Journal of Pharmaceutical and Biomedical Analysis 61 (2012) 93–99

Contents lists available at SciVerse ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Determination of piperazine-type stimulants in human urine by means of microextraction in packed sorbent and high performance liquid chromatography-diode array detection

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ARTICLE INFO

Article history:
Received 6 October 2011
Received in revised form 4 December 2011
Accepted 6 December 2011
Available online 13 December 2011

Keywords:
Microextraction by packed sorbent
Piperazines
HPLC-DAD
Urine

ABSTRACT

A method using microextraction by packed sorbent (MEPS) and high performance liquid chromatography-diode array detection (HPLC-DAD) is described for the determination of piperazine-type stimulants in human urine. The studied compounds were 1-benzylpiperazine (BZP), 1-(3-trifluoromethylphenyl) piperazine (TFMPP), 1-(3-chlorophenyl) piperazine (mCPP) and 1-(4-methoxyphenyl) piperazine (MeOPP); 1-(2-chlorophenyl)-piperazine (oCPP) was used as internal standard (IS).

The factors which might influence the extraction were screened previously using the fractional factorial design approach, and none of them influenced significantly the process.

The procedure was linear for concentrations ranging from 0.1 (lower limit of quantitation – LLOQ) to 5 μg/mL, with determination coefficients (R^2) higher than 0.99 for all analytes in all runs. The limits of detection were 0.1 μg/mL for BZP and TFMPP, while for MeOPP and mCPP 0.05 μg/mL was obtained. Intra- and interday precision ranged from 1 to 14%, and accuracy was within a ±15% interval for all analytes, fulfilling the criteria normally accepted in bioanalytical method validation. Under the optimized conditions, extraction efficiency was higher than 80% for all analytes, except BZP (50%).

MEPS showed to be a rapid (<2 min) and simple procedure for the determination of piperazine-type stimulants in human urine, allowing reducing the handling time and costs usually associated to this type of analysis. Furthermore, the fact that only 0.1 mL of sample is required make this method a valuable and powerful tool for drug monitoring in human urine in situations where those compounds are involved, for instance in forensic scenarios.

1. Introduction

The term "designer drugs" refers to modified substances, both natural and synthetic, or completely designed molecular structures presenting psychotropic effects [1]. These new "designer drugs" can be divided into several subgroups, including piperazine derivatives [1], from which the most prominent are 1-benzylpiperazine (BZP), 1-(3-trifluoromethylphenyl)piperazine (TFMPP), 1-(3-chlorophenyl)piperazine (mCPP) and 1-(4-methoxyphenyl)piperazine (MeOPP) [2,3] (Fig. 1). Piperazine was originally used in veterinary medicine to treat parasitic infections, while its derivatives are extensively used as recreational drugs all over the world, despite their prohibition in several countries [4]. The mixture of BZP and TFMPP produces effects similar to those of MDMA [5,6]. The action of piperazine-type compounds involves serotonin (5-HT) uptake inhibition and 5-HT1 receptor agonistic effects [3,6], and they may act as stimulants producing euphoria [7]. The best pharmacologically characterized piperazine is mCPP, a known metabolite of the antidepressant drugs trazodone and nefazodone, which acts by increasing the extracellular levels of dopamine, serotonin and noradrenalin [7–9].

The elimination of piperazines occurs mainly through the kidney, and for the most lipophilic drugs (mCPP and TFMPP) less than 1% of the dose is eliminated unchanged in urine [10,11]. TFMPP is largely metabolized by the body and excreted in urine [8,12], with 4-hydroxy-TFMPP as its main metabolite [5,10]. Concerning BZP, it is not extensively metabolized, and is excreted unchanged in the urine [10].

Staack et al. [12] reported that some metabolites are excreted as sulphuric and/or glucuronic acid conjugates, and have been detected in urine 48 h after administration.

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doi:10.1016/j.jpba.2011.12.004

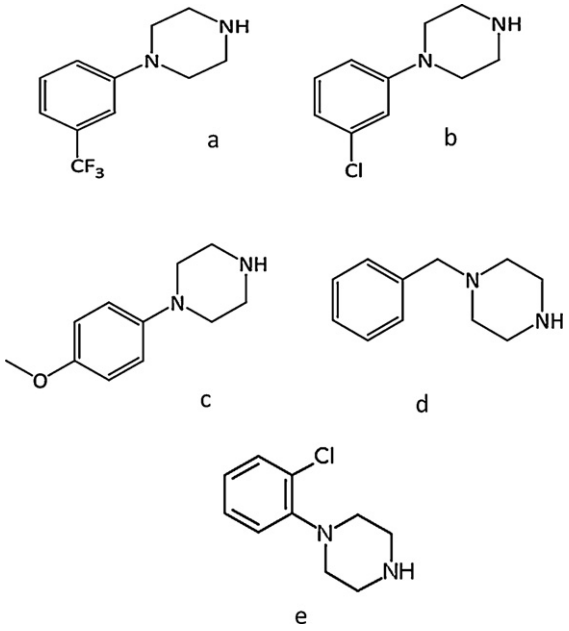

Fig. 1. Structural formulae of the studied piperazines: TFMPP (a), mCPP (b), MeOPP (c), BZP (d), and IS (e).

