Original article

Validation and application of hair analysis for the detection of methamphetamine in young Thai adults

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Background: To date, blood and urine analyses have been the major methods of detecting methamphetamine (MA) use in Thailand. Recently, hair analysis has been introduced as an alternative and complementary procedure.

Objectives: To validate a simple, rapid, and economical method of analyzing hair for MA and its metabolite, amphetamine (AM).

Methods: The analysis employed solid-phase microextraction (SPME) coupled with gas chromatography and mass spectrometry (GC-MS). Simplicity, rapidity, and economy were achieved, in part, by optimizing SPME conditions and by eliminating deuterated reagents and the derivatization step in GC-MS. The validated method was employed to detect and quantify MA in 1,111 hair samples of young Thai research volunteers who reported using MA in the form of so-called “yaba” tablets at least 3 times during the previous 3 months.

Results: Validated data from the study indicated that the method’s linearity, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ) met international standards for hair analysis of MA. Expressed as ng of MA per mg of hair (ng/mg), the LOQ was 0.5 ng/mg (102.39% accuracy, 5.46% precision). The method was less sensitive for quantification of AM in hair, with a LOQ of 2.5 ng/mg (103.41% accuracy, 6.95% precision). Using 0.5 ng/mg as a cutoff in the analysis of samples provided by admitted MA users, the study yielded an overall detection rate for MA of 35.5%, with a range of 0.51–54.61 ng/mg; the mean MA level was 4.90 ± 6.78 ($\pm$ SD) ng/mg. Thirty-one percent of the participants reported they had consumed fewer than 10 yaba tablets during the previous 3 months. Of this group, 29% had hair samples that tested positive for MA. Detection rates trended upwards with increased yaba use. No relationship was found between the number of yaba tablets reportedly consumed and the concentration of MA detected in hair.

Conclusions: This study validated a rapid, simple, and economical SPME/GC-MS technique for quantification of MA in hair. The method was less sensitive for quantification of AM.

Keywords: Hair analysis, methamphetamine, solid phase microextraction-gas chromatography/mass spectrometry, Thailand, yaba abuse

Drug abuse is a major problem in Thailand, as it is worldwide. In Thailand, the most frequently abused illicit drug is methamphetamine (MA), which is customarily mixed with caffeine and sold in tablet form under the general name of “yaba” (the literal translation from Thai is crazy drug). Chemical analysis of yaba tablets seized by Thai police reveals an average content of 21.76 ± 6.39 mg% MA and $62.43 \pm$

9.15 mg% caffeine [1]. Use of yaba is widespread in both rural and urban areas and has become a fashion trend for some young people.

As drug abuse has proliferated, methods of detecting illicit drug use have grown in importance for clinical, legal, and judicial purposes. The most commonly used techniques are blood and urine analysis. Both techniques have certain drawbacks for forensic purposes, which often require a wider window of detection and sometimes a less intrusive, more practical sampling method. For these reasons, hair analysis has become both an alternative and a complementary

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approach for detecting drug abuse. In hair analysis, the sampling method is relatively simple, and the detection window is theoretically limited only by the length of the hair sample, with each hair strand preserving a history of drug use that can be measured in terms of weeks, months, or even years. The method's chronological reach is especially useful in establishing extended or chronic drug abuse [2-4].

In Thailand, hair analysis for detecting drug abuse is not yet widely practiced. The most recently reported procedure follows international protocols in using deuterated reagents, solid-phase microextraction (SPME) and gas-chromatography mass-spectrometry (GC-MS) with derivatization [5]. However, we believe that a simpler more economical and less time-consuming procedure might be of value for routine forensic and clinical work, both for qualitative and quantitative detection. The purpose of this study was to validate such a method. Specifically, we sought to develop hair analysis for detecting MA and its metabolite, amphetamine (AM), using readily accessible economical reagents and employing SPME and GC-MS without derivatization. The method was then used to analyze MA content in the hair of young Thai adults who reported consuming MA, usually by communally smoking yaba tablets.

