Chiang Mai Med J 2011;50(2):31-41.

Original article

Method Validation of Methamphetamine and Amphetamine in Hair Analysis with Its Application to Yaba Abusers

Pongpichan Horcharoen, B.Sc., Anongphan Junkuy, M.Sc., Pongruk Sribanditmongkol, M.D., Ph.D.

Department of Forensic Medicine, Faculty of Medicine, Chiang Mai University

Abstract

Objective The purpose of this study was to validate the method of determining methamphetamine (MA) and amphetamine (AP) in hair, using derivatization coupled with solid-phase microextraction (SPME) and Gas chromotography-Mass spectrometry (GC-MS). Then, applying this method to the examination of hairs from drug abusers.

Method Hair specimens were collected from YABA abusers who participated in a double-blind control behavior intervention. Negative control hairs were collected from drug-free volunteers. Hair was washed with distilled water and acetone. Twenty mg of hair-extracted under alkaline conditions was derivatized with heptafluorobutyric anhydride (HFBA) mixed with heptafluorobutyric chloride (HFBCl). After validation, this protocol was used for measuring MA and AP from 45 abusers.

Results The standard curves (0.2-10 ng/mg of hair) showed linearity with $r^2 > 0.99$. The precision was less than 15% for both MA and AP. The accuracy was within 110.36% for both analytes. The limit of detections (LODs) for MA and AP were 0.10 and 0.15 ng/mg of hair, and the limit of quantitation (LOQ) were 0.15 and 0.20 ng/mg of hair, respectively. Using this method, MA was detected in 46.67% of abusers and the concentration was 0.20-20.06 ng/mg of hair. AP was detected in 33.33% of cases, with a concentration of between 0.22 and 2.76 ng/mg of hair. When compared to hair analysis without reported sample derivatization, there was excellent agreement MA analysis between both protocols, but not for AP.

Conclusion This method is more sensitive and complies with international hair analysis guidelines. It is not so complicated and the automated SPME makes this protocol suitable for simultaneous hair analysis of MA and AP. **Chiang Mai Medical Journal 2011;50(2):31-41.**

Keywords: methamphetamine, amphetamine, hair analysis, derivatization, SPME

Address correspondence to: Pongruk Sribanditmongkol, Ph.D., Department of Forensic Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand: E-mail:psriband@yahoo.com

Received 5 April 2011, and in revised form 2 June 2011.

Drug abuse is still a significant problem in Thailand. The data from the Office of Narcotics Control Board of Thailand showed that the number of arrested drug trafficking suspects had increased each year from 36,854 in 2006 to 48,693 in 2008.[1] The most important type of drug abuse in Thailand is still YABA, which contains methamphetamine (MA) and caffeine. Some tablets also contain amphetamine (AP) and ephedrine.[2]

The detection of MA or its metabolites in the body is crucial for diagnosis of MA abuse. Biological specimens commonly used in the laboratory test are blood and urine. Generally, a biological specimen from a subject has to be screened by a sensitive laboratory technique such as colour test, immunoassay test or thin layer chromatography. If a screening test shows a positive result, that specimen is subjected to a more specific confirmation test such as high performance-liquid chromatography (HPLC) or gas chromatography-mass spectrometry (GC-MS).[3]

A blood sample is the specimen of choice in forensic work, since it represents acute use, and its concentration correlates very well with clinical manifestation.[4] However, its window of detection is narrow and collection of blood specimens is not always convenient. Urine is used most widely for drug abuse monitoring. The detection time of substance is longer in urine than in blood. Urine collection is less invasive than taking a blood specimen. However, MA and AP derivatives can be detected in urine for approximately 2-3 days depending on dosage, urine pH and analytical techniques.[5]

These limitations lead to the exploration of other biological matrixes to monitor drug abuse. Hair is a tissue in which many substances are deposited and it can be used to detect many compounds.[6,7] Considering drug abuse, hair analysis has been applied to forensic and drug monitoring work for a prolonged period of time. However, the introduction of hair analysis in Thailand has not been customary and still needs more laboratory verification to support its application. In 1979, Baumgartner *et al,* published the first report on the detection of morphine in the hair of heroin abusers by using a radioimmunoassay (RIA) test.[8] Since then, many techniques and applications of hair analysis for drug abuse have been reported. Recently, gas chromatography coupled with mass spectrometry (GC-MS) has been a popular technique for hair analysis. The GC-MS method is quite sensitive for detection of low amounts of drugs in hair, and this instrument is available in many Thai forensic laboratories. The sensitivity of GC-MS analysis also depends on the property of the target compound. The more the compound volatization, the greater the sensitivity of detection. Finding a technique to increase MA and AP volatization was the main purpose of this study.

Headspace solid-phase microextraction (HS-SPME) coupled with a GC-MS applied to hair analysis of drugs has been published by Gentili *et al*.[9] Their study increased the sensitivity of drug abuse detection in hair. Later, derivatization procedures were developed extensively, resulting in increased sensitivity for detection. Derivatization is the process of modifying a target substance to produce a more volatile and stable compound, which can be analyzed properly by a GC.[10] Skender *et al*, used heptafluorobutyric anhydride (HFBA) for the derivatization of AP and MA in hair, and their limit of detections (LODs) of AP and MA were 0.20 and 0.05 ng/mg of hair, respectively.[11] Lee

and co-workers reported the determination of AP and MA in hair using derivatization with trifluoroacetic anhydride (TFAA).[12]

