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Matrix solid-phase dispersion on column clean-up/pre-concentration as a novel approach for fast isolation of abuse drugs from human hair

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ABSTRACT

A simple and fast sample pre-treatment method based on matrix solid-phase dispersion (MSPD) for isolating cocaine, benzoylecgonine (BZE), codeine, morphine and 6-monoacethylmorphine (6-MAM) from human hair has been developed. The MSPD approach consisted of using alumina (1.80 g) as a dispersing agent and 0.6 M hydrochloric acid (4 mL) as an extracting solvent. For a fixed hair sample mass of 0.050 g, the alumina mass to sample mass ratio obtained was 36. A previously conditioned Oasis HLB cartridge (2 mL methanol, plus 2 mL ultrapure water, plus 1 mL of 0.2 M/0.2 M sodium hydroxide/boric acid buffer solution at pH 9.2) was attached to the end of the MSPD syringe for on column clean-up of the hydrochloric acid extract and for transferring the target compounds to a suitable solvent for gas chromatography (GC) analysis. Therefore, the adsorbed analytes were directly eluted from the Oasis HLB cartridges with 2 mL of 2% acetic acid in methanol before concentration by N_2 stream evaporation and dry extract derivatization with N-methyl-tert-butylsilyltrifluoroacetamide (BSTFA) and chlorotrimethylsilane (TMCS). The optimization/evaluation of all the factors affecting the MSPD and on column clean-up procedures has led to a fast sample treatment, and analytes extraction and pre-concentration can be finished in approximately 30 min. The developed method has been applied to eight hair samples from poli-drug abusers and measured analyte concentrations have been found to be statistically similar (95% confidence interval) to those obtained after a conventional enzymatic hydrolysis method (Pronase E).

1. Introduction

Among different human materials used for toxicological and forensic analysis, hair offers the advantage of a substantially longer detection window (months to years) which enable retrospective investigation of chronic consumption. In addition, hair is a durable and stable matrix in which toxic substances are pre-concentrated and remain for a long time without significant alterations. Therefore, hair analysis for assessing drugs is a well established and recommended methodology in the forensic field. As reviewed by Pragst and Balikova [1], and by Hansen [2], there are numerous applications of hair analysis for assessing abuse drugs, and standardized hair testing approaches and official guidelines [3] are available for those laboratories dealing with toxicological studies.

Hair is considered a non-homogenous fiber with a complex structure [4] which determines the selective incorporation of certain compounds. In addition to the melanin content of the hair, which is one of the key factors controlling drug incorporation, the lipophilicity and the basicity of the drug also plays an important role [5]. This key factor affects the passive diffusion of drugs from blood capillaries into the growing cells, and uncharged (lipophilic) organic molecules penetration and diffusion in matrix cells is favored. However, it must be said that drug incorporation into the hair follows a multi-compartment model [5] and other important drugs incorporation ways into the hair such as absorption from sweat or sebum secretions, and also from deep skin compartments during hair shaft contribute significantly to the drugs incorporation into hair [6,7]. Other drugs, such as hydrophilic substances (molecules or ions), can reach the matrix cells after protonation (basic compounds) or deprotonation (acid compounds) [1,5]. Therefore, drug incorporation is a function of the pK_a of the compound and its melanin affinity [8,9], and it is facilitated at lower pHs of the matrix cells for basic drugs incorporation, and at higher pHs of the matrix cells for retaining acid drugs [10].

Because drug incorporation in hair is dependent on the lipophilicity and basicity of the drug, there are several extraction methods which are focused for isolating certain groups of substances from hair. Reviews on this topic show numerous extraction

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treatments mainly based on methanol, aqueous acids or buffer solutions as extracting solvents, alkaline digestions with aqueous sodium hydroxide, or enzymatic digestions (hydrolysis) [1,11,12]. Methods based on acidic hydrolysis have been reported to offer high yields for cocaine, opiates and their metabolites [13], although enzymatic hydrolysis are also recommended, mainly because the moderate pH and temperature conditions inherent to these procedures [14–19]. In addition, the possibility of speeding up the enzymatic hydrolysis procedures by using ultrasound irradiation has offered important practical advantages for shortening the whole analytical procedure [20]. Most of these sample treatment methods require a clean-up procedure for removing co-extracted substances present in the extracts. This is quite important mainly when using GC–MS as an analytical technique [21], and although several liquid–liquid and solid-phase extraction (SPE) procedures are available [1,21], the over-all procedure is long and difficult when analyzing large number of samples.

Other reported extractions for drugs are those based on supercritical fluid extraction (SFE) [22–24]. Although SFE is a quite expensive technique [1], it main advantage is the possibility of simultaneous extraction and clean-up stages [25,26]. This advantage is also offered by other modern extraction techniques such as pressurized liquid extraction (PLE) [25,26], sub-critical water extraction (SWE) or pressurized hot water extraction (PHWE) [27], and matrix solid-phase dispersion (MSPD) [28,29]. Among these extractive procedures, MSPD is simpler and cheaper because specific equipment is not required. In addition, this sample preparation procedure allows for reducing of solvent consumption, exclusion of sample component degradation, and improvement of extraction efficiency. Since the introduction of MSPD by Barker et al. [30], this technique has been used for extracting numerous organic compounds in quite different samples [28,29]. MSPD consists of sample architecture disruption by mechanical blending with a solid support bonded-phase [28,29,31], which leads after blending to a new sample matrix solid support phase in which analytes tend to be less strongly bonded. Therefore, analyte extraction can be easily performed by using less-toxic reagents/solvents (at low concentration and/or using low volumes), and under mild operating conditions (atmospheric pressure and room temperature). Therefore, integrity of target compounds is enhanced, and the procedure can be considered as an environmentally friendly method.