Materials and methods

Chemicals

The target analytes of methamphetamine (MA) and amphetamine (AM) were purchased from Alltech-Applied Science Labs (State College, PA, USA). Both chemicals were of 99% purity. The following compounds, supplied by Lipomed (Arlesheim, Switzerland), were included in the study for selectivity assessment: 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxy-N-ethylamphetamine (MDE), and ketamine. Purities were 99.94%, 99.72%, 99.58%, and 99.25%, respectively. Analytical grade benzaldehyde, used as the internal standard, was obtained from B.D.H. (London, UK), which also supplied acetone. Potassium carbonate (K_2CO_3) and methanol were acquired from Fisher Scientific (Loughborough, Leicestershire, UK). Hydrochloric acid (HCl) was obtained from Merck (Darmstadt, Germany).

Standard solutions

Stock solutions (1 mg/mL) of standards were prepared in methanol and kept at 4°C. Working

solutions of 1 µg/mL were prepared in 1 mol/L HCl and diluted to 250 ng/mL for a selectivity test. The working standards were diluted in 1 mol/L HCl and added to 20 mg of drug-free hair from laboratory staff to yield final concentrations of 0.5, 1, 2.5, 5, and 10 ng/mg of hair for MA, and 2.5, 4, 5.5, 7, 8.5, and 10 ng/mg of hair for AM. These hair-analyte solutions were vortexed for 30 s. Stock solution of benzaldehyde (0.01% v/v) was prepared in methanol and diluted in distilled water to yield a working solution of 0.0001% v/v. Stock and working standard solutions used for other aspects of method validation were prepared in the same manner as for curve calibration.

Participants and hair specimens

The study's objectives and procedures were reviewed and approved by the Thai Ministry of Public Health Ethical Committee, the Human Experimentation Committee at the Research Institute for Health Sciences at Chiang Mai University, and the Institutional Review Board at John Hopkins Bloomberg School of Public Health (USA). All participants received a modest monetary payment for their enrollment in the larger study and provided their written informed consent for participation.

Hair specimens were donated for analysis by young Thai adults ($n = 1,111$) residing in the Chiang Mai region of northern Thailand. These participants were voluntarily participating in a larger research program that attempted to evaluate the effects of peer-network intervention on drug abuse and on risk-taking sexual behavior [6]. The hair specimens were collected from participants who reported consuming MA at least 3 times during the previous 3 months. The preferred method of ingestion was by smoking yaba tablets with friends and associates. Trained interviewers recorded the history of the participants' yaba use, and trained field staff collected the hair samples, which were cut from the vertex posterior region of the scalp with root ends marked, and kept in a clean plastic bag.

Sample preparation

Sample preparation procedures were adapted from those described by Gentili et al. [7]. Each hair sample was cut into 3-cm lengths from the root end, approximately corresponding to drug use during the prior 3 months. The specimens were washed 4 times by vortexing for 1 minute. The first 3 washings were with 5 mL of distilled water and the final washing with 5 mL of acetone. The hair was then dried at 50°C and

cut into pieces of approximately 1 mm in length. Twenty milligrams of each hair sample was extracted with 200 μ L of 1 mol/L HCl in a closed headspace vial at 60°C for 1 hour. After cooling to room temperature, 150 μ L of benzaldehyde (104 μ g/mL) was added to the vial as an internal standard. Next, the extract was pipetted into a new 10 mL vial containing 1,650 μ L of 1 mol/L K_2CO_3 , making the final volume 2 mL. The vial was then rapidly sealed. As reported by Gentili, the addition of the potassium salt increased the ionic strength of the solution, thereby decreasing the solubility of the analytes and optimizing their extraction by SPME.

