Determination of Amphetamines in Hair by GC/MS after Small-volume Liquid Extraction and Microwave Derivatization

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We report here on the results of a procedure for the determination of amphetamine drugs in hair. The procedure is simple and sensitive. The results from the procedure using small-volume extraction matches perfectly with those either from using the derivatization method or selected ion monitoring (SIM) detection. We validated our method using four different amine drugs, including amphetamine, methamphetamine, methylenedioxy-amphetamine and methylenedioxy-methamphetamine. The detection limit for these drugs is about 50 ± 7.5 pg/mg in hair and the intra-day and inter-day reproducibility are within 15% at most drug concentrations. Moreover, we also showed the utility of the procedure in analyses of authentic hair samples taken from amphetamine abusers, and demonstrated that the method meets the requirement for the analysis of a trace amounts of amphetamines in human hair.

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Introduction

Hair has been recognized as an important biological specimen, along with urine and blood, for detecting drug abuses. It has several advantages, such as a long surveillance time window, easy sample collection, availability of repetitive sampling, and an excellent correlation between the "degree of addiction" and the actual drug consumption, over other types of specimen.^{1,2} Amphetamine (AM) and its derivatives are powerful stimulants of a central nervous system. The abuse of amphetamine, methamphetamine (MAM) and their methylenedioxy-derivatives, such as methylenedioxy-amphetamine (MDA), methylenedioxy-methamphetamine (MDMA) and methylenedioxyethyl-amphetamine (MDE) has increased enormously during the last few years, causing serious health and social problems. In response to a growing demand for reliable detection, a number of methods have been developed to provide reliable results for the identification and quantification of drugs and related compounds in biological matrices.³⁻⁶ However, detecting trace amounts of these substances in biological matrices remains to be a challenge for analytical chemists.

Analyses of drugs in hair involve a number of steps, any one of which is critical for the sensitivity of drug detection. First, hair is processed under an alkali, acidic condition,⁶⁻⁸ or with an enzymatic treatment.² This step is followed by either liquid-phase extraction (LPE),^{6,7,9} solid-phase microextraction (SPME),¹⁰⁻¹³ or supercritical fluid extraction (SFE).¹⁴ Second, concentrating and derivatizing the analytes, in order to increase the detection sensitivity, are performed.^{9,10} Lastly, the analytes are detected using gas chromatography/mass spectrometry (GC/MS),^{15,16} gas chromatography/mass spectrometry and mass spectrometry (GC/MS/MS),¹⁷ high-performance liquid chromatography (HPLC),^{6,9,18} and capillary electrophoresis

(CE).^{4,7} These methods require certain instrumentation setups, such as SPME or CE or HPLC, and are therefore limited for their wide applications, especially in routine case or field work.

The aim of this study was to develop a sensitive, low-cost and simple procedure to analyze trace amounts of amphetamines in human hair. We applied simply low-cost small-volume liquid extraction, followed by applying rapid and complete derivatization under microwave heating,¹⁹ and by using GC/MS for detection using selected ion monitoring (SIM) mode. With the perfect compatibility of small-volume liquid extraction and derivatization, both of which were conducted in the same solvent, as well as the combination of derivatization and SIM detection. This method was simple and of sufficiently high sensitivity with limits of detection (LOD) suitable for the determination of drugs below the cut-off usually adopted for hair analysis (0.1 ng/mg).⁷

In our procedure, we used a small volume (50 – 100 μ L), compared to a 10-fold volume in the reported procedure, of an organic solvent;^{4,5,9} We used microwave heating to shorten the derivatizing time to within 3 min, which is not applied widely, as reflected from the literature. Furthermore, in our procedure, there is neither a pH adjusting step that was used in acid extraction,^{5,8} nor phase-separating and solvent-evaporating steps, which were used in most hair-analysis procedures. Those steps are not only time-consuming, but also result in sample losses.^{4,5,9} Moreover, we demonstrated the utility of our procedure for analyzing hair samples from amphetamine abusers using small amounts of hair samples. The procedure is sensitive, and can be used to analyze trace amounts of amphetamines in human hair.

