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Hybrid silica monolith for microextraction by packed sorbent to determine drugs from plasma samples by liquid chromatography–tandem mass spectrometry

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ABSTRACT

The present study (1) reports on the synthesis of two hybrid silica monoliths functionalized with aminopropyl or cyanopropyl groups by the sol–gel process; (2) evaluates these monoliths as selective stationary phase for microextraction by packed sorbent (MEPS) to determine drugs in plasma samples via liquid chromatography–tandem mass spectrometry (LC–MS/MS) in the multiple reactions monitoring (MRM) mode; and (3) discusses important factors related to the optimization of MEPS efficiency as well as the carryover effect. The prepared hybrid silica monoliths consisted of a uniform, porous, and continuous silica monolithic network. The structure of the aminopropyl hybrid silica monolith was more compact than the structure of the cyanopropyl hybrid silica monolith. The Fourier-transform infrared spectroscopy (FTIR) spectra of the hybrid silica monoliths displayed readily identifiable peaks, characteristic of the cyanopropyl and aminopropyl groups. Compared with the aminopropyl hybrid silica phase, the cyanopropyl hybrid silica phase exhibited higher binding capacity for most of the target drugs. The developed method afforded adequate linearity at concentrations ranging from the lower limit of quantification (0.05–1.00 ng mL^{-1}) to the upper limit of quantification (40–10,500 ng mL^{-1}); the coefficients of determination (r^2) were higher than 0.9955. The precision of the method presented coefficients of variation (CV) lower than 14%; the relative standard error (RSE) of the accuracy ranged from −12% to 14%. The developed method allowed for simultaneous analysis of five antipsychotics (olanzapine, quetiapine, clozapine, haloperidol, and chlorpromazine) in combination with seven antidepressants (mirtazapine, paroxetine, citalopram, sertraline, imipramine, clomipramine, fluoxetine), two anticonvulsants (carbamazepine and lamotrigine), and two anxiolytics (diazepam and clonazepam) in plasma samples from schizophrenic patients, which should be valuable for therapeutic drug monitoring purposes.

1. Introduction

Schizophrenia is recognizably a devastating neuropsychiatric disorder that affects about one percent of the world's general population [1,2]. Antipsychotic medications constitute the primary pharmacological therapy of schizophrenia. To lessen the symptoms associated with the disease, most schizophrenic patients use other classes of drugs such as antidepressants, anxiolytics, and anticonvulsants [3] concomitantly with antipsychotics.

There are two generations of antipsychotics. First-generation antipsychotics (FGAs) (e.g., chlorpromazine, haloperidol, and thioridazine) have been prescribed since the 1950s, whereas second-generation antipsychotics (SGAs) (e.g., clozapine, risperidone, quetiapine, and olanzapine) were introduced in the early 1990s [4]. The therapeutic response of these medications depends on the drug plasma concentration, and the established optimal levels are based on the therapeutic outcome [5,6]. Although SGAs are more efficacious and better tolerated than FGAs, they induce extrapyramidal side effects such as weight gain [7], metabolic disturbance [8], and hyperprolactinemia [9]. Most of these adverse effects are dose-related [10]. Therefore, therapeutic drug monitoring in schizophrenic patients is necessary to adjust doses, minimize adverse effects, and check patient adherence to the therapy.

Over the last years, researchers have developed several liquid chromatography–tandem mass spectrometry (LC–MS/MS) methods to determine antipsychotics in plasma samples [11–14]. However, none of the proposed methods has enabled concomitant analysis of antipsychotics, antidepressants, anxiolytics, and anticonvulsants.

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Sample preparation of biological matrixes is an important step in analytical processes: it removes endogenous components from the sample and pre-concentrates trace-level analytes. Proteins and other endogenous compounds may negatively affect the performance of separation columns, increase column backpressure, and suppress electrospray ionization (ESI) during LC–MS/MS analysis.

Regarding miniaturization and automation, MEPS is a promising sample preparation method. MEPS uses only 1–4 mg of sorbent directly incorporated into the barrel of a syringe and therefore requires small volumes of the sample and the elution solvent. The development of commercial MEPS sorbents (C_{18}, C_8, C_2, C_8-SCX, silica, polystyrene-divinylbenzene (PS-DVB), and porous graphitic carbon) has allowed for successful application of MEPS to extract a wide range of drugs, such as antidepressants [15,16], antipsychotics [17], antibiotics [18], anticonvulsants [19], and immunosuppressives [20], from different biological matrices. The complexity of biological samples has called for the design of selective sorbents, like molecular imprinted polymers (MIP) [21], restricted access materials (RAM), and monolithic phases.

The unique properties of monolithic phases—low backpressure, high mass transfer rate, and high permeability—have made them more attractive than conventional particulate-packed phases. The hybrid silica monolithic phase has gained prominence due to its mechanical and pH stability, solvent resistance, and facile preparation [22]. The direct incorporation of organic functional moieties into inorganic silica monolithic matrixes via the sol–gel process has afforded a wide variety of organic–inorganic hybrid monoliths with different organic moieties and distinct macro-mesoporous structures for application in capillary electrochromatography [23], solid phase extraction [24], and in-tube solid phase extraction [25].

This study reports on the preparation of two hybrid silica monoliths functionalized with cyanopropyl or aminopropyl groups. It also describes the use of these monoliths as sorbents for MEPS, to accomplish selective isolation of 16 drugs from plasma samples for further analysis by LC–MS/MS and simultaneous determination of five antipsychotics (olanzapine, quetiapine, clozapine, haloperidol, and chlorpromazine) in combination with seven antidepressants (mirtazapine, paroxetine, citalopram, sertraline, imipramine, clomipramine, fluoxetine), two anticonvulsants (carbamazepine and lamotrigine), and two anxiolytics (diazepam and clonazepam) in plasma samples. The developed method should constitute a valuable tool for therapeutic drug monitoring in schizophrenic patients.