SPME/GC-MS analysis

SPME/GC-MS analysis for MA and AM followed, with modification, a method described by Gentili et al. [7]. The hair-sample solution was analyzed using a 6890N Series Gas Chromatograph and a 5973 Series Inert Mass Selective Detector (Agilent Technology, Wilmington, DE, USA). This equipment was coupled with a Multi-Purpose Sampler 2 (MPS2) SPME system (Gerstel, Mulheim an der Ruhr, Germany). SPME extraction was performed at 90°C with 65 μ m polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber (Supelco, Bellefonte, PA, USA).

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 25 μ m film thickness. The column temperature was held initially at 60°C for 2 min, then gradually increased by 20°C/min to 250°C, and finally held at 250°C for 1 min. The temperature of the injection port was set at 250°C. A splitless injection mode was used (purge flow 60 mL/min, purge time 0.5 min). Helium served as the carrier gas at a flow rate of 1.0 mL/min. Quadrupole, ion source, and interface temperatures were set at 150°C, 230°C, and 280°C, respectively. MS was conducted in the selected ion monitoring (SIM) mode with the following results: m/z 58, 65, 91 (MA); m/z 44, 65, 91 (AM); m/z 77, 105, 106 (benzaldehyde); m/z 44, 135, 136 (MDA); m/z 58, 77, 135 (MDMA); m/z 44, 72, 135 (MDE); m/z 180, 182, 209 (ketamine). The retention times of these compounds were well separated. For MA, AM, and benzaldehyde, the underlined ions were used for quantitative assessment and the other two were used as qualifier ions.

Method validation

Five-point calibration curves for MA and 6-point curves for AM ranged from 0.5–10 and 2.5–10 ng/mg of hair, respectively. Three sets of calibration curves were plotted each day for 3 consecutive days. The accuracy and precision of the method were analyzed in 3 different concentrations within the calibration curve. Intraday analysis was performed in 7 replicates for each concentration, whereas interday analysis was conducted each day in triplicate for each evaluated concentration for 4 consecutive days. Limit of detection (LOD) and limit of quantitation (LOQ) values were selected based on the accuracy and precision of the 3 lowest concentrations of MA and AM.

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare abundance (counts) responses for different SPME conditions. In analyzing MA-detection results for hair samples of groups reporting different intensities of yaba consumption, a Chi-square test was used to compare percentages of MA positives and a Kruskal–Wallis test to compare median ranks of MA concentration.

Results and discussion

SPME fiber selection

Sample preparation is a critical step that greatly influences the reliability and accuracy of analytical results. SPME is a simple and efficient solventless method of sample preparation. With its automated apparatus, SPME is highly suitable for the routine analysis of large numbers of samples, and it is widely used in the detection of amphetamine compounds [7–10]. Several kinds of SPME fibers are commercially available, but only two types, 100 μ m polydimethylsiloxane (PDMS) and 65 μ m PDMS/divinylbenzene (PDMS/DVB), are in general use for the analysis of drugs and poisons. PDMS fiber is a common choice for many drugs [11–15]. Because it can detect a variety of volatile compounds of low molecular weight, it often serves to analyze multiple illicit drugs in a single specimen [7, 8]. However, prior findings have indicated that PDMS/DVB fiber is more specific for volatile polar compounds such as nitroaromatics and amines [13, 16, 17]. Therefore we selected PDMS/DVB fiber for MA and AM analysis in this study.

