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The development of a new disposable pipette extraction phase based on polyaniline composites for the determination of levels of antidepressants in plasma samples

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ABSTRACT

In the present work, a new stationary phase for disposable pipette extraction (DPX) based on composites of polyaniline and a styrene–divinylbenzene (SD) copolymer was applied to the analysis of fluoxetine and norfluoxetine in plasma samples using liquid chromatography and fluorescence detector (LC–FD).

The DPX variables, such as number of draw/eject cycles, sample pH, type and volume of the desorption solvent, were optimized to established the sorption equilibrium and shorten the analysis time. Among the DPX evaluated variables, the higher extraction efficiency were obtained with 200 μL of plasma mixed with 200 μL of borate solution (pH 9), followed by liquid desorption of the drug with 200 μL acetonitrile in a single cycle. The DPX/LC–FD method demonstrated a linear response over the dynamic range from 10 to 1000 ng mL^{-1} for fluoxetine and from 80 ng mL^{-1} (LOQ) to 1000 ng mL^{-1} for norfluoxetine with r^2 = 0.997 and 0.998, respectively. The limit of quantification (LOQ) was 10 ng mL^{-1} for fluoxetine and 80 ng mL^{-1} for norfluoxetine. Based on the analytical validation results, the proposed method can be a useful tool for determining the fluoxetine and norfluoxetine levels in plasma samples from patients receiving therapeutic dosages.

1. Introduction

Therapeutic drug monitoring (TDM) of antidepressants may safeguard against drug–drug interactions, can be used to control compliance, and may increase the therapy efficacy in patients suffering from major depressive illness. Several chromatographic methods have been developed for the determination of antidepressants in plasma and serum, but they require complex isolation procedures to improve their sensitivity and specificity [1–4].

Among the sample preparation techniques, liquid–liquid extraction (LLE) [5,6] and solid-phase extraction (SPE) [7,8] are the most frequently used for drug extraction from biological fluids. However, these procedures are time-consuming and tedious and use a large amount of organic solvents.

Disposable pipette extraction (DPX) is a solid-phase extraction (SPE)-based device in which a small amount of SPE sorbent is placed inside a pipette tip fitted with a screen at the narrow bottom end and a barrier near the top of the tip [9,10]. DPX has become an essential tool for the purification and concentration of proteins and peptides in genomics, proteomics, and metabolomics [11–13]. This technique has also been successfully employed in environmental, toxicological, and drug analyses [9–11,14]. Although DPX is a technique derived from SPE, the extraction efficiency is based on the sorption equilibration time between the sample solutions and the dispersive sorbent; consequently, this process is not dependent of the sample flow-rate. Furthermore, the miniaturized format for DPX results in smaller solvent elution volumes than the conventional SPE technique [9]. The main advantages of DPX could be the adaptability to high-throughput parallel sample processing while still maintaining flexibility of sorbents and procedures [14].

The first commercially available micropipette tip was based on chromatographic media, C18 microparticulates, embedded in the scaffold of a polymer (ZipTip, Millipore, Bedford, MA, USA). Since then, different phases with different interaction modes, such as hydrophobic, ion exchange, and affinity, have been introduced [15–17]. The advantages of the developed phases include easy preparation and control of permeability and surface charge. Using

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monolith phases, frits are not necessary and often result in greater pH stability [18].

The conducting polymer coatings, such as polypyrrole, polyaniline, and polythiophene, have been used as extraction and separation phases for miniaturized techniques [1–3,19], mainly because their multifunctional properties result in assorted interactions between the analyte and phase. Polyaniline (PANI) is a conducting polymer that has attracted much interest in separation science due to its permeability (porous structure) and multifunctional properties, which result in intermolecular interactions, such as acid–base, π–π, dipole–dipole, hydrophobic, and hydrogen bonding and ion exchange between the polymer and analytes [1]. PANI is a promising alternative sorbent to ionizing compounds due to its easy synthesis from an inexpensive monomer, good conductivity, and remarkable stability under ambient conditions [20].

A conducting polymer, such as PANI, could be polymerized in the presence of supports to produce materials with defined shape. The polymerization of PANI in the presence of polystyrene (PS) latex can be carried out to produce nearly mono-dispersed PANI-PS composites with core–shell morphology, where the conducting polymer forms the shell. Moura and co-workers successfully applied PANI and a sulfonated styrene–divinylbenzene (SD) copolymer as an acid catalyst in the esterification of stearic acid with methanol [20]. Their easy synthesis and ability to control shape, size and porosity made composites of PANI and macroporous sulfonated styrene–divinylbenzene (SD) copolymer very attractive to employ as the extraction phase of DPX.

In this study, the potential of the SD copolymer/PANI composites as sorbents for the DPX technique to determine antidepressants (fluoxetine and norfluoxetine) in plasma samples by LC–FD was evaluated.

