to Wu and Pawliszyn [26], extraction by the PPY film decreased dramatically with decreasing sample solution

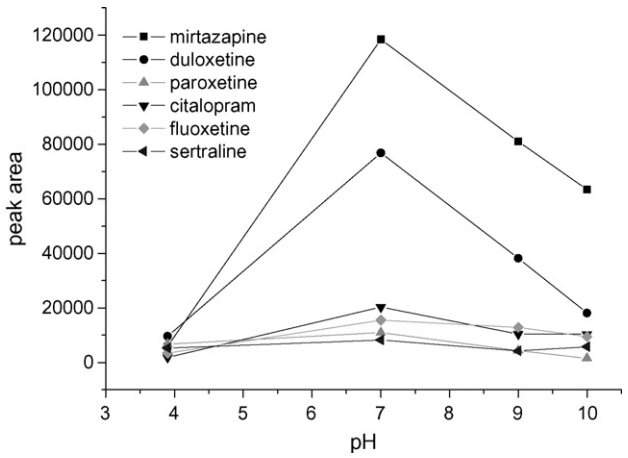

Fig. 5. Effect of the matrix pH on the SPME efficiency.

pH due to electrostatic repulsion between the basic analytes and the film, since the analytes and the film are positively charged at low pH values [26]. The PPY polymeric surface, and ionizable drugs should be partially or un-ionized at pH values higher than 7.0.

The extraction temperature plays an important role in analyte adsorption because it influences the mass transfer rate, and the partition coefficient of the analyte [32]. When the extraction temperature was increased from 25 to 50 °C, the average peak area of the extracted drugs also increased, but the PPY films did not exhibit thermal stability. Thus, room temperature (25 °C) was selected for the subsequent studies, to ensure stability and robustness of the PPY films. Fig. 6 shows representative time extraction profiles (15–60 min) at 25 °C. The extraction time at 40 min was selected because it led to the best results for most drugs.

Desorption conditions were tested to ensure effective removal of the extracted analytes from PPY film. The mobile phase gave the best results among the evaluated solvents (acetonitrile and mobile phase). The liquid desorption equilibrium was established at 15 min, but remained nearly constant for desorption time of 15–40 min, which corresponds to the complete desorption of drugs from the SPME phase, as no detectable carryover was observed.

The fiber-to-fiber reproducibility was also investigated, so as to evaluate the reproducibility of the electrochemical coating procedure. Three different fibers were coated under the same conditions. The average SPME/LC results from different fibers were very similar with coefficient of variation (CV) ranging from 6 to 11%. These assays were carried out with plasma samples spiked with drugs

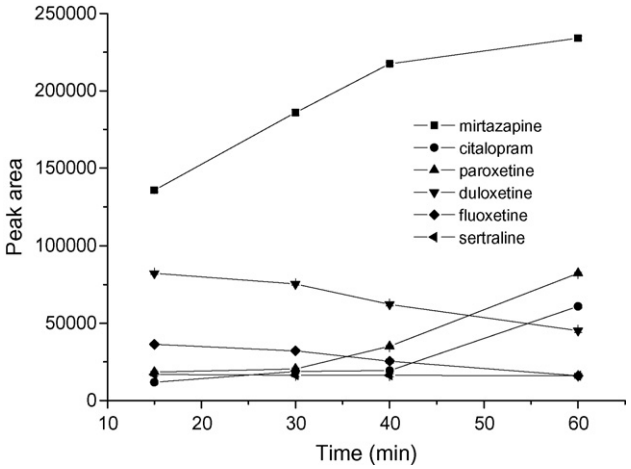

Fig. 6. Extraction time profile for SPME–PPY/LC at 25 °C with mobile phase.

Table 1
Linearity and limit of quantification (LOQ) of the SPME–PPY/LC method.

Drugs	Linear regression (LOQ: 1200 ng mL^{-1})[a]	r^2	LOQ (ng mL^{-1})
Mirtazapine	$y = 7384.05 + 61.76x$	0.998	16
Citalopram	$y = 2702.01 + 7.55x$	0.999	20
Paroxetine	$y = 1719.29 + 10.09x$	0.999	20
Duloxetine	$y = 8914.69 + 58.67$	0.998	16
Fluoxetine	$y = 2069.17 + 10.77x$	0.998	25
Sertraline	$y = 2454.18 + 8.94x$	0.998	25

[a] Based on area values.

(300 ng mL^{-1}). Robustness of the PPY films was confirmed after 30 extractions were carried out minimum loss of extraction efficiency, and there was no significant difference in the porous surface of the PPY film either.

Because of ionic change properties, and the adsorptive of PPY films, the salt addition was not evaluated. According to Tamer et al. [39], PPY films exhibit poor extraction efficiency in highly concentrated saline solution (upper to 1.5 mol L^{-1}), due to the competition between the salt cations and the analytes in the surface of the phase. In low saline solution concentration, there are no changes in the extraction efficiency.

