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Journal of Chromatography B, 670 (1995) 162–166

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Short communication

Simultaneous determination of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine in human hair by gas chromatography–mass spectrometry

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First received 24 January 1995; revised manuscript received 4 April 1995; accepted 4 April 1995

Abstract

A procedure is presented for the simultaneous identification and quantification of amphetamine (AP), methamphetamine (MA), methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) in human hair. The method involves decontamination of hair with dichloromethane and warm water, heat-alkaline hydrolysis in the presence of deuterated internal standards, liquid–liquid extraction and gas chromatography–mass spectrometry after derivatization with pentafluoropropionic anhydride–pentafluoropropanol. The limit of detection for AP, MA and MDA was 0.05 ng/mg using a 50-mg hair sample; for MDMA it was 0.1 ng/mg. Coefficients of variation ranged from 7 to 18%. This assay has been successfully utilized in the evaluation of the deposition of the drugs in hair obtained from various parts of the anatomy of a stimulant abuser.

1. Introduction

Whether or not drugs of abuse are present in the body is generally ascertained by testing a urine sample. Recently, hair testing was proposed as a basis for documenting chronic or repeated drug exposure [1–9]. The major practical advantage of hair testing compared with urine testing for drugs is its larger surveillance window: weeks to months, depending on the length of the hair shaft, versus 1 to 3 days. In fact, for practical purposes, the two tests complement each other. Urine analysis provides short-term information of an individual's drug use, whereas long-term histories are accessible through hair analysis. Moreover, in contrast to the qualitative information obtained from urine analysis, hair analysis provides quantitative information on the severity and pattern of an individual's drug use. The number of drugs which can be detected in hair is growing every day. However, only a few major drugs are of concern. The drug on which the most hair analysis work has been reported is cocaine, followed by morphine or opiates. Of the articles which address analyses of hair for the major drugs of abuse, almost all of those dealing with amphetamine have been written by Japanese researchers. In many cases, amphetamine (AP) and methamphetamine (MA) have been the target drugs [10–14]. Only one paper reports

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0378-4347/95/$09.50
SSDI 0378-4347(95)00160-3

the detection of methylenedioxymethamphetamine (MDMA) in hair [15]; not one mentions methylenedioxyamphetamine (MDA) testing. Moreover, as far as we are aware, there are no reports on the simultaneous detection of these drugs.

The purpose of this work was to develop a method based on gas chromatography–mass spectrometry (GC–MS) for the screening of AP, MA, MDA and MDMA in hair samples. Due to its sensitivity and specificity, GC–MS is the state-of-the-art for analysis for drugs of abuse.

2. Experimental

2.1. Chemicals

Methanol, ethyl acetate and dichloromethane were HPLC grade (Merck, Darmstadt, Germany). All other chemicals were analytical grade and supplied by Merck. AP, MA, MDA and MDMA were purchased from Sigma (St. Louis, MO, USA). AP-d_5, MA-d_5, MDA-d_5 and MDMA-d_5 were purchased from Radian (Austin, TX, USA). Pentafluoropropionic anhydride (PFP) and pentafluoropropanol (PFPOH) were purchased from Aldrich (Steinheim, Germany).

2.2. Sample extraction

Hair samples weighing at least 30 mg were cut as close as possible to the skin from the posterior vertex. The hair was decontaminated by washing the specimen according to the following procedure: in 5 ml dichloromethane for 2 min at room temperature, 5 ml warm water for 2 min and 5 ml dichloromethane for 2 min.

The protein matrix of the hair was destroyed by incubation in 1 ml of 1 *M* sodium hydroxide for 10 min at 95°C in the presence of 100 ng of the following internal standards: AP-d_5, MA-d_5, MDA-d_5 and MDMA-d_5. After cooling, the drugs were extracted with 10 ml ethyl acetate. After agitation (10 min) and centrifugation (10 min, 2000 *g*), the organic phase was purified by an additional acid extraction (5 ml of 0.2 *M* HCl) and the aqueous layer was re-extracted with 2 ml 1 *M* sodium hydroxide and 5 ml ethyl acetate. After agitation (10 min) and centrifugation (10 min, 2000 *g*), the organic phase was removed. A 20-μl aliquot of methanol–hydrochloric acid (99:1, v/v) was then added to ensure non-volatility of the drugs. After evaporation of the ethyl acetate, 50 μl of PFP and 30 μl of PFPOH were added to the dry extract, which was sealed and heated at 70°C for 30 min. After derivatization, the mixture was dried under nitrogen and reconstituted in 30 μl of ethyl acetate. Using an autosampler (HP 7673), 1.5 μl of the derivatized sample was injected into the GC–MS system.

