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Duration of detection of methamphetamine in hair after abstinence



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ABSTRACT

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Keywords: Methamphetamine Yaba Hair analysis Prospective sampling Duration of detection Abstinence chemical compounds remain positive for a considerable period of time after subjects have reported cessation of use. These findings were generally based on small sample populations or individual case studies. Within the last decade, hair analyses of larger populations have investigated the phenomenon of residual positives in abstinent individuals in order to determine the period of time required for various compounds to present negative hair test results at internationally accepted cutoff levels. Such data has primarily been used to establish guidelines for retesting former abusers of illicit drugs in order to evaluate claims of abstinence. To date, research has focused on cocaine and opiates. The present study is the first to examine the duration of detection of methamphetamine (MA) and its metabolite amphetamine (AP) in the hair of chronic MA users who recently ceased their consumption of the drug. The study population (n = 63) consisted of inpatients at a hospital drug rehabilitation program in Chiang Mai, Thailand. Drug taking behavior was collected by personal interview at the time of enrollment. Subjects provided hair samples at approximately monthly intervals for MA and AP analysis by gas chromatography-mass spectrometry at 0.2 ng/mg cutoff levels. The correlation of baseline MA and AP concentrations in hair at the beginning of abstinence with corresponding duration of detection indicated great individual variability for the rate of clearance of MA and AP from hair. In regard to duration of detection, the majority of chronic MA users remained MA positive for up to about 90 days of reported abstinence, but by 120 days, the detection rate had fallen to about 16%. All subjects tested negative for MA after 153 days of abstinence. For AP, the limit of the duration of detection was reached at 106 days. With the adoption of a margin of safety to compensate for outlier individual variability, the present study affirmed that hair analysis of chronic MA abusers should test negative for MA after 6 months of claimed abstinence.

Researchers in the field of hair analysis have known for at least two decades that test results for many

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1. Introduction

The analysis of chemical compounds in hair has become an accepted and valuable tool for researchers in several disciplines. Because hair analysis usually provides a much wider window of detection than the analysis of other biological matrices, the technique has played a significant role in the identification of chronic drug abuse for clinical, judicial and forensic purposes [1,2]. Human scalp hair typically grows at the rate of about 1 cm per month. By analyzing sequential 1 cm segments of hair strands cut close to the scalp, researchers can create approximate calendars of

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http://dx.doi.org/10.1016/j.forsciint.2015.06.030 0379-0738/© 2015 Elsevier Ireland Ltd. All rights reserved. drug use, where segments located proximal to the scalp represent recent drug-taking behavior and more distal segments represent more remote behavior [3,4].

Hair analysis to detect illicit drug use in a general population will yield a much greater number of negative results than positive results. Therefore, it might be said that greater analytical effort has gone into the detection of abstinence than into the detection of use. However, in the terminology of drug abuse research, "abstinence" does not mean merely refraining from drug taking in the first place. Rather, it means the cessation of active use followed by prolonged and ultimately permanent disuse. When abstinence is studied in these terms, it is usually in the context of monitoring a chronic drug abuser's compliance with rehabilitation requirements, as mandated by legal or medical authorities [5,6]. Since the beginning of the twenty-first century, methamphetamine (MA) has been the most widely abused illicit drug in Thailand [7]. Typically, the drug

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is ingested by smoking an illegally manufactured tablet known in the Thai language as "yaba" (crazy drug), which contains approximately 10–20% MA and 50–70% caffeine. To a lesser extent, MA is also consumed in the form of crystalline methamphetamine, known locally as "ice" [7]. The Thai penal code deals severely with those who are convicted of possessing and/or consuming MA-containing substances, but the Narcotics Addict Rehabilitation Act of 2002 allows the appropriate authorities to place MA users into compulsory rehabilitation programs before proceeding with criminal prosecution [8,9]. If an individual's compliance with program requirements satisfactorily passes medical and legal review, the individual can rejoin society without penalty. MA users can also voluntarily enter such programs to receive treatment. Abstinence is monitored both during and after rehabilitation by periodic drug testing by urinalysis.