Optimizing SPME conditions

The SPME procedure for the sample consisted of incubation, extraction by fiber adsorption, and fiber desorption. An attempt was made to optimize each of these steps (**Figure 1**). First, an MA sample of 250 ng/mL was incubated at 90°C for different durations. Results showed no significant differences in the MA response. A 5 min incubation period was selected because its MA yield was marginally higher than the only other period of shorter duration. Next, PDMS/DVB fiber was inserted into the headspace, allowing the volatile MA to be adsorbed in the SPME system. The quantity of extracted MA was calculated for adsorption times ranging from 1 to 30 min. Although there were significantly lower yields at the shortest durations ($P < 0.001$), there were no significant differences between durations of 10, 20, and 30 min. The two lengthiest adsorption times produced slightly higher MA values, but this potential benefit seemed annulled by prolongation of the procedure, which might render it unsuitable for the realities of daily laboratory work. Therefore the extraction period was set at 10 min. Desorption was performed at 250°C to release MA from the fibers into the GC injection port. There were no significant differences in MA response among durations ranging from 1 to 10 min. A 5 min desorption time was selected because it yielded higher MA values without significantly prolonging the procedure. An additional consideration was that a shorter desorption might cause a carry-over effect to the next sample [18]. To avoid this effect, the SPME fiber was kept in a bake out station at 270°C for an additional 5 min before the next cycle of analysis. With a 5 min desorption, there was no carry-over effect if a sample contained 10,000 ng/mL of MA. However, there was 0.08% carry-over effect if the sample contained more than 20,000 ng/mL of MA. It is therefore necessary to be aware of a possible carry-over effect when dealing with sample concentrations of 20 ng/mg or more.

Method validation

Protocol selectivity was demonstrated by a lack of interference from other amphetamine type stimulants and from blank hair samples eluting with the target analytes during GC-MS analysis. Retention times were well separated (**Figure 2**). As **Table 1** indicates, calibration curves for both MA and AM were substantially linear; the coefficients of determination (r^2) were greater than 0.99 for MA and greater than

0.98 for AM. **Tables 2** and **3** show the accuracy and precision for MA and AM in both intra- and interday analyses. Accuracy, as expressed in the relative recovery, and precision, as expressed in the relative standard deviation, were both within FDA guidelines for such analytical procedures [19]. The selection of values for limit of detection (LOD) and limit of quantitation (LOQ) was based on the accuracy and precision of the 3 lowest concentrations of MA and AM. For MA, these values were 0.25, 0.3, and 0.5 ng/mg of hair. The corresponding values for AM were 1.5, 2, and 2.5 ng/mg. Although the lowest of the three values for MA and AM could be detected, the ratio between quantifier and qualifier ions exceeded the recommended level ($\pm 20\%$) [20]. Consequently, the LOD and LOQ values for MA were set at 0.3 ng/mg and 0.5 ng/mg, respectively. For AM, the selected LOD and LOQ were 2.0 and 2.5 ng/mg, respectively (**Table 1**).

Validated data from this study indicate that the method's linearity, accuracy, precision, LOD, and LOQ meet international standards for the hair analysis of MA, but not for AM. The Society of Hair Testing (SoHT) [21] recommends 0.2 ng/mg as the cutoff for the analysis of MA in hair, but it acknowledges the standards of the French Society of Analytical Toxicology (SFTA), which recommends a cutoff of 0.5 ng/mg [22]. In our study, the LOQ of 0.5 ng/mg for MA complied with the SFTA guideline and was used as the cutoff for our analysis of MA in hair. Both organizations recommend the same cutoff levels for AM as for MA. Although our results for AM did not meet these specifications, our protocol has qualitative and quantitative applications for analyzing concentrations greater than its LOD and LOQ.

The reliability of quantitative analysis by GC-MS greatly depends on the choice of the internal standard. The best compounds have characteristics and properties similar to the intended analyte. A deuterated compound of the analyte is often recommended, but such reagents are very expensive, especially for routine analysis of numerous samples. Because our goal was to develop a method using readily accessible economical reagents, we looked elsewhere, experimenting first with benzylamine as the internal standard. This compound, however, proved unstable in an acid environment, changing to its metabolite, benzaldehyde. We therefore used benzaldehyde as our internal standard, and it showed good accuracy and precision (**Tables 2 and 3**).

