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Determination of cocaine and its metabolites in plasma by porous membrane-protected molecularly imprinted polymer micro-solid-phase extraction and liquid chromatography–tandem mass spectrometry

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ABSTRACT

A selective molecularly imprinted polymer synthesized for the selective retention of cocaine (COC) and its metabolites [benzoylecgonine (BZE), ecgonine methyl ester (EME), and cocaethylene (CE)] was used as a solid adsorbent for assessing cocaine abuse by plasma analysis. The MIP beads (50 mg) were loaded inside a cone shaped device made of a polypropylene (PP) membrane for micro-solid-phase extraction (μ-SPE). High performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) was used for quantifying the analytes after MIP-μ-SPE. The best retention capabilities were reached when loading plasma samples (within the 0.1–5.0 mL range), previously adjusted to pH 5.5 by orbital-horizontal shaking (150 rpm, 50 °C) for 10 min. Analyte elution was achieved by subjecting the MIP-μ-SPE device to ultrasound (37 kHz, 325 W) with 10 mL of dichloromethane/2-propanol/ammonium hydroxide (76:20:4) for 8 min. After eluate evaporation to dryness and re-dissolution in 100 μL of mobile phase, the MIP-μ-SPE method yielded a pre-concentration factor of 50. Precision was assessed by intra-day and inter-day assays, and accuracy (intraday and inter-day analytical recovery, as well as the analysis of a BTMF 1/11-B control serum sample) show that the developed method is highly precise and accurate. In addition, the limits of detection, ranging from 0.061 ng mL^{-1} for COC to 0.87 ng mL^{-1} for BZE, were low enough for confirmative conclusions regarding cocaine abuse. The method was used for screening/quantifying cocaine and metabolites in plasma samples from poly-drug abusers.

1. Introduction

The assessment of organic compounds in clinical/forensic samples requires pre-concentration and clean-up stages before applying the most convenient instrumental technique. This is mainly attributed to the presence of large biomolecules and high concentrations of salts that interfere in the chromatographic separation/detection step. Regarding drugs of abuse, some substances/metabolites can occur at very low concentrations depending on the detection window (the time frame within which a drug can be detected since last use), and on other factors such as the preparation and route of administration, the duration of use (acute or chronic), the molecule or metabolite looked for, the pH and concentration of the matrix (urine, oral fluid), and the inter-individual variation in metabolic and renal clearance [1]. In general, blood (plasma/serum) offers better lower limits of detection and quantification than hair and urine, and most drugs of abuse must be detected at the low nanogram per milliliter level for 1 or 2 days [1]. Developed methods in the toxicological laboratory should therefore offer limits of detection lower than the cut-off values for confirming the abuse of a drug. As far as driving under the influence of drugs (DUID) is concerned, cut-off levels for cocaine (COC) in whole blood have been established at 61 ng mL^{-1} [2].

Improved extraction/pre-concentration sample pre-treatments involving small amounts of sample and hazardous organic solvents are therefore needed.

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Micro-solid-phase extraction (μ-SPE) techniques offer the high analyte separation/pre-concentration yields and clean-up inherent to conventional solid phase extraction (SPE), while permitting the use of small volume/mass of sample and extracting phase. Although SPE is widely used when assessing cocaine and its metabolites in biological samples [3], the development and application of μ-SPE techniques is scarce.

An appealing μ-SPE approach has been proposed by Basheer et al. [4]. It consists on holding the adsorbent material in a polypropylene (PP) membrane (porous membrane protected μ-SPE). The PP membranes are typically rectangular (2.0 × 1.5 cm) in shape, and the edges of the PP membrane are heat-sealed after each successive fold and after packaging the adsorbent material [4]. Analyte enrichment occurs by placing the μ-SPE device into a vial containing the sample and magnetically stirring. Proposals based on this design can be found in the literature for isolating contaminants from environmental and food samples. Solid sorbents such as multiwalled carbon nanotubes (MWCNTs) [4], C18 [5–7], and ethylsilane (C2) modified silica [8], rice husk modified to silica–Fe [9], amino and urea-grafted silica gel [10], SBA-15/polyaniline *para*-toluenesulfonic acid nanocomposite [11], synthetic zeolite imidazolate framework 8 (ZIF-8) [12–14], and metal-organic framework (MOF) MIL-101(Cr) [15] have been proposed.

Exploring new materials for enrichment is an exciting and active area of development in SPE, and selectivity of new materials is one of the key factors. Molecularly imprinted polymers (MIPs) have been shown to offer excellent selectivity for those compounds used as template molecules during the MIP synthesis [16,17]. Based on MIP technology, MIP-μ-SPE has been reported for triazines isolation from water by using MWCNTs coated with MIPs [18], for ochratoxin A extraction from coffee, grape juice and urine using a commercial MIP material [19], and for hyperoside and isoquercitrin assessment in rat plasma [20]. MIPs as adsorbent result adequate when analyzing complex samples such as clinical/forensic materials, and a synthesized MIP exhibiting high selectivity for cocaine and metabolites has recently been used as adsorbent in MIP-μ-SPE when analyzing urine [21]. The latter MIP-μ-SPE device is, however, a modification of the μ-SPE approach designed by Basheer et al. [4], in which the typical rectangular in shape device is changed to a conical configuration with only one heat-seal on the upper part of the cone. Drawbacks associated to PP membrane break-down through the heat-sealing by action of solvents such as dichloromethane when reusing the μ-SPE device is therefore avoided [21].