Urinalysis has several drawbacks for monitoring MA abstinence, the most serious being the technique's narrow window of detection (typically 1-3 days), which offers recidivists many opportunities for circumventing detection. But the verification of abstinence by hair analysis also has difficulties. Prior research concerning drug use has established that a considerable period of time can elapse before the cessation of consumption is substantiated by negative hair test results [10]. As there was little information concerning the clearance of drugs from hair after the discontinuance of use [11], these residual positives mainly served as cautionary flags for the interpretation of hair test results [3,10,12]. The findings themselves were derived from the hair analyses of only a few individuals [10,11,13–16]. Recently, two investigations have applied segmental hair analysis to larger populations in order to study more systematically the declining concentrations of residual positives [17,18]. This work has targeted opiates and cocaine. There has been no corresponding study of methamphetamine (MA), despite its widespread abuse [14]. The primary objective of the present study is to use validated quantitative analysis protocols in order to determine the duration of detection of MA and its metabolite amphetamine (AP) in the hair of chronic MA users at the beginning of abstinence. An associated objective is to establish time-frame guidelines for retesting former MA users in order to evaluate more clearly claims of abstinence.

2. Methods and materials

2.1. Subjects

The results of this study are based on analyses of biological samples provided by in-patients at a MA rehabilitation program at Chiang Mai Thanyarak Hospital. The study enlisted only subjects who admitted repeatedly using MA during a 90-day period prior to enrollment. This information, along with other drug-taking data, was elicited by means of a personal interview with a single trained researcher who was responsible for conducting all of the study's interviews according to the same format. The study enrolled a total of 81 subjects, but it retained only those enrollees who were willing and capable of providing the required urine and hair specimens and who satisfactorily followed the hospital's treatment protocols. Duration of treatment, and therefore duration of participation in the study, ranged from 62 days to 105 days. The final number of subjects was 63. Sixty were in treatment by legal mandate; three by voluntary self-admission. Test results from those who were eventually excluded from the study were discarded. All enrollees received modest monetary payments after furnishing each hair sample. The 63 subjects who comprised the final sample received an additional lump-sum payment at the end of their participation for having furnished the required number of urine samples. This study's objectives and procedures were reviewed and approved by a Research Ethics Committee of the Faculty of Medicine of Chiang Mai University, as recorded in Document Number 193/2555. All enrollees gave written informed consent concerning their participation.

2.2. Chemicals and reagents

Methamphetamine hydrochloride (MA·HCl, 99.42% purity), and amphetamine hydrochloride (AP·HCl, 99.84% purity) were purchased from Lipomed (Arlesheim, Switzerland). Lipomed also supplied pentadeuterated methamphetamine hydrochloride (MA d_5 ·HCl, 99.04% purity), which was used as an internal standard in the hair analysis protocol. Derivatizing reagents for hair analysis were heptafluorobutyric chloride (HFBCl, 98% purity) and heptafluorobutyric anhydride (HFBA, 99% purity). Both were purchased from Sigma–Aldrich (St. Louis, MO, USA). Phenethylamine (99% purity), which was used as an internal standard in the urinalysis protocol, came from Fluka (Buchs, Switerland). Potassium carbonate (K₂CO₃, AR grade) was acquired from Fisher Scientific (Loughborough, Leicestershire, UK). Sodium hydroxide (NaOH, ACS grade) and acetone (AR grade) were obtained from Merck (Darmstadt, Germany).

2.3. Hair samples

The collection of hair specimens served two analytical functions. The first was to permit the quantification of baseline MA concentrations that reflected actual consumption patterns of chronic users at the beginning of abstinence, which was defined as the date of "last reported use". The second was to permit the comparison of MA concentrations over time after the last reported use. In both cases, the hair sampling rationale was based on the findings of previous research that scalp hair, despite some individual variation, typically grows at a rate of about 1 cm per month [4]. A single trained researcher followed the same protocol for collecting all hair specimens used in the study. Hair was cut close to the scalp from the vertex posterior region, with root ends marked, and kept in a clean plastic bag.

As determined by the interview process, 47 of the study's 63 participants reported that they had stopped using MA 11-30 days before the first collection of hair, which occurred on the same day as enrollment in the study. For this group, the 1 cm segment most proximal to the scalp was judged to represent active MA consumption before abstinence. The quantification of MA from these samples provided a baseline of initial concentration for comparison with MA values derived from 1 cm segments of "new growth" hair subsequently collected from the same individuals in the same fashion at approximately monthly intervals until the subjects exited from the study. Because 16 of the study's participants reported that they had last used MA more than 30 days prior to enrollment, the most proximal 1 cm segment of hair collected during their first hair cutting was judged not to represent active drug taking behavior. These hair segments, as well as the 1 cm segments of new growth hair subsequently collected at approximately monthly intervals, provided MA values that reflected a lengthening period of abstinence. For purposes of linguistic convenience, we have described the hair collection schedule as "approximately monthly." But for purposes of comparing MA concentrations over time, we tracked MA values in terms of intervals enumerated in days. These intervals were calculated by adding the number of days between hair cuttings to the number of days since the last reported use of MA.

