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Forensic Science International xxx (2009) xxx-xxx



Contents lists available at ScienceDirect

### Forensic Science International



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

### Rapid communication

## Distribution of methamphetamine and amphetamine in drug abusers' head hair

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#### ARTICLE INFO

Article history: Received 19 February 2009 Received in revised form 27 April 2009 Accepted 6 May 2009 Available online xxx

Keywords: Hair Methamphetamine Amphetamine Concentration Metabolite-to-parent drug ratio

#### 1. Introduction

Recently, analysis of hair for drugs has been expanded its application in forensic and clinical toxicology. Hair has been noted as a valuable specimen for diagnosis of drug abuse, tolerance, compliance and gestational drug exposure, etc. However, the interpretation of hair results is not as straightforward as that of other specimen's results such as blood. This is due to individual variations not only in drug metabolism but also in physiology (e.g. hair growth rate, melanin contents) and life style differences such as shampoo washing, cosmetic treatment (e.g. dyeing, bleaching), exposure to sunlight and so on. Due to these properties the relationship between the actual amount of drug taken and the quantitative hair results is very complicated [1,2].

In Korea methamphetamine (MA) is the most abused illegal drug and its analytical result in hair is routinely submitted to court as evidence; however, the hair result is often an issue in court due to complication and ambiguity of interpretation. Moreover, the recent introduction of a jury into the Korean legal system means that forensic analytical results require reasonable explanation to non-professionals.

In order to conclude findings from hair analysis as "detected" or "not detected" concentrations of parent drugs and metabolites and their ratios should be considered first. Many studies have been conducted mainly on the metabolite-to-parent drug ratios [2–5]. For MA abusers Han et al. [5] reported the concentrations and

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ABSTRACT

In order to aid the interpretation of hair results from methamphetamine (MA) abusers the MA and amphetamine (AP) concentrations in 2070 hair samples were statistically evaluated. The MA and AP concentrations in hair were put into three groups arbitrarily representing low, medium and high ranges and the metabolite-to-parent drug ratios of each group were examined. The concentration ranges proposed here were also applied to the interpretation of five authentic cases. The low, medium and high ranges of MA were 0.5–4.2, 4.2–24.5 and 24.5–608.9 ng/mg and those of AP were 0.1–0.4, 0.4–1.7 and 1.7–41.4 ng/mg. The AP-to-MA ratios showed large variation but a tendency that it decreased as the MA ranges increased. This evaluation was very useful to presume the severity of individuals' MA abuse and to provide law enforcement agencies more understandable information. It could also facilitate the court's decision regarding specific circumstances surrounding the drug-related crimes.

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ratios of MA and amphetamine (AP) in 2444 cases. The concentrations of MA and AP ranged from 0.51 to 193.75 ng/mg and from 0.13 to 13.39 ng/mg, respectively in head hair. The ratios showed large variation and ranged from 0.004 to 1.16, decreasing as the MA concentration increased.

Until now, any reliable reference for drug concentrations in hair has not been published except a statistical evaluation for illegal heroin consumers suggested by Jurado and Staub [6]. They proposed that every laboratory should establish concentration ranges based on its own experimental method because quantitative hair results are affected by the difference of methods. Another group has recently suggested reference ranges for heroin abusers with its own data using the same statistical evaluation. This group concluded that the reference ranges give a good indication for the presumed severity of drug abuse, but other factors including drug concentrations, the ratio of metabolite-to-parent, the possibility of absorption from sweat and environmental contamination should also be considered [2].

In the current study hair results collected for last three years were evaluated using the same statistical manner. The MA and AP concentrations in hair were put into three groups arbitrarily representing low, medium and high ranges and the metabolite-toparent drug ratios of each group were examined. Furthermore, the concentration ranges proposed here were applied to the interpretation of five actual cases.

#### 2. Materials and methods

#### 2.1. Data collection

According to our SOP, a MA concentration greater than the proposed cut-off value (0.5 ng/mg) [7] and an AP concentration greater than the limit of detection

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Please cite this article in press as: S. Lee, et al., Distribution of methamphetamine and amphetamine in drug abusers' head hair, Forensic Sci. Int. (2009), doi:10.1016/j.forsciint.2009.05.004

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(LOD), with appropriate compound ion ratios, are required to prove MA use by an individual. Data from 2070 head hair samples from cases of illegal MA use from 2006 to 2008 were collected, which corresponded with these criteria. Some of the head hair samples were segmented according to police request. In total 2355 segments of head hair of various lengths were analyzed for this study.

#### 2.2. Sample preparation

All hair samples were analyzed using a fully validated method described previously [8]. In summary, the hair samples were washed, cut, weighed accurately (ca. 10 mg) and agitated in 1% HCl in methanol for 20 h at 38 °C with MA-d<sub>5</sub> and AP-d<sub>5</sub> as internal standards. Then, the hair extract was evaporated to dryness at 45 °C under N<sub>2</sub> gas and the residue was derivatized with 100  $\mu$ l of trifluoroacetic anhydride (TFAA)/ethyl acetate (1:1) and incubated at 65 °C for 15 min. The excess derivatizing reagent was removed under N<sub>2</sub> gas at 45 °C and the residue was reconstituted in ethanol for gas chromatography–mass spectrometry (GC–MS) analysis.

