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Determination of amphetamines in human urine by headspace solid-phase microextraction and gas chromatography

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Abstract

Solid-phase microextraction (SPME) is under investigation for its usefulness in the determination of a widening variety of volatile and semivolatile analytes in biological fluids and materials. Semivolatiles are increasingly under study as analytical targets, and difficulties with small partition coefficients and long equilibration times have been identified. Amphetamines were selected as semivolatiles exhibiting these limitations and methods to optimize their determination were investigated. A 100-μm polydimethylsiloxane (PDMS)-coated SPME fiber was used for the extraction of the amphetamines from human urine. Amphetamine determination was made using gas chromatography (GC) with flame-ionization detection (FID). Temperature, time and salt saturation were optimized to obtain consistent extraction. A simple procedure for the analysis of amphetamine (AMP) and methamphetamine (MA) in urine was developed and another for 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxy-*N*-methamphetamine (MDMA) and 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA) using headspace solid-phase microextraction (HS-SPME) and GC–FID. Higher recoveries were obtained for amphetamine (19.5–47%) and methamphetamine (20–38.1%) than MDA (5.1–6.6%), MDMA (7–9.6%) and MDEA (5.4–9.6%).

Keywords: Amphetamines

1. Introduction

Within only a decade of trials, solid-phase microextraction (SPME) has been established as a powerful method for sample preparation. SPME was originally developed (1989) aimed at the extraction of organic compounds from environmental samples and the subsequent analysis by GC. SPME offers significant advantages such as simplicity, low cost, compatibility with analytical systems, automation and solvent-free extraction. Furthermore, SPME has also been coupled to liquid chromatography and capillary electrophoresis and thus has been applied to the analysis of macromolecules in various biological samples such as, for example, urine, plasma and hair. As a result, in the last few years, SPME has been extensively applied in a broad field of analysis including food, biological and pharmaceutical samples. An ever-broader range of analytes has been analyzed by SPME: drugs of abuse, tricyclic antidepressants, steroids, alcohols, analgesics and so forth [1].

Amphetamines are a major class of central nervous system stimulants. Abuse of amphetamines and derivatives has increased dramatically recently, thus

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analysis of amphetamines becomes of increased interest in toxicology, occupational medicine and law enforcement. Amphetamines are frequently monitored in very complex matrices. HS-SPME minimizes interactions between the sample and the fiber and has proven useful for these analyses.

A number of publications have appeared recently addressing the usefulness of SPME for the analysis of amphetamines in biological matrices and most of them use headspace sampling but HS-SPME is still under investigation to optimize analytical conditions [2–7]. In this paper, several factors affecting analyte recovery were evaluated and the findings were applied to the optimization of amphetamine extraction from human urine by HS-SPME.

2. Material and methods

2.1. Materials

All reagents (Methanol, K_2CO_3, NaCl and KOH) were of analytical grade (Merck, Darmstadt, Germany). Stock solutions of AMP, MA, MDA, MDMA and MDEA were obtained from the United Nations International Drug Control Programme (Vienna, Austria) and prepared in methanol at concentrations, as bases, 0.62, 0.65, 0.69, 0.71 and 0.72 mg/ml, respectively.

SPME devices and 100-μm bonded PDMS fiber assemblies were purchased from Supelco (Bellefonte, PA, USA).

Drug-free urine samples collected from a healthy adult male were used to make amphetamine standard urine samples and used as control urine.

All samples were extracted from 9-ml clear glass vials, sealed with silicone septa and aluminium caps (Alltech, Deerfield, IL, USA).

2.2. Gas chromatography

Chromatographic analysis was carried out on a CE Instruments GC 8000 Top (ThermoQuest Italia, Rodano (MI), Italy) instrument equipped with a 30 m×0.25 mm I.D. AT-5 column (0.25 μm film thickness) (Alltech, Deerfield, IL, USA) and a FID. Data were stored and analysed on a C-R6A Chromatopac integrator (Shimadzu, Kyoto, Japan). Splitless injection was used and the carrier gas was helium at a flow-rate of 1 ml/min. The injector and detector temperatures were 220 and 280 °C, respectively. The oven temperature was held at 40 °C for 1 min and then increased to 280 °C at a rate of 20 °C/min, where the temperature was held for 5 min.

2.3. SPME sampling

The sample vial was prepared first by weighing an amount of solid salt into the empty vial. A urine sample was placed into the vial which was sealed rapidly with a silicone septum and an aluminium cap and heated at the appropriate temperature for a certain time using an aluminium block heater. The septum piercing needle of the SPME device was introduced into the vial and the fiber was exposed in the headspace for 15 min. Finally, the needle was removed from the vial and inserted into the heated injection port of the gas chromatograph (220 °C) for the desorption step, for 1 min. Fibers were thermally cleaned for 1 h at 250 °C daily.