2.4. Hair analysis

Quantitative hair analysis for MA and AP followed a previously published validated protocol involving solid-phase microextraction (SPME) in-line with gas-chromatography/mass-spectrometry (GC–MS) [19]. SPME used a Multi-Purpose Sampler 2 (Gerstel, Mulheim an der Ruhr, Germany). GC–MS relied on a 6890N Series Gas Chromatograph coupled with a 5973 Series Inert Mass Selective Detector (Agilent Technology, Wilmington, DE, USA).

Each 1 cm hair sample was vortex washed for 1 min, 3 times with distilled water and finally with acetone. The hair was dried at 60 °C and cut into approximately 1 mm lengths. Twenty milligrams was combined in an extraction vial with 200 μ L of 0.5 M NaOH and 150 μ L of 300 ng/mL MA-d5, the internal standard. The vial was capped and incubated at 70 °C for 30 min. After cooling to 40 °C, the extract was separated into a new headspace vial, derivatized by adding 50 μ L of HFBCI:HFBA (8:2, v/v), combined with 1650 μ L of 1 M K₂CO₃ to optimize SPME, and then rapidly sealed.

In the SPME procedure, the vial of derivatized hair was incubated for 5 min at 90 °C. Vaporized analytes were adsorped for 10 min at 90 °C by polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber (Supelco, Bellefonte, PA, USA). The extracts were then desorped into the injection port of the GC-MS instrument for 5 min at 250 °C. GC utilized an Agilent HP-5MS fused silica capillary column measuring 30 m \times 0.25 mm (i.d.). The stationary phase employed (5%-phenyl)-methylpolysiloxane with 0.25 µm film thickness. The column temperature was held initially at 60 °C for 2 min, gradually increased by 20 °C/min to 250 °C, and finally held at 250 °C for 1 min. Splitless injection mode was used, with helium serving as the carrier gas at a flow rate of 1.0 mL/min. MS was conducted in the selected ion monitoring (SIM) mode, with the following results: *m*/*z* 240, 118 and 91 for AP; *m*/*z* 254, 210 and 118 for MA: and m/z 258 and 213 for MA-d5. Ions used for quantitation were m/z 240 for AP. m/z 254 for MA and m/z 258 for MA-d5. The limit of detection (LOD) and limit of quantitation (LOQ) for MA analysis were 0.10 and 0.15 ng/mg of hair, respectively. The LOD and LOQ for AP analysis were 0.15 and 0.20 ng/mg of hair, respectively.

2.5. Urine samples

Subjects provided urine samples under hospital staff supervision on a weekly basis (intervals of 6–8 days) until their discharge from the hospital. Each sample, consisting of approximately 50 mL, was collected in a clean plastic container without preservative. The samples were then refrigerated at 4 °C until analysis about 1–2 days later.

2.6. Urinalysis

Urinalysis adapted the protocol for hair analysis. Modifications in the procedure reflected the fact that urinalysis was used only for qualitative detection in this study. Briefly, 1 mL of urine was combined with 100 μ L of phenethylamine (100 μ g/mL), which served as an internal standard. After 1 mL of 5 M K₂CO₃ was added to the mixture, the solution was subjected to the SPME and GC conditions described above for hair analysis, except that polydimethylsiloxane (PDMS) fiber (Supelco, Bellefonte, PA, USA) was substituted for PDMS/DVB fiber.

In MS analysis, urine samples were run in full scan mode (25-550 amu), adopting basic parameters and procedures employed by Cordero and Paterson in a GC-MS urine-screening test developed for the simultaneous detection of MA, AP and other illicit drugs [20]. Blank urine samples were spiked with 9 concentrations of MA and AP, ranging from 0 to 1000 ng/mL. These were then run in triplicate to identify instrument-specific retention times $(\pm 0.15 \text{ min})$ for the target analytes, as well as to generate peak mass/charge data for comparison with the Wiley 275 spectral database (version W8N05ST). The lowest concentration of analyte to record a match of at least 80% with the Wiley database was accepted as a reliable limit of determination (LOD) and cutoff for qualitative screening. For MA and AP, the lowest concentrations to fit this criterion were 50 ng/mL (90% match) and 200 ng/mL (81% match), respectively. Accordingly, these concentrations served as LODS and cutoffs for qualitative screening.