Figure 1. Optimized solid-phase microextraction parameters. **A:** incubation time, **B:** extraction time and **C:** desorption time for methamphetamine (250 ng/mL) ($n = 3$ at each data point, mean $\pm$ SD)

Figure 2. Chromatograms. **A:** methamphetamine (MA), amphetamine (AM), methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), methylenedioxy-N-ethylamphetamine (MDE), and ketamine. **B:** drug free sample from laboratory staff with internal standard (250 ng/mL of benzaldehyde) added. **C:** hair sample from subject #00223 0089 0001 (only MA was detected).

Table 1. Calibration curves, LOD and LOQ for MA and AM in hair.

Analyte	Equation ^a	r^2	LOD ^b (ng/mg)	LOQ ^c (ng/mg)
MA	$y = 1.8388(0.13)x + 0.3259(0.23)$	0.9974 (± 0.0022)	0.3	0.5
AM	$y = 0.4789(0.02)x + 1.1378(0.09)$	0.9865 (± 0.0056)	2.0	2.5

^aData as mean ($\pm$ SD); n = 9, ^bLimit of detection; n = 10, ^cLimit of quantitation; n = 10, y = abundance ratio, x = concentration of analyte, MA = methamphetamine, AM = amphetamine.

Table 2. Intraday precision and accuracy for MA and AM detection in spiked hair (n = 7).

Analyte	Expected concentration (ng/mg)	Observed concentration (mean $\pm$ SD) (ng/mg)	Precision (%RSD)	Accuracy (%)
MA	1	1.04 $\pm$ 0.09	8.54	103.75
	2.5	2.46 $\pm$ 0.23	9.53	98.39
	5	4.94 $\pm$ 0.44	12.92	98.90
AM	2.5	2.62 $\pm$ 0.20	7.68	104.68
	5	5.05 $\pm$ 0.51	10.08	101.08
	10	10.18 $\pm$ 0.93	9.18	101.79

MA = methamphetamine, AM = amphetamine, RSD = relative standard deviation.

Table 3. Interday precision and accuracy for MA and AM detection in spiked hair (n = 12).

Analyte	Expected concentration (ng/mg)	Observed concentration (mean $\pm$ SD) (ng/mg)	Precision (% RSD)	Accuracy (%)
MA	1	0.93 $\pm$ 0.08	9.07	93.50
	2.5	2.42 $\pm$ 0.20	10.72	96.71
	5	4.90 $\pm$ 0.55	11.23	98.06
AM	2.5	2.78 $\pm$ 0.09	3.14	111.22
	5	4.92 $\pm$ 0.64	13.09	98.49
	10	10.26 $\pm$ 1.18	11.45	102.30

MA = methamphetamine, AM = amphetamine, RSD = relative standard deviation.

Analysis of hair samples for MA

The validated method was applied to detect MA in 1,111 hair samples of young Thai adults, 18–25 years old, who admitted using yaba at least 3 times within the 3 months before having their hair cut for the study [6]. Seventy-five percent of the participants were male. The median age of all participants was 19 years.

All participants received monetary compensation for their participation in the study. The participants self-reported their yaba use to trained interviewers, who questioned them about the frequency of their drug taking, the number of tablets usually consumed on each occasion, and the date of last use. **Table 4** shows the study population's demographics and reported history of yaba use.

Table 4. Profile of yaba using study participants.

	Male (n = 833)			Female (n = 278)		
	Range	Mean ($\pm$ SD)	Median	Range	Mean ($\pm$ SD)	Median
Age (years)	18–25	19.47 (± 1.85)	19.00	18–25	19.13 (± 1.69)	18.00
Duration of use (years)	0.08–12	4.59 (± 2.35)	4.00	0.08–11	3.58 (± 2.17)	3.00
Use intensity ^a	0.25–900	46.22 (± 75.69)	24.00	0.50–600	45.12 (± 66.75)	20.10
Last occurrence (days)	1–90	14.98 (± 16.18)	9.00	1–87	14.95 (± 16.67)	9.00

^aTablets reportedly consumed in 3 months.

