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Interlaboratory comparison of quantitative determination of amphetamine and related compounds in hair samples

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Abstract

Testing human hair for drugs of abuse is a relatively new technique which requires control before being fully accepted in justice applications. Laboratories must be able to demonstrate that they can accurately determine what drugs are present in unknown hair samples and at what levels. To date few exercises have been organized in USA, Germany and France, all devoted to opiates, cocaine and cannabis. However, the number of drugs which can be detected in hair is growing every day. Among them, amphetamine and related compounds, such as MDMA, are of major interest due to increasing abuse. At the initial stage of this work, four different preparation procedures were used to test amphetamine, MDA and MDMA. Direct methanol extraction, acid (HCl 0.1 N), alkaline (NaOH 1 N) and enzymatic (β-glucuronidase/arylsulfatase) hydrolyses were compared. Best recoveries were observed after alkaline hydrolysis. The same hair sample was powdered and sent to 16 laboratories, in USA (4), Germany (6), France (3), Spain (1), Japan (1) and Korea (1) to test amphetamine, methamphetamine, MDA and MDMA. All laboratories returned results within 3 months. Amphetamine tested positive 13 times with concentrations ranging from 3.3 to 17.5 ng/mg. Only 2 laboratories identified methamphetamine, using GC/MS, at low concentration (0.8 and 1.8 ng/mg), which appears to be a false positive. MDA and MDMA both tested positive in 14 cases, with concentrations ranging from 1.8 to 19.5, and 8.9 to 100.0 ng/mg for MDA and MDMA, respectively. These scattered results clearly indicated that new exercises are needed to ensure quality in hair testing. This is one of the major aims of the Society of Hair Testing.

Keywords: Hair; Amphetamine; Methylene-dioxymethamphetamine; Quality control

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1. Introduction

A critical element in the acceptance of hair analysis for detection of drugs of abuse is laboratory performance. Laboratories must be able to demonstrate that they can accurately determine what drugs are present in unknown hair samples and at what levels.

To date, few exercises have been organized in USA [1–3], Germany [4–6], Spain [7] and France [8,9]. Interlaboratory comparisons of hair analysis have been published for opiates, cocaine and cannabis. In most cases, GC/MS was used for the analyses. However, no extraction method could be identified as being superior to the others. To the best of our knowledge, no report documents an international comparison of amphetamines testing results nor a comparison between analytical procedures. The present paper presents data for these two subjects.

2. Experimental

2.1. Specimen

Hair samples from the head were collected from a male subject, aged 24 years, known to be a stimulant abuser and deceased from fatal methylenedioxymethamphetamine overdose. The strands of hair were cut as close as possible to the skin from the posterior vertex, and stored at room temperature. Ten grams constituted the hairpool to assure sufficient material for the comparisons and a reserve stock for re-analysis.

The hair was decontaminated by washing the specimen two times in 5 ml dichloromethane for 2 min at room temperature. The strands of hair were then pulverized in a ball mill, in order to obtain 5 g of homogenous material.

2.2. Materials

Methanol, ethylacetate and dichloromethane were HPLC grade (Merck, Germany). All other chemicals were analytical grade and supplied by Merck. Amphetamine (AP), methamphetamine (MA), methylenedioxyamphetamine (MDA), and methylenedioxymethamphetamine (MDMA), along with their deuterated (d_5) analogous were purchased from Radian (Austin, USA). Heptafluorobutyric anhydride and β-glucuronidase (12 units/ml)/arylsulfatase (60 units/ml) were purchased from Sigma (St-Louis, USA) and Merck (Germany), respectively.

2.3. GC / MS analysis

For GC/MS analysis, the samples were derivatizated with 90 μl heptafluorobutyric anhydride/ethyl acetate (2:1, v/v), 20 min at 70°C. The samples were evaporated under a stream of nitrogen, then dissolved in 30 μl ethyl acetate. A 1-μl portion was injected into a Hewlett Packard GC/MS system, which consisted of a HP 5890 chromatograph coupled with a mass selective detector (5989 B). The

flow-rate of carrier gas (helium, purity grade N 55) through the column (HP-5 MS, 30 m × 0.25 mm i.d.) 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 was employed (off-time: 0.75 min). The injector temperature was 260°C.

For quantitative determinations, the following ions were used: m/z 240 (AP), 244 (AP-d_5), 254 (MA), 258 (MA-d_5), 375 (MDA), 380 (MDA-d_5), 254 (MDMA), and 258 (MDMA-d_5)

2.4. Alkaline hydrolysis [10]

A 30-mg quantity of powdered hair was incubated in 1 ml of 1 M sodium hydroxide for 10 min at 95°C in the presence of 100 ng of 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 and centrifugation, the organic phase was removed. A 50-μl aliquot of isopropanol–hydrochloric acid (99:1, v/v) was then added to ensure non-volatility of the drugs and the mixture evaporated to dryness.