3. Results

3.1. Characteristics of study participants

All participants (n = 63) had naturally black scalp hair. About 16% (n = 10) had dyed their hair another color, but none of these cosmetic treatments occurred within 60 days of the first hair cutting. During the course of the study, participants practiced normal hair hygiene, such as combing, brushing and washing with hospital-supplied shampoo. All subjects consumed MA in the form of yaba. One individual also occasionally ingested crystal methamphetamine. Except for one subject who took yaba tablets orally, MA consumption was by means of smoking. Three participants reported using one other illicit drug (toluene, cannabis, or heroin) in addition to MA. None reported taking any other amphetamine-type-stimulant.

Table 1 presents data concerning the participants' age, gender and MA consumption. There was no statistically significant difference between males (n = 26) and females (n = 37) in terms of age, duration of reported MA use, or period of time elapsed since last reported use before enrollment. But there was a significant difference in reported frequency of MA use per week, with females claiming more frequent consumption.

3.2. Urinalysis for MA

All urine specimens from all subjects tested negative for MA (<50 ng/mL), supporting participant reports of total abstinence during the course of the study.

3.3. Hair analysis: detection and quantitation of MA and AP after last reported use

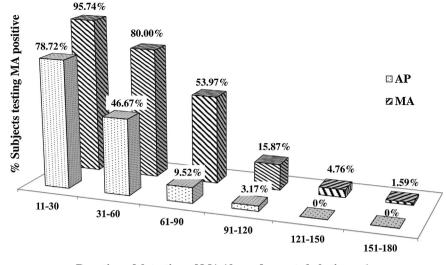
Detection of MA and AP was based on a cut-off level of 0.2 ng of each analyte per mg of hair, as recommended by the guidelines of

Table 1	1
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Subject profile: age, gender and reported MA use.

		All (<i>n</i> =63)	Female $(n=37)$	Male (<i>n</i> =26)
Age (years)	Mean ± SD Min-max	$\begin{array}{c} 29.22 \pm 7.92 \\ 18 - 50 \end{array}$	$29.73 \pm 8.41 \\18 49$	$\begin{array}{c} 28.5\pm7.28\\ 1850\end{array}$
Duration of reported MA use (years)	Mean \pm SD	4.93 ± 4.07	5.31 ± 4.20	$\textbf{4.38} \pm \textbf{3.9}$
Interval between last reported MA use and first hair collection (days)	Min-max Mean \pm SD	$\begin{array}{c} 0.33{-}21 \\ 27.06 {\pm}15.49 \end{array}$	$\begin{array}{c} 0.5{-}21 \\ 28.73 \pm 18.69 \end{array}$	$\begin{array}{c} 0.33 {-} 15 \\ 24.69 {\pm} 9.09 \end{array}$
Reported frequency of use per week	Min-max Mean \pm SD	$\begin{array}{c} 11 {-} 85 \\ 4.73 {\pm} 8.34 \end{array}$	$\begin{array}{c} 11 {-} 85 \\ 6.42 \pm 10.53 \end{array}$	$\frac{13-53}{2.33 \pm 1.60^{\circ}}$
	Min-max	1-35	1–35	1-7

p < 0.05 compared between female and male using independent-simple *T*-test.



Duration of detection of MA (days of reported abstinence)

Fig. 1. Percentage of MA and AP detection in hair after last reported use of MA (*n* = 63).

the Society of Hair Testing [21]. After cessation of MA use, the detection rate and concentration levels of MA and AP continuously declined (Fig. 1, Table 2). Although hair test results for MA remained positive for a majority of subjects (about 54%) for up to 90 days after last reported use, detection rates and concentration levels dropped dramatically in successive 30 day intervals. No MA positives were detected in any subject after 153 days of reported abstinence. AP concentrations declined even more rapidly than MA values so that a majority of the subjects (about 53%) tested negative within 31–60 days. However, the limit of duration of detection for AP was not reached until 106 days of reported abstinence.