On the basis of these data, the best SPME experimental conditions among those investigated for antidepressant assays (Figs. 5 and 6) were as follows: 250 μL of plasma modified with 4 mL phosphate buffer (pH 7), extraction temperature at 25 °C, 40 min, followed by drug liquid desorption on mobile phase at 25 °C for 15 min.

3.3. Analytical validation of SPME–PPY/LC

The specificity of the developed method is demonstrated by representative chromatograms of a drug-free human plasma sample and of a drug-free human plasma sample spiked with antidepressants in the therapeutic interval concentration (Figs. 7 and 8). These chromatograms evidence the ability of the method to unequivocally measure the drugs in the presence of endogenous plasma components. Drug-free human plasma from several individuals were tested, and showed no significant interferents at the retention times of the analytes.

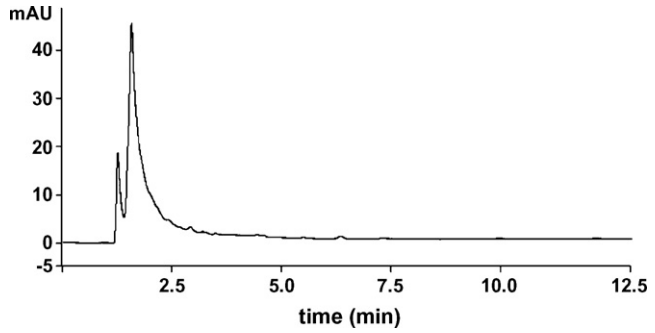

Fig. 7. SPME–PPY/LC chromatogram of drug-free plasma sample.

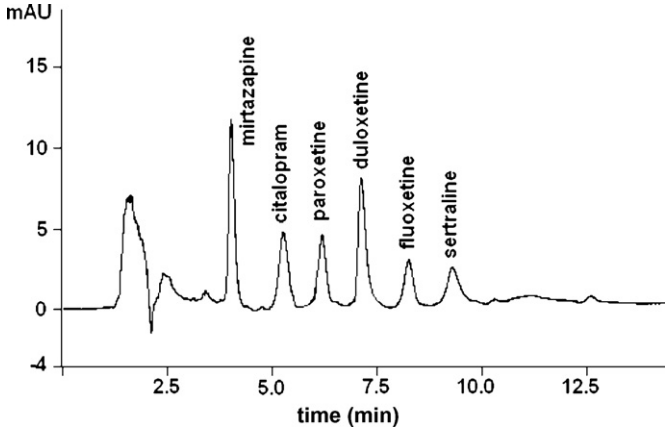

Fig. 8. SPME–PPY/LC chromatogram of drug-free plasma sample spiked with antidepressants at 300 ng mL^{-1}.

Table 2
Inter-day precision (coefficient of variation, CV) and absolute recovery of the SPME–PPY/LC method.

Drugs	Added concentration (ng mL^{-1})	Recovery (%) ($n = 5$)	Accuracy (%) ($n = 5$)	CV (%) ($n = 5$)
Mirtazapine	16.0	89	82	12
	200.0	68	68	10
Citalopram	20.0	89	84	13
	200.0	70	70	11
Paroxetine	20.0	46	80	14
	200.0	37	77	12
Duloxetine	16.0	82	76	14
	200.0	66	65	13
Fluoxetine	25.0	91	88	14
	200.0	79	78	12
Sertraline	25.0	72	92	14
	200.0	70	70	10

The linearity of SPME–PPY/LC method was determined with plasma samples spiked with analytical standards that result in a concentration ranging from the LOQ up to 1200 ng mL^{-1}. The regression equations and the corresponding correlation coefficients for all the drugs are given in Table 1.The LOQ values were determined as the lowest concentration in the calibration curve in which the CV was lower than 15% and based on a signal-to-noise ratio about 10.

The absolute recoveries, accuracy and inter-day precision of the SPME–PPY/LC method were assessed by replicate analysis ($n = 5$), with plasma samples spiked with standards at different concentrations (Table 2).

The PPY film thickness is approximately 20.0 μm. The higher surface area resulted in higher volume (19.6 μL) compared with commercial SPME coatings. This could explain the obtained recovery values.

Table 3
Retention time of the drugs studied as possible interferents.

Drugs	Retention time (min)
Methyldopa	1.00
Ranitidine	1.15
Cafeine	1.73
PEMA	1.77
Primidone	1.92
Moclobemide	2.00
Diclofenac	2.12
Diazepam	2.16
Flurazepam	2.70
Propanolol	2.95
Phenobarbital	3.12
Clonazepam	3.77
Carbamazepine	3.92
Phenytoin	4.00
Mirtazapine	**4.15**
Desipramine	4.60
Citalopram	**5.26**
Paroxetine	**6.16**
Amitryptiline	6.65
Duloxetine	**7.13**
Fluoxetine	**8.23**
Sertraline	**9.41**
Lidocaine	9.72
Clomipramine	10.43

Analytes in boldface.