2.5. Enzymatic hydrolysis [11]

A 2-ml Soerensen-buffer solution (pH 7.6) was added to about 30 mg of powdered hair together with the internal standards and 75 μl β-glucuronidase/arylsulfatase. The solution was incubated for 2 h at 40°C, and centrifuged. The supernatant was extracted as in Section 2.4.

2.6. Acid hydrolysis

About 30 mg of the powdered hair sample was incubated in 1 ml 0.1 M hydrochloric acid for 18 h at 50°C in presence of the deuterated standards. After centrifugation, the supernatant was alkalinized with NaOH and extracted as in Section 2.4.

2.7. Methanol sonication [12]

A 100-mg quantity of the powdered hair and the internal standards were extracted with 5 ml methanol in an ultrasound bath at 45°C for 4 h. After centrifugation, the methanol was decanted and evaporated.

3. Results and discussion

Testing human hair for amphetamine and related compounds is generally achieved after alkaline hydrolysis. In 1995, Nakahara [13] presented an excellent review on all the analytical procedures described in the literature.

Hair findings, following the analysis of the same specimen with four different procedures are presented in Table 1. The specimen was positive for AP, MDA and

Table 1
Comparison ($n = 3$) of four extraction procedures to test amphetamine and related compounds in the same hair specimen (all concentrations are in ng/mg)

Procedure	AP	MDA	MDMA
Alkaline hydrolysis	7.29 ± 0.59	7.49 ± 0.34	41.33 ± 0.24
Enzymatic hydrolysis	7.08 ± 0.41	5.64 ± 0.64	35.35 ± 1.41
Acid hydrolysis	6.79 ± 0.51	6.62 ± 0.20	40.33 ± 0.84
Methanol incubation	6.12 ± 1.28	3.75 ± 0.17	33.03 ± 0.84

MDMA. All the experiments were done in triplicate. Although not statistically different, higher concentrations were observed after alkaline hydrolysis. Lower concentrations were observed after methanol direct incubation, but the latter procedure allows a toxicological screening of the sample [12]. Based on the results, it is not possible to determine which method performed best.

The same powdered sample was sent to 16 international laboratories (Table 2), in France, Germany, Spain, Japan, Korea and United States. Laboratories were asked to test quantitatively the sample for AP, MA, MDA and MDMA and to send back their results along with the analytical procedure. All of them returned results within 3 months. Results are summarised in Table 3.

Two participants detected MA, which, obviously is a false positive. Three laboratories failed to identify AP, however, in detectable amount. AP is generally found in hair in the range 0.5–120 ng/mg [13].

Table 2
List of participating laboratories

Laboratory	Location
Institut de Médecine Légale	Strasbourg, Fance
Toxlab	Paris, France
Institut de Recherche Criminelle de la Gendarmerie	Rosny sous Bois, France
Instituto Nacional de Toxicologia	Sevilla, Spain
Bayerisches Landeskriminalamt	Munchen, Germany
Zentrum der Rechtsmedizin	Frankfurt, Germany
Institut für gerichtliche Medizin	Berlin, Germany
Institut für Rechtsmedizin	Heidelberg, Germany
Institut für Rechstmedizin	Munchen, Germany
Institut für Rechtsmedizin	Homburg, Germany
National Institute of Health Sciences	Tokyo, Japan
National Medical Services	Willow Grove, USA
Department of Health and Human Services	Baltimore, USA
Psychemedics Corporation	Culver City, USA
Federal Bureau of Investigation	Quantico, USA
National Institute of Scientific Investigation	Seoul, Korea

Table 3
Results of the international comparison

Parameter	Amphetamine	Methamphetamine	MDA	MDMA
Number of participants	16	16	16	16
Number of positive results	13	2	14	14
Number of quantification	13	2	13	13
Mean ± S.D. of positive results	7.6 ± 4.1	1.3 ± 0.7	6.9 ± 5.4	39.5 ± 23.6
Median of positive results	10.4	1.3	11.0	51.6
Range of positive results	3.3–17.5	0.8–1.8	2.5–19.5	3.3–100.0

Table 4
Summary of analytical procedures used and concentrations measured (ng/mg)

Procedure	Number of laboratories	AP	MDA	MDMA
Alkaline hydrolysis	4	5.5–13.4	4.9–16.4	8.9–45.0
Acid hydrolysis	3	ND–10.2	3.6–6.2	41.0–100.0
Enzymatic hydrolysis	1	5.0	4.6	34.0
Methanol incubation	4	ND–17.5	1.8–19.5	3.3–54.4
Methanol-HCl	2	3.3–6.8	2.5–7.2	45.1–49.5

ND, not detected.

All the participants used GC/MS, with various analytical procedure (Table 4). Only two laboratories did not present their method.

The results demonstrate that neither approach consistently provided more precise results. However, the spread of the results was worse when compared with results published for opiates or cocaine [1–6,8]. These scattered results clearly indicated that new exercises are needed to ensure quality in hair testing. This is one of the major aims of the Society of Hair Testing.

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