Forty-seven of the 63 participants were recent abstainers from MA. According to interview data, their last use of the drug occurred 11–30 days before their first hair cutting. Following the hair sampling methodology discussed previously, the 1 cm segment of these samples that were most proximal to the scalp were analyzed to establish baseline concentrations of residual MA and AP in the hair of abstinent individuals. The purpose of this analysis was to investigate the relationship between drug concentrations at the beginning of abstinence and the duration of MA and AP detection. The correlation coefficient (r) for initial drug concentrations and duration of detection was 0.527 (p < 0.05) and 0.682 (p < 0.05) for MA and AP, respectively. These data indicated a moderately positive correlation between the two variables.

We also divided the baseline drug concentrations values of MA into low, medium and high ranges defined, respectively, as minima to 25th percentile, 25th percentile to 75th percentile, and 75th percentile to maxima, as described by Lee et al. [22]. These MA ranges corresponded to 0.2-4.2, >4.2-24.5 and >24.5-608.9 ng/ mg of hair. For the low-range concentration group, MA was detected in hair up to approximately 90 days of reported abstinence, although the majority of low-range subjects (8/14 cases) remained positive for only up to about 30 days (Fig. 2). These findings were significantly different than the hair test results of the MA medium-range and MA high-range concentration groups (p < 0.5, using Z-test). For the vast majority of medium-range subjects (24/26 cases), the duration of MA detection was between 30 and 90 days of reported abstinence, while all of the high-range subjects (n = 5) remained positive between 60 and 120 days. There was no significant difference in the duration of detection between the medium-range and high-range groups.

When the same procedure was applied to AP hair test results, the low, medium and high ranges were defined, respectively, as 0.2-0.4, >0.4-1.7 and >1.7-41.4 ng/mg of hair [22]. However, the study sample was considerably smaller than for MA, as 21% of the subjects (10/47 cases) tested negative at the first hair cutting (Fig. 3). For all subjects in the low-range concentration group (n = 3), the limit of AP detection was reached within 30 days of reported abstinence. For the medium-range group (n = 24), AP positives were spread almost continuously over the first 60 days of abstinence. Positive results for the high-range group (n = 10) were also widely distributed, reaching the limit of duration of detection at about 90 days of reported abstinence.

Table 2
Concentration of MA and AP in hair and AP/MA ratio for positive cases.

		Days of reported abstinence							
		11-30	31-60	61-90	91-120	121-150	151-180		
MA (ng/mg)	n	45	48	34	10	3	1		
	$Mean \pm SD$	$\textbf{9.97} \pm \textbf{9.67}$	3.62 ± 5.44	1.80 ± 4.97	1.52 ± 3.33	$\textbf{0.40} \pm \textbf{0.53}$	0.40		
	Min-max	$\textbf{0.36} \pm \textbf{40.64}$	0.26-31.92	0.20-26.12	0.21-10.96	0.20-1.00	0.40		
AP (ng/mg)	п	37	28	6	2	0	0		
	$Mean \pm SD$	1.52 ± 1.23	0.65 ± 0.51	1.03 ± 1.24	$\textbf{0.91} \pm \textbf{0.64}$	N.D.	N.D.		
	Min-max	0.24-5.76	0.22-2.16	0.27-3.30	0.46-1.36	N.D.	N.D.		
AP/MA	п	37	28	6	2	0	0		
	$Mean \pm SD$	$\textbf{0.15} \pm \textbf{0.09}$	0.15 ± 0.07	$\textbf{0.17} \pm \textbf{0.10}$	$\textbf{0.25}\pm\textbf{0.19}$	N.D.	N.D.		
	Min-max	0.06-0.55	0.07-0.37	0.11-0.35	0.12-0.39	N.D.	N.D.		

n = number of subjects who tested positive for MA or AP in hair; N.D. = not detected.

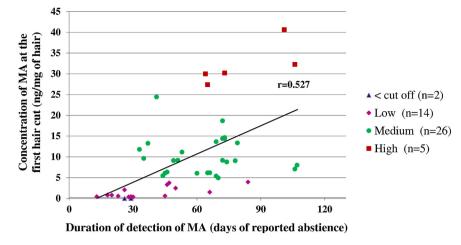


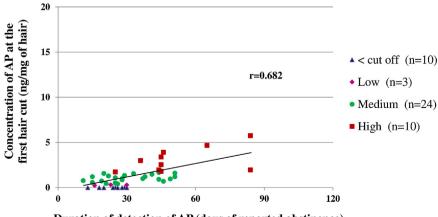
Fig. 2. Duration of detection of MA in hair of subjects whose last reported MA use was within 30 days of testing (n = 47).