2. Materials and methods

2.1. Reagents and standards

Haloperidol, olanzapine, clonazepam, mirtazapine, paroxetine, citalopram, sertraline, chlorpromazine, imipramine, clomipramine, quetiapine, diazepam, fluoxetine, clozapine, carbamazepine, and lamotrigine standards were purchased from Cerilliant (Round Rock, TX, USA). Stable labeled internal standards (ISs) haloperidol-d4, clonazepam-d4, paroxetine-d6, citalopram-d6, sertraline-d3, imipramine-d3, clomipramine-d3, quetiapine-d8, diazepam-d5, fluoxetine-d6, clozapine-d4, and carbamazepine-d10 were also acquired from Cerilliant (Round Rock, TX, USA). Tetraethyl orthosilicate (TEOS) (98%), 3-cyanopropyltriethoxysilane (CN-TEOS) (98%), (3-aminopropyl) triethoxysilane (APTES) (98%), and N-dodecylamine (99%) were obtained from Sigma-Aldrich (St. Louis, USA). Acetonitrile, ethanol (HPLC grade), methanol (HPLC grade), ammonium acetate, and formic acid were supplied by JT Baker (Phillipsburg, USA). Cetyltrimethylammonium bromide (CTAB, 95%) was purchased from Aldrich. The working standard drug solutions were prepared by diluting the stock solutions of these drugs (1.0 mg mL^{-1}) to an appropriate volume of methanol, on the basis of their therapeutic intervals (haloperidol: 5–20 ng mL^{-1}, olanzapine: 1–20 ng mL^{-1}, clonazepam 10–80 ng mL^{-1}, mirtazapine 30–100 ng mL^{-1}, paroxetine 10-70 ng mL^{-1}, citalopram 10–200 ng mL^{-1}, sertraline 30–200 ng mL^{-1}, chlorpromazine 30–150 ng mL^{-1}, imipramine 150–250 ng mL^{-1}, clomipramine 20–250 ng mL^{-1}, quetiapine 20–400 ng mL^{-1}, diazepam 200–500 ng mL^{-1}, fluoxetine 100–500 ng mL^{-1}, clozapine 300–800 ng mL^{-1}, carbamazepine 2000–12,000 ng mL^{-1}, and lamotrigine 3000–15,000 ng mL^{-1}). A mixed standard solution (100 ng mL^{-1}) was prepared for MEPS optimization. These solutions were stable for 45 days, at a temperature of −20 °C. The water used to prepare the mobile phase had been previously purified in a Milli-Q system (Millipore, São Paulo, Brazil).

2.2. Preparation of hybrid silica monoliths

The preparation procedures were based on the procedures reported in the literature [25,26] with some modifications.

The organic–inorganic hybrid silica monolith functionalized with cyanopropyl groups was prepared via a two-step catalytic sol–gel process as follows: the precursors, 55 μL of cyanopropyl-TEOS and 55 μL of TEOS, were added to a solution consisting of 90 μL of ethanol and 13 μL of acetic acid (2 mol L^{-1}) in an Eppendorf vial (1.5 mL); hydrolysis was performed at 60 °C, for 5 h. After that, 7 mg of N-dodecylamine was added to the solution, at room temperature. Then, 50 μL of this mixture was quickly inserted in a polyethyene conical tube (200 μL), for in situ polymerization. For the polymerization and condensation step, both ends of the tube were sealed with two pieces of rubbers, and the tube was incubated at 40 °C, for 15 h. Subsequently, the tube was extensively rinsed with ethanol, to remove N-dodecylamine and soluble hydrolysis products, which was followed by drying at 60 °C, for 48 h. This conical tube was properly connected to a glass syringe (3 mL) for MEPS.

The organic–inorganic hybrid silica monolith functionalized with aminopropyl groups using TEOS and APTES as precursors was prepared via one-step catalysis without any additional changes in pH. First, 56 μL of TEOS, 56 μL of APTES, and 5 mg of CTAB were dissolved in ethanol/water solution (107 μL/18 μL) and stirred at room temperature, for 30 s. Next, 50 μL of the pre-condensation mixture was quickly inserted in a polyethyene conical tube, sealed at both ends with two pieces of rubbers, and incubated at 40 °C, for 15 h. After that, the tube was flushed with ethanol and water, to remove porogen and residues, and dried at 60 °C for 48 h.

A frit (diameter of 3 mm, pore size of 2.0 μm) was placed on the top of the tube to prevent the sorbent from moving during the extractions. This conical tube (200 μL) was properly connected to a glass syringe (3 mL) for MEPS.

2.3. Characterization of hybrid silica monoliths

Scanning Electron Microscopy (SEM) helped to evaluate the morphological and structural aspects of the monolithic sorbent. To this end, samples were coated with gold in a Bal-Tec SCD050 Sputter coater instrument (Fürstentum Liechtenstein), for 180 s, and were then analyzed under a Zeiss EVO 50 scanning electron microscope (Cambridge UK). Identification of the chemical groups by Fourier Transform Infrared Spectroscopy (FTIR) was conducted on a Shimadzu- IRPrestige-21 spectrometer, in KBr pellets. Nitrogen sorption experiments were carried out at 77 K in a Micrometrics ASAP 2010 nitrogen sorption porosimeter. Specific surface areas were determined by the BET method.