3. Results and discussion

In preliminary experiments using HS-SPME sampling conditions from literature ([1] and references within), differences in recovery were found between urine samples spiked with AMP or MA on the one hand and those spiked with MDA or MDMA or MDEA on the other hand. So it was decided to develop an HS-SPME sampling procedure for AMP and MA and another procedure for MDA, MDMA and MDEA.

3.1. Development of the HS-SPME sampling procedure for AMP and MA

The sample vial was prepared by weighing 1 g K_2CO_3 into a 9-ml vial and 1 ml of urine spiked with AMP (1559 ng/ml, as base) was placed into the vial. The sample was heated at 60, 70 and 80 °C, respectively for 30 min. During the last 15 min, the metallic needle of the SPME device was introduced into the vial through the septum and the SPME fiber was exposed in the headspace. The spiked samples

Table 1
Recovery of AMP (1559 ng, as base) in spiked urine using HS-SPME at different temperatures

Temperature (°C)	Recovery of AMP (%)
60	25.9
70	15.9
80	6.8

were analysed in duplicate at each temperature. The average recoveries of AMP are shown in Table 1.

A heating temperature of 60 °C was adopted for further experiments, because of the higher analyte recovery.

The suitable time for exposing the fiber in the headspace above the sample was found to be 15 min, after testing three different times (5, 10 and 15 min). The sampling time of 15 min was adopted because of the improved precision.

Amphetamines are all semivolatile compounds and their volatilisation from a liquid matrix depends strongly on some basic parameters such as pH or salt saturation. Repeatable extraction recoveries can be obtained when these parameters are standardized. K_2CO_3 and NaCl (with NaOH) were tested for their salting-out effect. The recovery of AMP in the presence of K_2CO_3 (1 g/ml, complete saturation) was higher than that of NaCl (0.7 g/ml+100 μl of 1 *M* NaOH) and that of K_2CO_3 (0.7 g/ml) (Table 2). Each experiment was carried out in duplicate.

3.2. Calibration curves, recoveries and limits of detection in the HS-SPME method for analysis of AMP and MA

Blank urine spiked with AMP or AM at the concentration ranges shown in Table 3, were analysed using the following procedure: 1 ml sample+1 g K_2CO_3, heating temperature 60 °C, heating time 30 min, adsorption time the last 15 min. Each spiked sample was analysed in duplicate.

Calibration curves were constructed by plotting the mean peak area of the two measurements.

The calibration graphs were linear in the concentration range tested (125–3742 ng/ml) for AMP and (129–3876 ng/ml) for MA, respectively (Table 3).

The minimum detectable levels of AMP and MA in urine were 30 ng/ml for both compounds. The detection limits were calculated as the concentration of analytes in the sample, which gave a signal-to-noise ratio of three.

The recovery ranges of AMP and MA in spiked urine at various concentrations are presented in Table 3. Additionally, the selected SPME protocol resulted in effective sample clean-up as illustrated in Fig. 1,

Table 2
Recovery of AMP in spiked urine (1559 ng/ml, as base) using HS-SPME in the presence of K_2CO_3 or NaCl (+NaOH)

Sample	Temperature (°C)	Recovery (%)
Spiked urine+1.0 g/ml K_2CO_3	60	25.9
Spiked urine+0.7 g/ml K_2CO_3	60	14.8
Spiked urine+0.7 g/ml NaCl (+100 μl 1 *M* NaOH)	60	19.7

Table 3
Linear regression data, recovery range and detection limits for AMP and MA

Compound	Concentration range (ng/ml)	Regression line	Recovery range (%)	Correlation coefficient (*r*)	Detection limit (ng/ml)
AMP	125–3742	$y=876.11x+72\,577$	19.5–47.0	0.99664	30
MA	129–3876	$y=896.67x+77\,180$	20.0–38.1	0.99765	30