4. Discussion

As Pragst observed in his discussion of segmental hair test results for various drugs after the cessation of use, "There is no sharp border between positive and negative hair sections but a more or less broad transition zone" [10]. Previous researchers have tended to describe this transition from positive to negative as a "disappearance" of the drug, which, in the words of one study, ultimately leads to "drug-free" hair [11,14,17,18]. Such language tends to obscure the fact, especially for the lay public, that positive and negative results are always tied to cut-off levels that reflect the sensitivity of a given assay. As is true for the present study, hair analysts often adhere to cut-off levels established by the Society of Hair Testing, which has created such standards in an attempt to impose quality control on hair-test assays and to bring uniformity to hair-test interpretation [21]. But if cut-off levels were to be lowered, then in many cases hair test results would no longer be negative and hair samples would no longer be considered drugfree. Therefore, in this study, we do not discuss the "disappearance" of MA and AP in hair after abstinence. Instead, we focus on the "duration of detection" of these compounds in the hair samples of our study population.

Most studies that compile time lines of drug use by means of segmental hair analysis rely on retrospective hair sampling techniques. Since hair samples are easily stored, retrospective sampling enables a researcher to compile a large study population that might represent months or even years of hair collection. Although hair samples appear to be chemically stable in storage, environmental degradation resulting from sunlight, climatic conditions, and cosmetic treatment may affect drugs incorporated into living scalp hair before hair cuttings end up in the researcher's collection bag [3,23]. To minimize environmental impairment, our study employed a prospective sampling technique that furnished a fresh hair segment, cut close to the scalp, on an approximately monthly basis. As for sample contamination by the drugs themselves in the hospital and/or laboratory, the stability of the AP/MA ratios (0.15–0.17) for the first 3 months (see Table 2) argues against the possibility [24]. Although the ratio increased to 0.25 in the fourth month, the spike was based on test results from only 2 subjects, which is too small a sample for meaningful interpretation. The increased ratio does not seem to invalidate the general conclusion about the absence of in-house contamination.

In describing our prospective sampling technique, we characterized the monthly hair cutting as collecting "new growth" hair. However, this description is not completely accurate. Unless harvested from a freshly shaved scalp (which was not the case in our study), all tufts contain strands of old hair that is in a telogenic or stationary phase. Representing 10–15% of a typical tuft, telogenic hair can remain in place for as long as 6 months [3,12]. It seems reasonable to assume that telogenic hair preserves a record of older drug use as compared to adjacent newly grown hair. Its persistence in scalp hair is perhaps the most commonly cited reason why freshly harvested hair segments from drugabstaining individuals continue to test positive for a considerable period of time after cessation of drug use. Another common explanation for these residual positives is that tissue depots in the



Duration of detection of AP (days of reported abstinence)

Fig. 3. Duration of detection of AP in hair of subjects whose last reported MA use was within 30 days of testing (n = 47).

scalp act as drug storage reservoirs that continue to release their contents into hair after the onset of abstinence [18]. Neither of these explanations has yet been the subject of systematic investigation. To date, the mechanisms causing positive hair test results for abstinent individuals, as well as the reasons for the gradual clearance of drugs from hair, are not well documented.

To evaluate the reliability of our findings concerning the clearance of residual positives from the hair of abstinent individuals, we entered data from our entire study sample (n = 63) into a Weibull Cox Proportional Hazards model [25] using SAS program with the aim of predicting the duration of detection of MA and AP at a cutoff of 0.2 ng/mg hair. The calculation relied on mean values of the following covariates: frequency of reported MA use per week; days of abstinence prior to the first hair collection; and concentration of MA and AP in the hair samples analyzed from the first hair collection. According to this modeling study, the median value for the duration of detection of MA at the prescribed cutoff was about 78 days, with a 95% confidence interval of 71.4-84.5 days. For AP, the median value for the duration of detection was about 42 days, with a 95% confidence interval of 38.5-46.8 days. For the majority of subjects, actual hair test results for MA and AP approximated the model's predictions.