2.3. GC–MS method

The GC–MS system consisted of a Hewlett Packard (5890) chromatograph with a mass selective detector (5989 B) operated at 70 eV with an ion source temperature of 250°C. The electron multiplier voltage was set at +400 V above the autotune voltage. The mass spectrometer was autotuned daily with perfluorotributylamine.

The flow-rate of carrier gas (helium, purity grade N 55) through the column (HP-5 MS capillary column, 5% phenyl–95% methylsiloxane, 30 m × 0.25 mm I.D., 0.25 μm film thickness) was 1.0 ml/min.

The column oven temperature was programmed from an initial temperature of 60°C to 290°C at 30°C/min and maintained at 290°C for the final 2 min. Splitless injection with a split valve off-time of 0.75 min was employed. The injector temperature was 260°C.

Table 1 shows the ions monitored for the different drugs and the deuterated internal standards (I.S.) and the retention times (t_R). Analytes were identified and quantified on the basis of comparison of retention times and the abundance of two confirming ions relative to the deuterated internal standards.

Standard calibration curves were obtained by adding 10 (0.2 ng/mg), 25 (0.5 ng/mg), 50 (1.0 ng/mg), 100 (2.0 ng/mg), 500 (10.0 ng/mg) and 2500 (50.0 ng/mg) ng of pure standards, prepared in methanol, to 50 mg of pulverized blank

Table 1
Selected ions and retention times

Compound	Retention time (min)	Ion (undeuterated) (*m/z*)	Ion (deuterated) (*m/z*)
Amphetamine–PFP	6.84	*190*, 118	*194*, 123
Methamphetamine–PFP	7.58	*204*, 160	*208*, 163
MDA–PFP	8.93	*325*, 135	*330*, 136
MDMA–PFP	9.59	*204*, 339	*208*, 341

The ions italicised were used for quantification.

control hair (obtained from laboratory personnel; previously tested and found to be drug-free).

Recovery and inter-day precision were determined by adding 100 ng of pure standards to 50 mg of pulverized blank control, corresponding to 2 ng/mg hair.

Correlation coefficients were typically >0.994 for all analytes. The inter-day precision ($n = 6$), recovery and limit of detection for each drug is presented in Table 2.

3. Results and discussion

Under the chromatographic conditions used, there was no interference with the drugs or the internal standards by any extractable endogenous materials present in hair. Responses for all compounds were linear in the range 0.2–50.0 ng/mg of hair.

The limits of detection for extracted AP, MA and MDA were 0.05 ng/mg (signal-to-noise ratio >3) using a 50-mg hair sample. The limit of detection of MDMA was 0.1 ng/mg.

The accuracy, expressed as the error relative to the least-squares equation, was assessed by passing six replicate samples of three concentrations of the four drugs through the entire procedure in one analysis day. The concentrations tested were 1.0, 2.0 and 10.0 ng/mg, respectively, and the accuracy of the method was in the range 6.1 to 17.9%.

Taking into account our previous considerations, the positive cut-off value for all the drugs was set at 0.5 ng/mg [16]. This positive cut-off was found to exclude external contamination satisfactorily.

Because of the use of deuterated internal standards, the deviations caused by the loss of sample during the extraction were low. As demonstrated by Moeller [5], the use of deuterated standards represents the state of the art in hair testing. Molecular ions were used for quantifying the target drugs with the exception of MDMA, where the molecular ion was not strong enough. The major ions observed in the spectra of MDA and MDA-d_5 were m/z 135 and 136, respectively, which were considered unsuitable for quantification due to the risk of overlap.

For pH adjustment, alkaline hydrolysis proved

Table 2
Inter-day precision, recovery and limit of detection of each drug

Drug	Inter-day precision (%)	Recovery (%)	Limit of detection (ng/mg)
Amphetamine	7.0	78.6	0.05
Methamphetamine	17.4	79.4	0.05
MDA	9.1	81.6	0.05
MDMA	14.6	75.9	0.1

more suitable than acid or enzymatic hydrolyses, leading directly to an optimum extraction pH.

Hair samples from the head, axillae, pubis and leg were collected simultaneously from a male subject, aged 24 years, known to be a stimulant abuser. AP, MDA and MDMA were detected in all hair samples, clearly indicating chronic exposure. Typical chromatograms are presented in Figs. 1, 2 and 3. Results are presented in Table 3. The highest drug concentration was found in leg hair, followed by (in descending order) hair of the head, pubic hair and axillary hair. In the literature, reports on opiates and cocaine in hair, the highest drug levels were observed in pubic hair, followed by head hair and the axillary hair [17,18]. No data have been published to date on drug detection in leg hair. Only Cone et al. [19] have demonstrated that cocaine concentrations tend to be higher in arm hair than in head hair samples.