#### 2.3. GC/MS analysis

Analysis was performed using an Agilent 6890/5973 GC–MS system. The MS was operated in selected ion monitoring (SIM) mode. The TFAA derivatized ions for MA, AP, MA-d<sub>5</sub> and AP-d<sub>5</sub> were as follows: MA, m/z 154, 118, 110, 91; AP, m/z 140, 118, 91; MA-d<sub>5</sub>, 158, 122; AP-d<sub>5</sub>, 144, 122 [8].

#### 3. Results and discussion

The present study included quantitative results from 2070 cases (2355 segments), which are sufficient to conduct a reliable statistical data analysis. As shown in Table 1 the concentrations of MA and AP were from 0.5 to 608.9 ng/mg (mean = 21.0 ng/mg, median = 10.1 ng/mg) and from 0.1 to 41.4 ng/mg (mean = 1.5 ng/ mg, median = 0.8 ng/mg), respectively. These concentrations ranged much wider than those in previous studies, probably due to the difference in populations and sample numbers. In the study by Miki et al. [9] the concentrations of MA and AP were from 0.9 to 56.4 ng/mg (mean = 17.8 ng/mg, median = 7.2 ng/mg) and from 0.1 to 3.5 ng/mg (mean = 0.9 ng/mg, median = 0.5 ng/mg), respectively, for 15 MA users. In another study of 40 volunteer subjects from drug rehabilitation programs [10] the concentrations of MA and AP were from 0.2 to 34.4 ng/mg (mean = 10.2 ng/mg, median = 6.2 ng/mg) and from 0.0 to 4.5 ng/mg (mean = 1.0 ng/mg) mg, median = 0.7 ng/mg), respectively.

In other studies, the low, medium and high ranges for 6-acetylmorphine and morphine in hair were defined using minimum to the 25th percentile, the 25th to 75th percentile and the 75th percentile to maximum, respectively [2,6]. Using the same classification for this study, the low, medium and high ranges of MA were 0.5–4.2, 4.2–24.5 and 24.5–608.9 ng/mg and those of AP were 0.1–0.4, 0.4–1.7 and 1.7–41.4 ng/mg in 2070 drug abusers' head hair.

The AP/MA ratios ranged from 0.01 to 1.04 (mean = 0.11, median = 0.08), which is very similar with those in the previous study [5]. As shown in Fig. 1 the ratios decreased as the MA ranges increased. This can also provide supplementary information to indicate heavy drug users where drug metabolism is saturated.

#### Table 1

MA and AP concentrations (ng/mg) in drug abusers' head hair (no. of cases = 2070, no. of segments = 2355).

	МА	AP	Interpretation
Cut-off	0.5	0.1	
Mean	21.0	1.5	
Standard error	0.7	0.1	
Minimum	0.5	0.1	Low range
Percentile 25	4.2	0.4	X
Median	10.1	0.8	Medium range
Percentile 75	24.5	1.7	¥ Ū
Maximum	608.9	41.4	High range

MA: methamphetamine; AP: amphetamine.



Fig. 1. Distribution of AP/MA ratios.

Table 2

Interpretation of MA and AP concentrations in drug abusers' head hair.

-			-		
Subject	Segment no.	Hair length (cm)	Concentrations (ng/mg)		Interpretation
			MA	AP	
A	1	3	1.0	0.1	Low
	2	3	5.9	0.6	Medium
	3	3	2.4	0.1	Low
	4	3	1.4	0.3	Low
В	1	2-9	124.5	5.6	High
С	1	6-12	6.9	0.3	Low to Medium
D	1	12	3.2	0.2	Low
Е	1	3-4	12.0	1.3	Medium

Hair samples from subject A and D, which were longer than 12 cm, were cut by 12 cm; segment numbers from 1 to 4 in subject A show segments from proximal to distal parts; hair lengths of subject B, C and D means from the shortest to the longest; MA: methamphetamine; AP: amphetamine.

The concentration ranges proposed here were used to interpret five authentic police cases, shown in Table 2, which were submitted into our laboratory in January 2009. Segmental analysis was only requested for subject A. For most cases the concentration ranges were clearly determined except for subject C, where the MA concentration was in the medium range but the AP concentration is in the low range. From the results shown in Table 2, it was presumed that subject A had ingested MA more often for the second three months from the point of time when hair was taken. Subject B and D were considered as a relatively heavy and light drug user, respectively. This evaluation was useful to provide the police with more understandable information on the presumed severity of drug use than quantitative values only. It could also facilitate the court's decision regarding specific circumstances surrounding the drug-related crimes.

#### Acknowledgement

The present work has been supported by funding from National R&D Program of the Ministry of Education, Science and Technology (MEST): Development of Forensic Identification Techniques for Crime Scene Investigation (Code number 0800-0820-0829).

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Please cite this article in press as: S. Lee, et al., Distribution of methamphetamine and amphetamine in drug abusers' head hair, Forensic Sci. Int. (2009), doi:10.1016/j.forsciint.2009.05.004

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