Experimental

Reagents and chemicals

N-Methyl-bis-trifluoroacetamide (MBTFA) was purchased from MACHEREY-NAGEL (Germany). 2-Methyl-benzylamine and heneicosane (C₂₁H₄₄) (internal standards) were purchased

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Table 1 Qualitative and quantitative selective ions in GC/MS detection

	AM-TFA	MAM-TFA	MDA-TFA	MDMA-TFA	2-Methyl-benzylamine-TFA (IS)
Retention time/min	6.17	7.61	10.34	11.67	7.51
SIM (<i>m/z</i>) Qualitative	91, 118, 140	110, 118, 154	77, 135, 162	135, 154, 162	91, 118
Quantitative	118	154	135	162	118

from ACROS ORGANICS (Belgium). AM, MAM, MDA, MDMA were all purchased from the Evidence Identification Center of China (Beijing, China). All reagents used in this work were of analytical grade.

Apparatus

GC/MS analysis was carried out on a GC-MS hyphenated instrument (Agilent Technologies). The ionizing voltage was set at 70 eV, and the scan range was from 40 to 450 *m/z*. A 30-m column with an internal diameter of 0.25 mm containing cross-linked and surface-bonded dimethyl-polysiloxane (DB-5) was used. The film thickness was 0.25 μm (Agilent). For gas chromatographic analyses, the temperature for both the injector and the detector were 250°C. The oven temperature was initially set at 100°C, holding steady for 1 min, followed by a ramp at a rate of 10°C/min to 210°C, then holding for 3 min. The solvent delay for a mass spectrometer was 3 min. Helium was used as a carrier gas at a flow rate of 1.0 mL/min. Splitless injection was used.

SIM application

Three selected ions with higher abundance and specificity in mass spectroscopy of derivatized standards were used for qualitative monitoring of the drugs. Among them, one ion for each drug was used for quantitative analyses. Table 1 lists the selective ions.

Hair sample preparation

Blank hair samples were obtained from a fifteen-year old male juvenile, who was free of illicit drug abuse. The hair samples were washed with a detergent to remove any lipids from the surface, and were rinsed with tap water, followed by distilled water to remove the detergent. The hair samples were then dried at room temperature and cut for the next steps. The blank hair samples were spiked with standard drugs with concentrations from 0.4 to 5 ng/mg, a typical range in drug users.²⁰ The same handling procedure was applied for hair samples taken from drug abusers.

Extraction procedures

About 20 mg of drug-spiked hair or real hair was accurately weighted, put into 4-mL glass vials and spiked with 2-methyl-benzylamine (IS), followed by the addition of 1 mL of 1 mol/L NaOH. The sealed vials were heated in a water bath at 70°C for 30 min to completely digest the hair and release the drugs. The digested hair solutions were transferred into 2-mL plastic centrifugal vials to remove any solid residuals, followed by the addition of 50 μL of chloroform into each solution. We also added a small amount of solid KCl to the solutions until they became saturated in order to increase the recovery and to eliminate emulsification. The solution mixtures were vortexed for 1 min, followed by centrifugation. The organic phases in the vials were drawn out directly with syringes for the analyses.

Preparation and derivatization of the samples

AM, MAM, MDA and MDMA were prepared at a

concentration of 1 mg/mL (calculated in term of the amount of free base) in methanol, and further diluted with methanol to 100 $\mu\text{g/mL}$ as stock solutions; heneicosane ($\text{C}_{21}\text{H}_{44}$), an internal standard (IS) solution used in derivatization examinations, was dissolved in methanol at a concentration of 100 $\mu\text{g/mL}$; 2-methyl-benzylamine (IS for hair analysis) was prepared at a concentration of 1 mg/mL in methanol as a stock solution. The derivatization was carried out in following steps: for standard samples, 5 ng of amphetamines were dissolved in 20 μL of chloroform in a 2-mL glass vial, followed by adding 10 μL of MBTFA. The vial was then sealed with sealing film and placed in a microwave oven to heat up under 300 W for 3 min. After derivatization, 1 μL of the reacted solution was drawn for the analyses using GC/MS.