A few papers have been published on the analysis of those compounds in biological specimens, namely the detection of BZP, 4-methylenedioxibenzylpiperazine (MDBP), TFMPP, mCPP, and MeOPP in blood, plasma and urine [1,9,12,13]; and of TFMPP, mCPP and MeOPP in hair [7]. In those articles, sample preparation was performed mainly by means of liquid-liquid extraction (LLE) [9] or solid-phase extraction (SPE) [1,7]. However, those sample preparation procedures are often time-consuming, laborious and expensive; in addition, they present deleterious effects on the environment as well, due to the high amounts of organic solvents that need to be used and discarded.

Microextraction by packed sorbent (MEPS) appears to be a good alternative to solve those problems. This recent technique for sample preparation is based on the miniaturization of conventional SPE, using a gas-tight syringe as the extraction device; this allows the online coupling to either gas or liquid chromatographic systems without modifications in the extracting device [14,15].

Several applications have been published on the use of this technique, namely the detection of 2,4,6-trichloroanisole and 2,4,6-tribromoanisole in wine [16], organic pollutants, parabens and triclosan in wastewater [17,18], and polycyclic aromatic hydrocarbons in water [19]. Biological samples have also been analysed using MEPS, namely blood, serum or plasma for risperidone, antidepressants, olomoucine and ropivacaine [20–23], and urine for the detection of cotinine [24]. However, its application to the analysis of piperazines in biological samples has not been published yet. High efficiencies are usually obtained (60–90%) using MEPS, and it is possible to use a wide range of sample volumes. The extraction time is reduced, and the use of organic solvents is minimal [15,21–23]; furthermore, the packing material can be reused, allowing several extractions to be performed using the same device [15,23]. One feature concerning MEPS is that the extraction conditions should be optimized previously, in order to obtain maximum efficiency and less interference by matrix constituents. The number of factors that need to be optimized is often high, and therefore factorial design appears to be a good way of designing the whole experiment; indeed, it allows planning the entire procedure, aiming at investigating the effect of the controlled factors on the response [25]. In addition, the extent at which the several variables interact with each other can be also documented using a minimal number of experiments [25].

This paper describes the use of MEPS for sample preparation in the quantitative determination of piperazines (BZP, TFMPP, mCPP and MeOPP) in human urine, allowing reducing the sample preparation time to less than 2 min.

2. Experimental

2.1. Standards and reagents

The analytical standards of BZP dihydrochloride, TFMPP hydrochloride and mCPP were purchased from Lipomed (Arlesheim, Switzerland) as 1 mg/mL solutions; MeOPP dihydrochloride and oCPP hydrochloride (internal standard, IS) were purchased from Sigma–Aldrich (Steinheim, Germany). Ammonium formate and acetic acid (50% purity) were acquired from Sigma–Aldrich (Switzerland). Methanol (HPLC grade) was obtained from Merck Co (Darmstadt, Germany). Ammonium hydroxide (analytical grade) was obtained from J.T. Baker (Holland). Ultrapure water was obtained from a Milli-Q System (Millipore, Billerica, MA, USA). MEPS 100–250 μL syringe and MEPS BIN (Barrel insert and Needle) M_1 (4 mg; 80% C_8 and 20% SCX) (SGE Analytical Science, Australia) were purchased from ILC (Porto, Portugal).

Stock solutions of MeOPP and oCPP were prepared at 1 mg/mL by weighing 10 mg of the compound in a 10 mL volumetric flask, and filling up to volume with methanol. Working solutions at 10 and 1 μg/mL were prepared for all analytes by proper dilution of the stock solutions with methanol. A working solution of the IS at 10 μg/mL was prepared also in methanol. All these solutions were stored in amber glass vials and light protected between 2 and 8 °C.

To prepare the 5 mM ammonium formate solution (pH 6.4), 315.3 mg of ammonium formate was weighed in a volumetric flask and a final volume of 1 L was obtained with ultrapure water.

2.2. Biological samples

Drug-free urine samples were provided by laboratory staff.

2.3. Liquid chromatographic conditions

Analyses were carried out using an HPLC system (Agilent 1290 Infinity LC) equipped with an Agilent 1290 Infinity Detector (G4212A DAD). The piperazines were separated in a Zorbax 300 SB-C_{18} (5 μm, 4.6 mm × 150 mm) column (Agilent) at 25 °C with a mobile phase consisting of 5 mM ammonium formate (pH 6.4)-methanol (55:45, v/v), using the isocratic mode at a flow rate of 0.8 mL/min. The mobile phase was filtered under vacuum (0.2 μm hydrophilic polypropylene filter) and degassed in an ultrasonic bath before use. The monitored wavelengths were 211 for BZP, 208 for mCPP, 236 for MeOPP, and 246 nm for TFMPP and IS, and their retention times were, in minutes, 6.0, 7.8, 4.8, 9.8 and 7.4 respectively.

2.4. Sorbent activation

The sorbent was manually activated with 100 μL of methanol followed by 100 μL of water before its first use.

2.5. Sample preparation

For the optimization study, 4-mL aliquots of urine were spiked with all compounds at 4 μg/mL, slightly agitated for 15 min and stored light protected until extraction by MEPS.

The extraction procedure has been previously optimized (see Section 3.1), and the final conditions are the following.