Using a cutoff of 0.5 ng/mg, MA was detected in 35.5% of the participants. **Table 5** shows the range, mean ($\pm$ SD), and median ranks of all positive participants. About 10% of our hair samples yielded MA-positive results above the upper limit of the calibration curve. In these cases, we reduced the amount of the hair sample and retested the diluted solutions. We then corrected the result using an appropriate factor. The percentage of positive hair samples was significantly different among different use-intensity groups, yet not the median of MA levels ($P = 0.177$) (**Table 5**). These data suggest that the variability of MA level in the analyzed hair samples cannot be explained by its relationship to the self-reported amount of yaba tablets consumed. The correlation between self-reported use intensity and the MA level detected in hair was also examined statistically. The results indicated no linear correlation ($r^2 = 0.01$), consistent with findings previously reported [23, 24]. Explanations include differences in individual metabolism, differences in hair melanin content, variations in the incorporation of the drug into hair, hair hygiene, and perhaps most importantly, the reliability of self-reported drug use [23, 25]. In animal studies, in which the delivered drug dose is controlled, a correlation between dosages and drug concentrations in hair has been demonstrated [26]. Recently, a similar correlation has been established for human subjects [25].

Limitations

Methodology

Almost all analytical procedures represent some sort of compromise involving equipment, staff, budgets, and results. At present, the state of the art for GC-MS analysis encourages the selection of an internal standard that is an isotopic analogue of

the analyte under investigation to achieve the closest approximation of chemical behavior. It is also customary to synthesize derivatives of certain analytes to improve their performance under GC-MS conditions [27]. However, as other researchers have pointed out [28, 29], the use of isotopic analogues is expensive compared with regular reagents, and derivatization of compounds can be “laborious, costly and time consuming” [9]. In eliminating both these practices, we recognized that we might be sacrificing a degree of sensitivity. This concern proved unjustified for the analysis of MA, because the protocol met international standards for its quantitative detection. Moreover, it also showed a high level of agreement ($k = 0.819$) for MA quantitation in hair with another validated protocol that uses both a deuterated internal standard and derivatization [5]. In terms of AM analysis, the protocol’s LOQ of 2.5 ng/mg did not meet international cutoff standards for quantitation. This consideration does not negate the protocol’s potential value, but it does require the analyst to understand the implications of its limitations. This will become clear by focusing on the protocol’s LOD for AM of 2 ng/mg.

AM is a metabolite of MA. Its concomitant detection when analyzing MA-positive hair samples is usually considered evidence that MA consumption did in fact occur, an important consideration for forensic investigations and judicial proceedings. Because of individual metabolic differences, the same detected concentrations of MA can be associated with divergent AM values, or different concentrations of MA with the same AM values. Two studies have analyzed hair samples collected from sizeable populations of MA users to ascertain the relationship between MA and AM concentrations. The first study divided its sample of hair-test results ($n = 2389$) into 6 ranges of increasing MA concentrations, with a range of 0.51–

Table 5. MA detection (%) and concentration in participant users’ hair samples.

Use intensity (tablets/3 months)	N	% positive ^a	MA in hair (ng/mg)		
			Range*	Mean*($\pm$ SD)	Mean rank ^b
<10	341	29.0	0.51–54.61	4.79 ($\pm$ 7.27)	187.11
10–30	397	37.5	0.53–34.70	4.10 ($\pm$ 4.96)	191.93
>30–90	240	36.7	0.51–39.96	5.67 ($\pm$ 7.85)	199.61
>90	133	43.6	0.75–48.50	6.02 ($\pm$ 8.05)	226.34
All	1111	35.5	0.51–54.61	4.90 ($\pm$ 6.78)	

^a $P = 0.012$, Chi-square test, ^b $P = 0.177$, Kruskal–Wallis Test, MA = methamphetamine, *calculated from only positive cases.