Results and Discussion

Optimization of the derivatization under microwave heating

We examined factors that might affect the derivatization efficiency, such as derivatizing solvents, amount of MBTFA, power and time of microwave heating. The area ratio of the derivatized products to IS ($\text{C}_{21}\text{H}_{44}$), not reactive in the derivatization, was used as a parameter to calibrate the results. The derivatizations were tested in three different organic solvents, or a mixed solvent, they were chloroform, ethyl acetate and chloroform:pyridine with a 5:1 ratio. The derivatizations were the most efficient in chloroform:pyridine (5:1) or in chloroform. We chose chloroform as the derivatizing solvent.

The optimal ratio of MBTFA (μL) to the analyte (μg) was about one to one, set by trial-and-error experiments. The microwave power level and the duration of heating under the microwaves were set at 300 W for 3 min to achieve the maximum efficiency of derivatization without decomposing the products.

Selection of extraction solvent

The hair samples spiked with four amine drugs and IS were digested under an alkali condition. The extractions with organic solvents were conducted directly in the basic aqueous solutions with four organic solvents (they are toluene, cycle hexane, ethyl acetate, chloroform). Among them, there were no obvious differences in the extraction efficiency. We chose chloroform as the solvent for both extraction and derivatization, partially because of its greater density than water, allowing it to remain at the bottom of a tapered vial, which is very convenient for extraction, especially when handling with a very small volume. The ethyl acetate as a solvent is highly volatile, which makes quantification very difficult; 20, 40, 50, 100 and 200 μL portions of chloroform containing 1 $\mu\text{g/mL}$ $\text{C}_{21}\text{H}_{44}$ as an IS were tested for extractions. The results showed that the smaller was the volume used in the extractions, the higher were the ratios of extracted drugs to IS. Therefore, the higher was the sensitivity of analysis achieved. Considering the ease of sampling from the extract, we chose 50 μL for extraction.

Fig. 1 Chromatograms of GC/MS-SIM of a blank (a) and spiked hair (0.5 ng/mg) (b) after MBTFA derivatization. 1, AM-TFA; 2, IS-TFA; 3, MAM-TFA; 4, MDA-TFA; 5, MDMA-TFA.

Calibration curves using blank hair samples spiked with standards

We obtained calibration curves using the following procedures. First, a series of different amounts (1, 10, 20, 50, 100 and 200 ng) of the four amphetamine standards and 20 ng of 2-methyl-benzylamine as an IS were spiked into the same amount (20 mg) of washed blank hairs to prepare 6 calibrators. Second, the hair samples were digested in solutions containing 1 mol/L NaOH. Third, the drugs from the dissolved hair solutions were extracted with 50 μ L of chloroform, and 20 μ L of the chloroform phases of extractions were transferred into new glass vials. Those samples were then derivatized with 10 μ L MBTFA under microwave heating. Lastly, the derivatized solutions were injected directly into a GC/MS, and the relative selected ion peak area (peak area of an analyte divided by that of the internal standard) vs. the concentration of the analyte in hair was plotted. The limits of detection (LODs) were determined based on the concentration in hair corresponding to a signal-to-noise ratio of 3:1. The limits of quantification (LOQ) were the lowest concentration over the linear range. Figure 1 shows the chromatograms and Table 2 lists the results.

Reproducibility

The daily reproducibility at different drug concentrations (5 replicates for each concentration) in hair was examined by spiking the same amount of drug standards and IS in five aliquots of blank hairs. The samples were processed with the

Table 2 Regression lines and detection limits for amphetamines spiked in hair

Drug	Calibration curve ^a $y = ax + b$	R^2	Linear dynamic range/ ng mg ⁻¹	LOD/ pg mg ⁻¹	LOQ/ pg mg ⁻¹
AM	$y = 0.9106x - 0.0697$	0.9992	0.05 - 10	20	50
MAM	$y = 1.3265x - 0.0322$	0.9996	0.05 - 10	20	50
MDA	$y = 1.6543x - 0.0905$	0.9995	0.05 - 10	20	50
MDMA	$y = 0.5075x + 0.0571$	0.9996	0.05 - 10	20	50

a. y, Peak-area ratio; x, concentration of drugs in hair (ng/mg).