Residual positives are not always detected in the hair of recently abstaining chronic MA users. In our sample of 47 subjects who had ceased MA consumption within 30 days of testing, 2 cases (about 4%) tested below the cutoff level for MA and 10 cases (about 21%) tested negative for AP (see Fig. 1 and Table 2). From a forensic perspective, the AP negatives are noteworthy because positive metabolite results are often required to substantiate the validity of positive MA tests. But as our study demonstrates, negative AP results do not always contradict recent MA abuse. It is an open question whether these negative results are related to varying MA drug concentrations before abstinence or to physiological variability affecting drug clearance during abstinence. Our data would seem to support either possibility. As Fig. 2 indicates, the majority of subjects (57%) who had the lowest baseline concentrations of MA at the beginning of abstinence presented negative hair samples within 30 days, which was significantly quicker than subjects having higher concentrations. But some members of this lowconcentration group remained positive for as long as 60–90 days, which overlapped the duration of detection for subjects who had the highest concentration of MA at the beginning of abstinence. Just as there is great individual variability concerning the incorporation of MA into human hair [26], there appears to be great individual variability regarding the drug's clearance from hair after abstinence.

Prior investigations have indicated duration of detection in hair of 3–4 months for opiates and for cocaine [17,18]. The opiate finding was based on the SoHT recommended cutoff, while the cocaine research used a cutoff lower than the SoHT guideline. Similarly, by the fourth month of reported abstinence, almost all subjects in the present study tested negative for MA, and there were no positives for AP at all (see Fig. 1 and Table 2). Yet in the case of 1 outlier subject, the limit of duration of detection for MA was not reached until 153 days. To avoid criminalizing chronic MA abusers who have discontinued their drug use, we recommend adopting a margin of safety for greater outlier variability by waiting 6 months before retesting for MA by hair analysis. After 6 months of claimed abstinence, appropriate segmental hair analysis of a former MA abuser should yield negative MA test results at a cut-off level of 0.2 ng per mg of hair.

5. Conclusion

For the vast majority of chronic MA users in our study (59/63), the limit of duration of detection for MA in hair was reached after 90–120 days of reported abstinence, using a cutoff of 0.2 ng of MA per mg of hair. All subjects tested negative for MA after 153 days of abstinence. At the same cutoff, the limit of duration of detection for AP for all subjects was 106 days of reported abstinence. Low-range MA concentrations at the beginning of abstinence reached a limit of duration of detection in a significantly shorter time than medium-level and high-level concentrations. But there was no significant difference between medium and high-range levels. For the metabolite AP, the duration of detection was shorter time than the duration of detection of MA.

Conflict of interest

The authors have no conflict of interest to report.