Although MSPD has been largely used for isolating numerous organic compounds [28,29,32], including organometallic species [33,34], the application for extracting drugs from forensic materials such as hair has not been tested yet. The objective of the current work has been the novel application of MSPD for extracting basic abuse drugs (cocaine, BZE, codeine, morphine and 6-MAM) from human hair samples. A clean-up procedure based on SPE was on column interfaced with the MSPD procedure for a fast abuse drugs isolation, clean-up and pre-concentration before GC–MS measurement. Variables affecting the MSPD process were fully studied by application of an experimental design approach.

2. Experimental

2.1. Apparatus

GC–MS analysis was performed with a Hewlett-Packard Model 6890 gas chromatograph (Hewlett-Packard, Avondale, PA), equipped with a HP-5 capillary column (30 m 0.22 mm I.D., 0.33 μm film thickness of cross-linked 5% phenyl methyl silicone) and a HP 5973 mass spectrometer as a selective detector. A Nahita glass mortar (50 mL capacity) with a glass pestle (Auxilab S.L., Beriáin, Navarra, Spain) was used for sample dispersion. Dispersed sample were packaged in 10 mL Injekt plastic syringes (Braun, Melsungen, Germany), between 10 mL polyethylene frits (Supelco, Bellefonte, PA, USA), and elution was forced by using a Visiprep TM DL vacuum manifold from Supelco (Bellefonte, USA). Other pieces of equipment were: 65 mm powder funnels from Barloworld Scientific (Stone, Staffs, UK), a Raypa UCI-150 ultrasonic cleaner bath (ultrasounds frequencies of 17 and 35 kHz and programmable for temperature and time) from R. Espinar S.L. (Barcelona, Spain), an ultracentrifuge Laborzentrifugen model 2K15 (Sigma, Osterode, Germany), an Orion 720A plus pH-meter with a glass–calomel electrode (Orion, Cambridge, UK), a Boxcult incubation camera (Selecta, Barcelona, Spain) coupled with an agitator Rotabit (Selecta), Univeba and Digiterm 3000542 thermostatic bathes (Selecta), a Reax 2000 mechanical stirrer (Heidolph, Kelheim, Germany), a VLM EC1 metal block thermostat and N_2 sample concentrator from VLM (Leopoldshöhe-Greste, Germany), and Oasis HLB syringes (3 cm^3, 60 mg) and Oasis HLB cartridges (225 mg) from Waters (Milford, MA, USA). Chemometrics package was Statgraphics Plus V 5.0 for Windows, 1994–1999 (Manugistics Inc., Rockville, MD, USA).

2.2. Reagents

Ultrapure water of resistance 18 MΩ cm^{-1} was obtained from a Milli-Q purification device (Millipore Co., Bedford, MA, USA). Pronase E, acetonitrile (gradient grade), methanol (gradient grade), 1,4-dithiothreitol (DTT), sodium hydroxide, potassium chloride, boric acid, acetic acid, chlorotrimethylsilane (TMCS) and N-methyl-tert-butylsilyltrifluoroacetamide (BSTFA) were from Merck (Poole, UK). Hydrochloric acid 37% was from Panreac (Barcelona, Spain). Ammonium hydroxide was from Scharlau (Barcelona, Spain). TRIS–hydroxymethyl-aminomethane (TRIS) was from Sigma–Aldrich (Stemheim, Switzerland). Diatomaceous earth, 95% SiO_2; C18 octadecyl-functionalized silica gel; and active magnesium silicate (Florisil), 60–100 mesh, used as dispersing agents, were from Aldrich Chemical Co. (Milwaukee, WI, USA). Alumina, aluminium oxide 90 active neutral (alumina N), 70–230 mesh (also used as a dispersing agent) was from Merck, while sea sand (washed) QP, SiO_2 was from Panreac (Barcelona, Spain). Drug stock standard solutions were prepared from cocaine, BZE, codeine, morphine and 6-MAM from Lipomed (Arlesheim, Switzerland). Deuterated drug stock standard solutions were prepared from cocaine-d_3 in acetonitrile, BZE-d_3 in methanol, codeine-d_3 in methanol, morphine-d_3 in methanol and 6-MAM-d_3 in methanol from Cerillant (Texas, USA).

2.3. Hair sample pre-treatment

Hair samples were obtained from poli-drug abusers from an addiction research centre in Santiago de Compostela. Hair about 2–3 cm long (approximately 0.5 g in weight) was cut with round-point scissors from the vertex posterior region of the scalp. To establish the limit of detection of the method drug-free scalp hair from laboratory staff volunteers was used.

All samples were decontaminated to remove residues of hair care products as well as sweat, sebum and dust typically present on hair, substances that can worsen the analytical noise/background ratio. In addition, the decontamination process also removes any drug potentially introduced through passive contamination [1]. Therefore, the decontamination procedure consisted of a mechanical stirring of hair in a diluted soap solution (physiological pH) for 30 min at room temperature, and finally, mechanical stirring with Milli-Q water several times. The successful removal of the external contamination of hair was proved through the negative result after the analysis of the last washing solution. The decontaminated hair samples were then oven dried at 40 °C for 24 h, and finally cut into small segments and pulverized in a vibrating zircon ball mill for 20 min. This last step ensures homogeneity of the sample. Pulverized hair specimens (mean particle size around 50 μm measured by