Table 3 Relative recoveries and reproducibility of amphetamines at different concentrations

Drug	Concentration/ ng mg ⁻¹ in hair	Relative recovery, %	Intra-day RSD, % (n = 5)	Inter-day RSD, % (n = 6, d = 3)
AM	5	96.5	2.6	6.6
	0.5	93.7	3.1	1.8
	0.1	96.6	2.2	6.4
MAM	5	95.8	7.2	4.1
	0.5	103.2	4.9	7.5
	0.1	94.24	11.2	18.2
MDA	5	102.4	1.8	5.1
	0.5	95.7	8.8	3.3
	0.1	102.7	13.5	6.9
MDMA	5	95.2	12.3	2.4
	0.5	97.1	10.0	3.7
	0.1	92.8	13.8	9

same procedures, and the peak-area ratios of the analytes to IS and the standard deviation of each analyte in hair were calculated. The inter-day reproducibility was examined within three days using 6 replicates of spiked hair samples at each concentration (Table 3). This procedure was repeated for different amounts of drugs to obtain the reproducibility at different drug concentrations. The relative standard variations (CV%) for all drugs at almost all concentrations were less than 15% except that at a concentration of 0.1 ng/mg in hair for methamphetamine; the RSD% was a slightly higher than the data (but less than 20%). The data generally met the requirement for trace amount analysis.

Relative recovery

Relative recoveries at different drug concentrations in hair were calculated using the area ratio of an analyte to IS in spiked hair samples divided by the ratio obtained from the same amounts of the drug standards; the results are given in Table 3. The results show that the data are around 100%, illustrating that the selection of IS was suitable for both the extraction and detection of the analytes. Losses in these processes were offset by the IS.

Real hair analyses of amphetamines abuser

Several hair samples from amphetamine drug abusers were collected and analyzed by the method presented in the paper. Methamphetamine was detected in most hair samples and its metabolite, amphetamine, was detected in some hair samples, depending on the amount of hair and the concentration of drugs in the hair. Amphetamine metabolized from methamphetamine was around 10%, varying from the degree of the methamphetamine addiction. The higher was the percentage, the higher was the

Table 4 Results of hair from abusers

No. of drug abuser	Hair amount/mg	Drug concentrations in abuser's hair/ng mg ⁻¹					
		MAM	AM	AM/MAM, %	MDMA	MDA	MDA/MDMA, %
1	20	12.26	0.77	6.28	—	—	—
2	20	33.8	1.09	3.2	—	—	—
3	1.5	2.1	0.22	10.5	—	—	—
4	4.6	3.2	0.29	9.1	—	—	—
5	7.7	0.24	—	—	—	—	—
6	8.9	1.52	—	—	—	—	—
7	22.2	1.11	—	—	—	—	—
8	6.5	1.08	—	—	—	—	—
9	21	0.45	—	—	—	—	—
10	4.6	21.98	1.52	6.91	1.49	0.33	22.1

Fig. 2 Chromatograms of GC/MS-SIM of a blank (a) and abuser's hair (b) after derivatization. 1, AM-TFA; 2, IS-TFA; 3, MAM-TFA.

degree of the addiction. The smallest amount of collected hair was only 1.5 mg (less than 10 cm in total length), in which the original drug of MAM, as well as its metabolite AM, were detected. The analysis results are listed in Table 4. Figure 2 shows chromatograms of analysis for a real hair abuser (No. 3 in Table 4).

Conclusions

We report here on a simple, economic and sensitive procedure

with good reproducibility for detecting amphetamines. Our procedure does not require laborious steps, such as pH adjusting, phase separation or solvent evaporation, used in most hair analysis procedures, which lead to sample losses and lower detection sensitivity. Hence, our procedure is more sensitive with a detection limit as low as 50 pg/mg in hair for the 4 amphetamines. In addition, our procedure is reliable, as demonstrated by its reproducibility with a relative standard deviation of less than 15%. Moreover, our procedure is simpler and much less solvent and time consuming, and is more practical for detecting trace amounts of the amine drugs in the hairs of addicts.

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