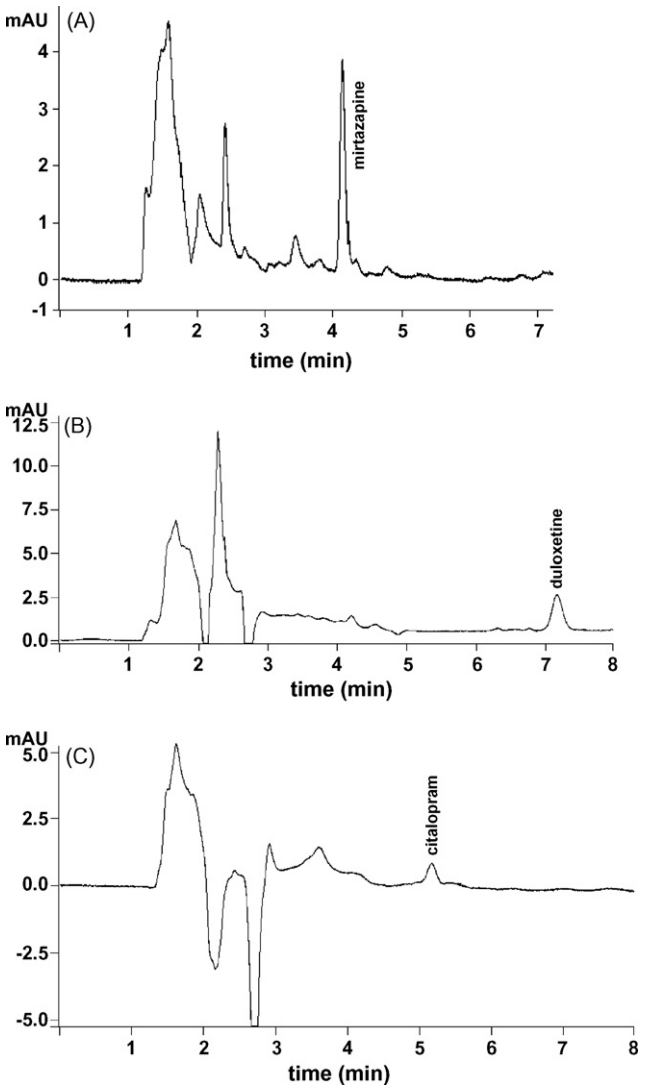

Fig. 9. SPME–PPY/LC analysis of plasma samples from elderly patients receiving therapeutic dosages. Drug concentrations: (A) 79.9 ng mL^{-1} for mirtazapine, (B) 76.5 ng mL^{-1} for duloxetine, and (C) 61.2 ng mL^{-1} for citalopram.

The recovery values obtained in the present study are better than those obtained by other SPME methods: SPME/LC-UV [18] using commercial phase (polydimethylsyloxane–divinylbenzene, 8–17%), and in-tube SPME/LC-UV [40] (14% cyanopropylphenyl-methylpolysiloxane, 5–43%) both methods used for determination of the same antidepressants from plasma samples. Moreover, the obtained recovery values are close to those described for the determination of the same analytes from plasma samples (52–110%) [41], and for the determination of methamphetamines (92–97%) [29] from human serum samples by stir bar sorptive extraction and LC-UV analysis, and SPME–PPY (thickness: 16 μm) and GC–MS analysis, respectively. However, the in-tube SPME/LC-UV method [40], compared with other on fiber SPME methods, allowed automation of the analysis, so, better precision with lower CV values were obtained.

Antidepressants may be prescribed in combination with different psychotropic agents and other drugs, so it was important to assess the probable interference from potentially coadministered compounds (Table 3). On the basis of retention times, the other drugs did not co-elute with the analytes.

4. Clinical application of the developed method

In order to evaluate the proposed method for clinical use, the described protocol was applied in the analysis of plasma samples from elderly depressed patients (Fig. 9). Peak shapes and resolution are very similar to those obtained using spiked blank plasma, and no interference is observed.

Drug concentrations found in these samples were 79.8 ng mL^{-1} for mirtazapine, 89.5 ng mL^{-1} for duloxetine, and 61.2 ng mL^{-1} for citalopram. The plasma samples were collected from elderly depressed patients under therapy with Cymbalta® (60 mg/day), Remeron® (45 mg/day) and Celexa® (40 mg/day). These patients were within therapeutic levels [42].

5. Conclusion

The SPME–PPY/LC method presents high sensitivity, precision, and accuracy, which allow the quantification of antidepressants in human plasma following oral administration. The PPY film displayed high extraction efficiency (selectivity and sensibility) toward the target analytes. Thus, the proposed SPME–PPY/LC method can be a useful tool for the determination of antidepressants in plasma samples from patients receiving therapeutic dosages. The method may also be applied in the evaluation of plasma levels in urgent toxicological analyses after the accidental or suicidal intake of higher doses.

Acknowledgments

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