2. Experimental

2.1. Reagents, standards and samples

1,4-Dioxane UV/HPLC grade, benzoyl peroxide 65%, acetone 99.5%, aniline PA, sulfuric acid 95–98%, nitric acid 65% and hydrochloric acid 37% were obtained from VETEC (Rio de Janeiro, Brazil). The 2,6-di-tert-butyl-4-methylphenoltoluene 99.5% and heptane 98% were obtained from LABIMPEX (São Paulo, Brazil). Methyl alcohol 99.8%, ethanol 99.5%, gelatin powder and sodium hydroxide 97% were obtained from SYNTH (São Paulo, Brazil). The hydroxyethylcellulose was acquired from Polithechno (São Paulo, Brazil), sodium chloride 99%, sodium dodecyl sulfate 99% were obtained from ISOFAR (Rio de Janeiro, Brazil), phenolphthalein 1% solution was acquired from Proquimios (Rio de Janeiro, Brazil), stearic acid 95% was acquired from INDUSLAB (Paraná, Brazil). Styrene was obtained from Lyondellbasell (Dallas, TX, USA) and divinylbenzene was obtained from PARCHEN (New York, USA) both were purified by washing with NaOH solution followed by reduced pressure distillation.

The fluoxetine and norfluoxetine analytical standards were donated by Lilly (São Paulo, Brazil). Citalopram, which was used as an internal standard (IS), was donated 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 volume of methanol, based on their therapeutic intervals. These solutions were stable for 45 days at −20 °C. Water purified in a Milli-Q system (Millipore, São Paulo, Brazil) was used to prepare the mobile phase. Drug-free plasma samples from patients who were not exposed to any drug for at least 72 h (blank plasma) were kindly supplied by the Hospital das Clínicas de Ribeirão Preto, University of São Paulo, Brazil. The studies were performed in accordance with the World Medical Association's "Ethical principles for medical research involving human subjects". These plasma samples were spiked with IS and target drugs and used to optimized the DPX process and validate the analytical method.

2.2. Instrument and chromatographic conditions

The LC–FD analyses were performed on a Shimadzu LC-20 AT (Kyoto, Japan) equipped with a fluorescence detector (RF-10 AXL, λ_{ex} = 230 nm and λ_{em} = 290 nm) and a CBM-20 A system controller. The separation was performed in a Lichrosphere 60® RP: Select B (250 mm × 4 mm, 5 μm particle size) (MERK, Darmstadt, Germany) 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 (55:45, 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.

2.3. Synthesis of the styrene–divinylbenzene (SD) copolymer

The styrene–divinylbenzene copolymer was synthesized based on the work previously published by Moura and co-workers [20]. The copolymer synthesis was carried out through aqueous suspension polymerization. The aqueous phase (AP) was composed of hydroxyethylcellulose at 0.26% (w/v), sodium chloride at 0.59% (w/v) and gelatin at 0.12% (w/v). The organic phase (OP) was prepared by dissolving 1% of the initiator, benzoyl peroxide, into a mixture containing styrene (16%, w/w) and divinylbenzene (84%, w/w) at room temperature; this copolymer was called SD84. Heptane and toluene were used as porogenic agents with a volume ratio of 85/15 and 150% dilution degree relative to monomer's volume. The organic phase was added to the aqueous phase, the system was stirred for approximately 15 min, followed by heating the solution to 70 °C while stirring at 250 rpm for 48 h. Finally, the copolymer beads were filtered and washed with water and then with ethanol. The copolymers were dried at 100 °C for 24 h, and the particles obtained were used to prepare the composites. A copolymer with styrene (71%, w/w) and divinylbenzene (29%, w/w) were prepared to compare the porosity with the extraction efficiency, this copolymer was called SD29. Except for the different proportions of styrene and divinylbenzene, the preparation procedure was the same for both copolymers.

2.4. Synthesis of the SD copolymer/PANI composites

Aniline was chemically oxidized into the copolymers. For this, 4 g of resins (SD84 and SD29) was placed into 40 mL of ethanol/aniline solution (80/20, v/v). Each system was mechanically stirred in a shaker for 3 h to swell the copolymer resin with the aniline. The reaction solution was prepared by mixing 2.3×10^{-3} mol of benzoyl peroxide in 20 mL of dioxane, 1.4×10^{-3} mol of sodium lauryl sulfate in 6 mL of water, and 0.06 mol of HCl. The swollen copolymer was filtered and added to the reaction solution.

The aniline polymerization was carried out under mechanical stirring in a temperature regulated bath at 25 °C for 24 h. Then, the composites were vacuum filtered and washed with methanol and ketone until the filtered solution became colorless. The composites were dried at 60 °C for 24 h. The procedure was repeated four times to prepare the composites from four deposition cycles.

2.5. Nitrogen adsorption measurements

The specific surface area and pore size distribution measurements were performed using a Micromeritics ASAP 2010 (Micromeritics Instrument Corporate, Norcross, GA, USA) nitrogen