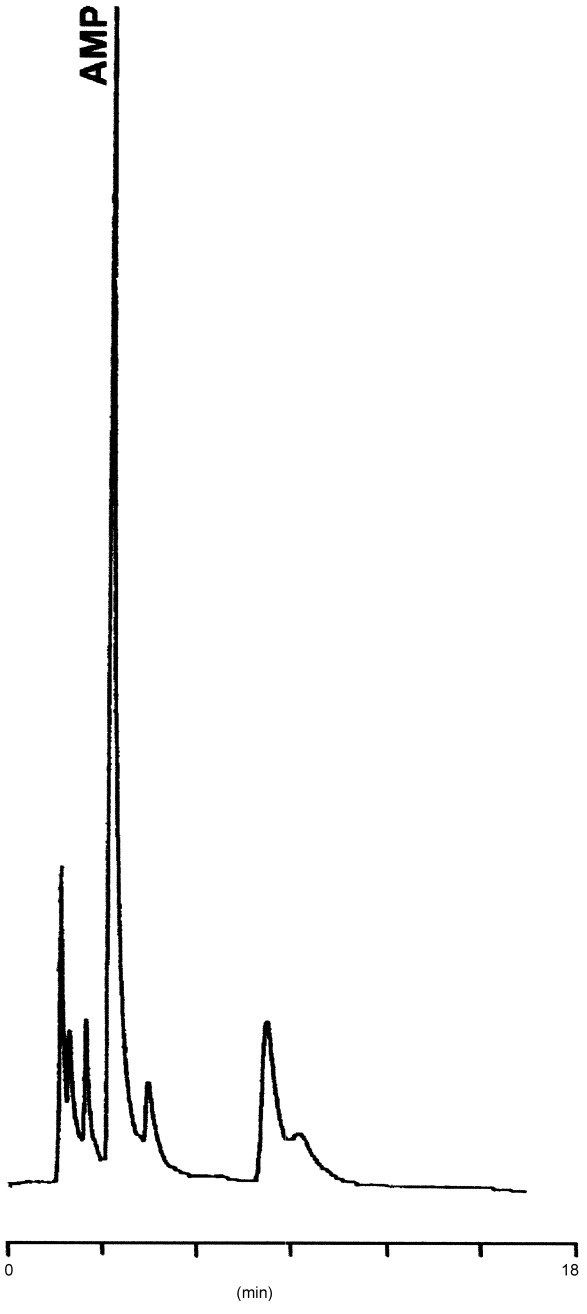

Fig. 1. GC–FID chromatogram of AMP. A urine sample containing 5000 ng AMP was analysed by the HS-SPME method. Conditions are given in the text.

where a representative chromatogram of a urine sample spiked with AMP is given.

3.3. Development of the HS-SPME sampling procedure for MDA, MDMA, and MDEA

Using the above method for AMP and MA, lower recoveries were found for MDA, MDMA and MDEA. The recoveries of the three compounds were very low but similar.

In order to establish the extraction conditions for these three compounds, a urine sample spiked with MDA was extracted under different conditions (heating temperature and sample volume) from those for AMP and MA.

Due to the very low recovery of MDA at 60 °C, a higher concentration of the urine spiked with MDA was used (2760 ng/ml, as base). The recovery was also low at 70 °C. So the heating temperature of the vial was set at 80, 90 and 100 °C for 45 min. The best results were obtained for 90 °C (Table 4). The spiked samples were analysed in duplicate at each temperature.

The sample volume was also checked in order to improve the sensitivity of the method. Thus, 1 g/ml K_2CO_3 was put into a 9-ml vial and 1, 2, 3 and 4 ml of urine spiked with MDA (2760 ng/ml, as base) were placed into the vial, respectively. Then, the vial was heated at 90 °C for 45 min. During the last 15 min, the metallic needle of the SPME fiber was exposed in the headspace. For each sample volume, two experiments were completed. The average recoveries of MDA are shown in Table 5.

The sample volume selected was 4 ml, because of the higher sensitivity. This sample volume also allowed enough room for the fiber to be positioned in the headspace above the sample, without contacting the sample.

Table 4
Recovery of MDA (2760 ng, as base) in spiked urine using HS-SPME at different temperatures

Temperature (°C)	Recovery (%)
80	3.3
90	6.6
100	2.6

Table 5
Recovery of MDA (2760 ng/ml, as base) in spiked urine using different sample volumes

Sample volume (ml)	Recovery of MDA (%)
1	6.6
2	4.1
3	2.9
4	5.5

3.4. Calibration curves, recoveries and limits of detection in the HS-SPME method for analysis of MDA, MDMA and MDEA

Blank urine spiked with MDA, MDMA and MDEA at the concentration ranges shown in Table 6, respectively, were analysed using the following procedure: 4 g K_2CO_3, 4 ml urine sample, heating temperature 90 °C, heating time 45 min, adsorption time the last 15 min. Each spiked sample was analysed in duplicate. The calibration curve was obtained by plotting the mean peak area.

Table 6 shows the calibration curves for MDA, MDMA, and MDEA. They showed linearity in the ranges tested. The minimum detectable levels of MDA, MDMA and MDEA in urine were 40 ng/ml for MDA and 35 ng/ml for MDMA and MDEA, respectively. The recovery ranges of MDA, MDMA and MDEA in spiked urine at various concentrations are presented in Table 6.

4. Conclusions

SPME is a rapid, solvent-free extraction technique and an alternative to traditional liquid–liquid and solid-phase extraction for sample preparation for analysis of amphetamines in biological samples. The objective of the present study was to achieve maximal analyte recovery using HS-SPME. So a simple method for the determination of AMP and MA in human urine has been developed with a separate method for the determination of MDA, MDMA and, MDEA.

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Table 6
Linear regression data, recovery range and detection limits for MDA, MDMA and MDEA

Compound	Concentration range (ng/ml)	Recovery range (%)	Regression line	Correlation coefficient (r)	Detection limit (ng/ml)
MDA	172.5–4312.5	5.1–6.6	$y=640.02x-82\,251$	0.99890	40
MDMA	177.0–4242.0	7.0–9.6	$y=829.10x+15\,943$	0.99885	35
MDEA	181.0–4519.0	5.4–9.6	$y=1173.60x-156\,690$	0.99709	35