4.99 ng/mg at the low end ($n = 950$) and 40.25–193.75 ng/mg at the high end ($n = 114$). Every range was associated with AM concentrations exceeding 2.0 ng/mg [30]. In the second study, researchers divided a sample of hair-test results ($n = 2355$) into “low, medium and high ranges,” defined by various percentiles of MA concentrations with corresponding AM values. The high category, representing the upper 25% of the sample, comprised MA and AM concentrations of 24.5–608.9 ng/mg and 1.7–41.4 ng/mg, respectively [31]. From these findings, it appears that our protocol’s AM analysis would have corroborated individual cases of MA use at all levels of detection. Its corroborative value would also appear to be greatest for the heaviest MA user groups, which often are of particular interest in forensic and judicial considerations. Nevertheless, the protocol’s AM LOD would have failed to corroborate a majority of MA positives. Further, this protocol’s AM LOQ would have been sensitive enough to provide data for the calculation of only a small percentage of AM/MA ratios, which can be a useful diagnostic tool for interpreting original MA dosages, the possibility of sample contamination, and the independent use of AM [30–32].

Participants and dosage

We analyzed hair samples provided by participants who reported consuming MA on at least 3 occasions during the 3 months before testing. Most participants consumed the drug by smoking yaba tablets with others. If a communal smoking episode involved several individuals and only a limited number of yaba tablets, participants honestly could have reported consuming yaba even though their consumption was minimal. The lowest reported estimate was a total consumption of 0.25 of a yaba tablet for the entire 3-month period (**Table 4**). Although the MA purity of yaba tablets is generally estimated at approximately 20% [1], the actual MA content of yaba tablets consumed by study participants is unknown. The study took place immediately following a severe crackdown on illicit drugs by Thai authorities [6], and it is possible that some participants ingested tablets containing no MA at all. These various circumstances may have resulted in unintentional over-reporting of drug use and the consumed dosages of MA may have been too low to yield test results above the protocol’s cutoff. In addition to unintentional over-reporting, some participants may have intentionally over-reported their

drug use to show solidarity with friends participating in the study and/or to receive the monetary payment that accompanied their own enrollment. Theoretically, 100% of the participants who provided hair samples consumed MA, yet our protocol yielded a hair-positive detection rate of 35.5%. Similarly, we found no linear correlation between self-reported use intensity and the MA level detected in hair. Confronted with similarly incongruent test findings, previous researchers have suggested that “self-reported dose patterns are poor approximations of the real amount of drug used” [23].

Conclusion

This study validated a SPME/GC-MS method for the analysis of MA and AM in hair. The method is rapid, simple, and economical, in part because it does not rely on derivatization or deuterated reagents. Using 20 mg of hair, this method demonstrated good accuracy, precision, and sensitivity, especially for the quantification of MA. Although the method lacked the sensitivity to quantify AM in hair according to international standards, it is suitable for qualitative detection for concentrations exceeding its LOD. Nevertheless, it also has quantitative application for concentrations exceeding the LOQ of AM.

The validated method was used to determine MA in the hair of people who had recently used yaba. With a cutoff of 0.5 ng/mg, MA was detected in 35.5% of the samples. MA concentrations ranged from 0.51–54.61 ng/mg; the mean level was 4.90 ± 6.78 ($\pm$ SD). With increased use of yaba tablets (>10), there was a tendency for the percentage of MA detection also to increase. However, there was no statistical correlation between the number of yaba tablets reportedly consumed and the MA concentration detected in hair. This inconsistency may call into question the validity of self-reporting as a means of measuring drug use. Further studies to improve the sensitivity of this analytical technique and to reduce the amount of hair needed for assay are needed.

Acknowledgements

This project was supported by a grant from the National Institutes of Health (1 R01 DA14702) to David D. Celentano, Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland. The assistance of Bangorn Sirojorn and Susan Sherman is appreciated, as is the help of Jeffrey Hess, who served as English language editor. We also would like to thank Professor Dr. Niwat Maneekarn

from the Faculty of Medicine, Chiang Mai University, for his critical review of the manuscript. The authors have no conflict of interest to report.

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