As yet, no literature data on the occurrence of stimulants in axillary, pubic and leg hair are available. Therefore, a comparison can only be made with data for head hair. AP concentration

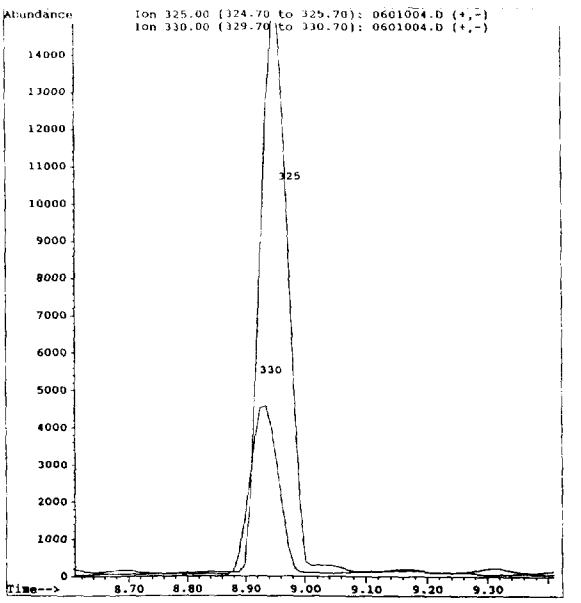

Fig. 2. SIM chromatogram of leg hair extract. MDA concentration: 12.50 ng/mg. Time in min.

was within the range of previously published concentrations [10–14]. Moeller et al. [15] reported a concentration of 0.6 ng/mg of MDMA

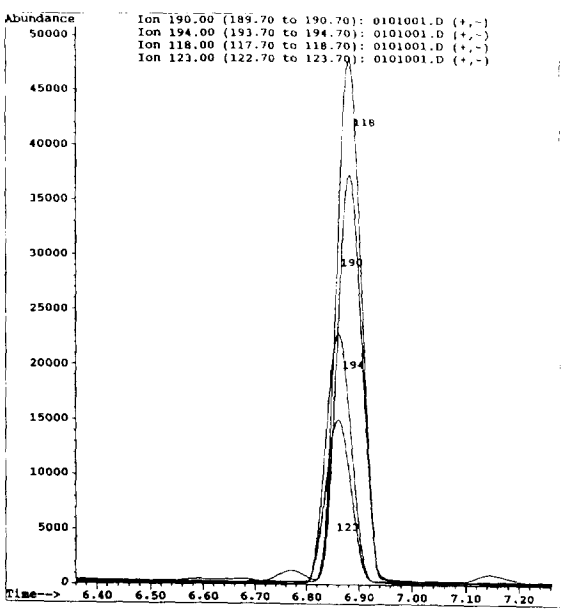

Fig. 1. Selected-ion monitoring (SIM) chromatogram of pubic hair extract. Amphetamine concentration: 6.35 ng/mg. Time in min.

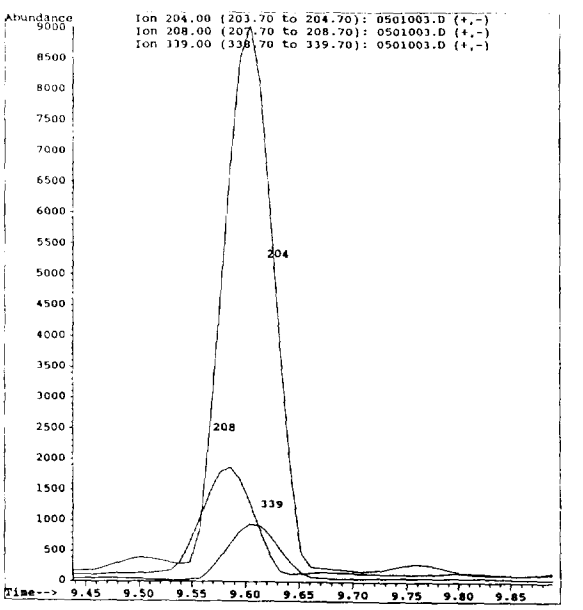

Fig. 3. SIM chromatogram of axillary hair extract. MDMA concentration: 16.17 ng/mg. Time in min.

Table 3
Stimulant concentrations in hair of the head, axillary, pubic and leg regions of one subject

Samples	Concentration (ng/mg)		
	Amphetamine	MDA	MDMA
Head	10.16	7.96	53.38
Axillae	2.65	2.07	16.17
Pubis	6.35	4.19	35.37
Leg	15.60	12.50	67.62

in a criminal case, where the suspect committed a murder under the influence of the drug. To the best of our knowledge, no data are available on MDA testing in hair.

The proposed method is highly specific, sensitive and precise for the simultaneous determination of AP, MA, MDA and MDMA in human hair. It is clear that analysis for the presence of stimulants in hair is a valid means of determining stimulant use history.

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