The aim of the current work has been to explore the possibilities of using a conical in shape MIP-μ-SPE device for the selective pre-concentration of COC and metabolites [benzoylecgonine (BZE), ecgonine methyl ester (EME), and cocaethylene (CE)] from blood samples before HPLC–MS/MS determination. Because the loading stage is assisted by mechanical stirring (orbital-horizontal shaking) instead of conventional magnetically stirring, up to 20 MIP-μ-SPE devices can be operated simultaneously, which increases the capability of the method when coping with a large number of samples. A pre-concentration factor of 50 and the use of HPLC–MS/MS lead to limits of detection lower than the cut-off values of COC in blood for confirmation analysis of cocaine abuse [2].

2. Materials and methods

2.1. Instrumentation

Analysis were performed with a 3200 Q TRAP LC/MS/MS system (ABSciex, Concord, Canada), equipped with a Flexar FX-15 UHPLC binary chromatographic pump (Perkin Elmer, Waltham, MA, USA), and a Flexar UHPLC autosampler (Perkin Elmer). COC, BZE, EME and CE separations were achieved by using a Phenomenex Kinetex 5 μ C18 100 Å (100 mm length × 2.10 mm i.d., 5.0 μm particle diameter) reverse phase column (Torrance, CA, USA) coupled to a Phenomenex C8 guard column (4 mm length × 3.0 mm i.d). Loading stage of the batch MIP-μ-SPE procedure was performed in a Rotabit orbital-rocking platform shaker (Selecta, Barcelona, Spain) placed inside a Boxcult temperature-controlled incubation chamber (Stuart Scientific, Surrey, UK). Elution was assisted by ultrasounds using a Raypa® Model UCI-150 ultrasonic cleaner bath (R. Espinar S.L., Barcelona, Spain) programmable for temperature and time (frequency of 35 kHz, and power of 325 W). MIP synthesis was performed using a low-profile roller from Stovall (Greensboro, NC, USA) placed inside the Boxcult temperature-controlled incubation chamber. The cone-shape PP envelope containing MIP beads was heat-sealed with a TN1010 heat-sealer from Siemens (Munich, Germany). A field emission scanning electron microscope Ultra Plus (Zeiss Oberkochem, Germany) was used for MIP characterization. Other laboratory devices were a Centromix centrifuge (Selecta), a Basic20 pH–meter with a glass–calomel electrode (Crison, Barcelona, Spain), a Reax 2000 mechanical stirrer (Heidolph, Kelheim, Germany), a vacuum pump (Millipore Co., Bedford, MA, USA), an oven model 207 (Selecta), a VLM EC1 metal block thermostat and N_2 sample concentrator from VLM (Leopoldshöhe-Greste, Germany), and an R-210 rotavapor equipped with a B-491 heating bath and a V-740 vacuum pump (Büchi Laboryechnik AG, Flawil, Switzerland).

2.2. Reagents

Ultrapure water, 18 MΩcm resistivity was obtained from a Milli-Q purification device from Millipore Co. Drug stock standard solutions were prepared from COC (1000 mg L^{-1} dissolved in acetonitrile), BZE (1000 mg L^{-1} dissolved in methanol), CE (1000 mg L^{-1} dissolved in acetonitrile), and EME (1000 mg L^{-1} dissolved in acetonitrile), purchased from Cerilliant (Round Rock, TX, USA). Deuterated cocaine (COC-d_3) stock standard solution in acetonitrile (100 mg L^{-1}) was from Cerilliant. Cocaine hydrochloride, used as a template, was from Sigma-Aldrich (Steinhelm, Germany). Ethylene dimethacrylate (EDMA), used as a monomer, and 2,2′-azobisisobutyronitrile (AIBN), used for free-radical polymerization, were purchased from Fluka (Buchs, Switzerland). Divinylbenzene-80 (DVB), used as a cross-linker, was from Sigma-Aldrich. MIP particles were enclosed inside a MIP-μ-SPE device made of PP prepared with ACCUREL® PP membrane (Membrana, Wuppertal, Germany). Acetonitrile and methanol (supragradient HPLC grade), ammonium acetate, neutral alumina, and sodium hydroxide were from Merck (Darmstadt, Germany). Potassium dihydrogen phosphate was from BDH (Poole, UK). Toluene, 2-propanol, ammonium hydroxide, and acetic acid 96% (m/m) were from Panreac (Barcelona, Spain). A control material, BTMF 1/11-B, for in vitro diagnostic use (drugs of abuse in serum, lyophilized) from ACQ Science (Rottenburg-Hailfingen, Germany) was used to assess accuracy. Other consumables used were: Durapore 0.20 μm membrane filters (Millipore), cellulose extraction thimbles (Filtros Anoia, Barcelona, Spain), and 0.20 μm cellulose acetate syringe filters (LLG, Meckenheim, Germany).

2.3. Plasma samples

Plasma samples were from polydrug abusers under control in an addiction centre in Santiago de Compostela, Spain (informed consent was obtained from the participants according to the approved protocol by the *Comité Ético de Investigación Clínica de Galicia*). Drug-free plasma samples (used for method validation) were obtained from the Blood Bank of Santiago de Compostela. For