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References

- [1] S. Pichini, O. Garcia-Algar, R. de la Torre, Clinical applications of hair analysis, in: P. Kintz (Ed.), Analytical and Practical Aspects of Drug Testing in Hair, CRC Press, Taylor and Francis Group, Boca Raton, FL, 2007, pp. 201–222.
- [2] P. Kintz, V. Spiehler, A. Negrusz, Alternate specimens, in: S. Jickells, A. Negrusz (Eds.), Clarke's Analytical Forensic Toxicology, Pharmaceutical Press, London, 2013, pp. 153–190.
- [3] F. Pragst, M.A. Balikova, State of the art in hair analysis for detection of drug and alcohol abuse, Clin. Chim. Acta 370 (2006) 17–49.
- [4] M.A. LeBeau, M.A. Montgomery, J.D. Brewer, The role of variations in growth rate and sample collection on interpreting results of segmental analyses of hair, Forensic Sci. Int. 210 (2011) 110–116.
- [5] R. Stanaszek, W. Piekoszewski, B. Karakiewicz, T. Kozielec, Using hair analysis to monitor abstinence in patients on a methadone treatment programme, Probl. Forensic Sci. 50 (2002) 17–34.
- [6] B. Dufaux, R. Agius, T. Nadulski, H.-G. Kahl, Comparison of urine and hair testing for dugs of abuse in the control of abstinence in driver's license re-granting, Drug Test. Anal. 4 (2012) 415–419.
- [7] Global SMARTE Programme, Patterns and Trends of Amphetamine-Type Stimulants and Other Drugs: Challenges for Asia and the Pacific 2013, UNODOC, Vienna, 2013.
- [8] Narcotics Addict Rehabilitation Act, BE 2545, 2002 Available online from http:// www.thailaws.com/law/t_laws/tlawo149_2.pdf (accessed 1.04.15).
- [9] Department of Probation, Ministry of Justice, Thailand. Probation work in Thailand, drug addicts rehabilitation in the compulsory treatment system. Available online from http://210.246.159.139/u3/probation_eng/ (accessed 1.04.15).
- [10] F. Pragst, Pitfalls in hair analysis, in: Conference paper presented at Joint GTFCh/ TIAFT Symposium, Analytical Pitfalls and Trends in Clinical Forensic Toxicology, Analytica Conference, 12 May 2004, Munich, Germany, 2004, Available from: https://www.gtfch.org/cms/images/stories/media/tk/tk71_2/Pragst1.pdf.
- [11] M. Felli, S. Martello, R. Marsili, M. Chiarotti, Disappearance of cocaine from human hair after abstinence, Forensic Sci. Int. 154 (2005) 96–98.
- [12] M. Moller, J. Gareri, G. Koren, A review of substance abuse monitoring in a social services context: a primer for child protection workers, Can. J. Clin. Pharmacol. 17 (2010) e177–e193.
- [13] M.R. Moeller, H. Sachs, F. Pragst, Application of hair in driving-license regranting, in: P. Kintz (Ed.), Analytical and Practical Aspects of Drugs Testing in Hair, CRC Press, Taylor and Francis Group, Boca Raton, FL, 2007, pp. 273–285.
- [14] E. Han, M.P. Paulus, M. Wittman, H. Chung, J.M. Song, Hair analysis and self-report of methamphetamine use by methamphetamine dependent individuals, J. Chromatogr. B 879 (2011) 541–547.
- [15] Y. Nakahara, K. Takahashi, Y. Takeda, K. Konuma, S. Fukui, T. Tokui, Hair analysis for drug abuse. Part II. Hair analysis for monitoring of methamphetamine abuse by isotope dilution gas chromatography/mass spectrometry, Forensic Sci. Int. 46 (1990) 243–254.
- [16] P. Xiang, Q. Sun, B. Shen, M. Shen, Disposition of ketamine and norketamine in hair after a single dose, Int. J. Legal Med. 125 (2011) 831–840.

- [17] F. Garcia-Bournissen, M. Moller, M. Nesterenko, T. Karaskov, G. Koren, Pharmacokinetics of disappearance of cocaine from hair after discontinuation of drug use, Forensic Sci. Int. 189 (2009) 24–27.
- [18] M. Shen, P. Xiang, Y. Sun, B. Shen, Disappearance of 6-acetylmorphine, morphine and codeine from human scalp hair after discontinuation of opiate abuse, Forensic Sci. Int. 227 (2013) 64–68.
- [19] P. Horcharoen, A. Junkuy, P. Sribanditmongkol, Method validation of methamphetamine and amphetamine in hair analysis with its application to yaba abusers, Chiang Mai Med. J. 50 (2011) 31–41.
- [20] R. Cordero, S. Paterson, Simultaneous quantification of opiates, amphetamines, cocaine and metabolites and diazepam and metabolite in a single hair sample using GC-MS, J. Chromatogr. B 850 (2007) 423-431.
- [21] G.A. Cooper, R. Kronstrand, P. Kintz, Society of hair testing guidelines for drug testing in hair, Forensic Sci. Int. 218 (2012) 20–24.

- [22] S. Lee, E. Han, Y. Park, H. Choi, H. Chung, Distribution of methamphetamine and amphetamine in drug abusers' hair, Forensic Sci. Int. 190 (2009) 16–18.
- [23] S.K. Baeck, E.Y. Han, H.S. Chung, M.Y. Pyo, Effects of repeated hair washing and a single hair dyeing on concentrations of methamphetamine and amphetamine in human hairs, Foresnic Sci. Int. 206 (2011) 77–80.
- [24] E. Han, et al., The study of metabolite-to-parent drug ratios of methamphetamine and methylenedioxymethanphetamine in hair, Forensic Sci. Int. 161 (2006) 124– 129.
- [25] D. Collett, Modeling Survival Data in Medical Research, 3rd ed., Chapman & Hall/ CRC Press, 2014.
- [26] A. Polettini, E.F. Cone, D.A. Gorelick, M.A. Huestis, Incorporation of methamphetamine and amphetamine in human hair following controlled oral methamphetamine administration, Anal. Chim. Acta 726 (2012) 35–43.