Sample preparation procedures using solvents are time-consuming, labour-intensive and multi-stage operations. SPME, which is another method of extraction introduced by Arthur and Pawliszyn in 1990, has been used widely in different fields of analytical chemistry.[13] It minimizes the sample preparation steps and potential sample contamination.[14] Derivatization of target substance followed by SPME also is introduced to the area of drug abuse analysis.[15]

In 2008, our forensic toxicology laboratory, Faculty of Medicine, reported hair analysis for MA and AP in YABA abusers.[16] The method used was HS-SPME coupled with GC-MS and the results showed that the LOQ for MA and AP were 0.5 and 2.5 ng/mg of hair, respectively. The LOQ for MA complied with the Société Française de Toxicologie Analytique (SFTA) guideline that states LOQ for hair analysis should be lower than 0.5 ng/mg of hair.[17] However, results showed that the sensitivity of AP was inadequate. Furthermore, this previous protocol still does not meet international standards, namely the Substance Abuse and Mental Health Services Administration (SAMHSA)[18] and the Society of Hair Testing (SoHT).[19]

The derivatization procedure is necessary for sample preparation, which can improve the chromatographic properties and detection sensitivity of hair in our laboratory. The purpose of this study was to develop a more sensitive method for the determination of MA and AP in hair by using derivatization coupled with SPME, and applying this verified method to examine 45 hair samples from other drug abuse intervention project.

MATERIALS AND METHODS

Chemicals and reagents

Amphetamine hydrosulfate ($AP{\cdot}HSO_4$), methamphetamine hydrochloride (MA·HCl) and pentadeuterated methamphetamine hydrochloride (MA-d5·HCl) were purchased from Lipomed (Arlesheim, Switzerland). The derivatizing reagents, heptafluorobutyric chloride (HFBCl) and heptafluorobutyric anhydride (HFBA), were purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan).

Equipment

The GC-MS instrument used in this study was an Agilent Technologies 6890N GC coupled with 5973 MSD (Agilent Technologies, USA). The GC column was an HP-5MS, 30 m × 0.25 mm (i.d.) × 0.25 μm film thickness fused silica capillary column (Agilent Technologies, USA). A fiber, coated with polydimethylsiloxane divinylbenzene (PDMS/DVB) was purchased from Supelco (Bellefonte, PA, USA). Automatic sampling was used with a GERSTEL multipurpose sampler (GERSTEL, Germany).

Samples

The study was approved by the Ethical Committee of the Faculty of Medicine, Chiang Mai University, according to document number 0515(015)/053. Forty five of 95 hair samples from subjects in the previous drug abuse intervention project, were selected randomly and they were analyzed for MA and AP by our previous analysis protocol.[16]

Negative control hair samples, collected from healthy subjects with no history of drug abuse, were used as blank hairs or spiked with MA and AP. Hair specimens were kept at room temperature until analyzed.

Standard solutions and internal standard (I.S.) solution

Stock solutions of MA and AP (500 µg/ml) and the internal standard (MA-d5) were prepared in methanol and stored at 4 °C until use. The standard substances were diluted with 0.5M sodium hydrochloric giving final concentrations of 0.2, 0.5, 1, 2.5, 5 and 10 ng/mg of hair in spiked hair samples. MA-d5 (2.25 ng/mg of hair) was used as an internal standard.

Chromatographic conditions

The GC operating condition was as follows: the oven temperature was held at 60 °C for 2 min, then increased to 250 °C at 20 °C/min and held for 1 min. The temperatures of the injection port and interface were set at 250 °C and 280 °C, respectively. A splitless injection mode was used. Helium was used as a carrier gas at a flow rate of 1.0 mL/min. Quantitation ions used for selected ion monitoring (SIM) mode were 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 (all as HFB derivatives). Ions used for quantitation were m/z 240 for the AP derivative, m/z 254 for the MA derivative and m/z 258 for the MA-d5 derivative.

Sample preparation

A 3 cm length of hair was collected from the vertex posterior region of the scalp from a subject experienced in YABA abuse over a three month period. The hair was washed 3 times with 5 ml of distilled water, then washed again for the last time with 5 mL of acetone. A 20 mg portion of washed hair was placed into a 10 mL volume extraction vial. Sodium hydroxide (0.5 M, 200 µL) and MA-d5 (300 ng/mL, 150 µL) were added into the vial, which was closed with a cap. The sample was heated at 70 °C for 30 minutes to dissolve the hair. After cooling to 40 °C, the extract was separated and placed into a new vial before adding to 50 µL of HFBCl:HFBA (8:2 v/v) for derivatization. Finally, 1,650 µL of 1 M potassium carbonate was placed in the vial, which was sealed immediately. The derivatized sample was analyzed by HS-SPME GC-MS, and started by incubating the vial in a heat tray for 5 minutes at 90 °C. The vapors of the analytes were diffused into the headspace of the vial. The compounds in the headspace were adsorbed by the extraction fiber in the needle of the SPME device for 10 minutes at 90 °C. After extraction, the fiber was pulled back into the needle, which was then inserted into the injection port of the GC–MS. The fiber was exposed for 5 minutes in the injection port at 250 °C for desorption of the analytes from it.

Validation of method

MA and AP in hair samples were measured by an HS-SPME GC-MS. The HFBCl:HFBA mixture of 8:2 v/v was used as a derivatizing reagent. The validation of this analytical method followed the US FDA guideline.[20] These parameters were evaluated: linearity, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ).

In order to demonstrate linearity, six sets of standard solutions (0.2, 0.5, 1, 2.5, 5 and 10 ng/mg of hair) were prepared and analyzed (n=15; 3 replicates per day). The accuracy and precision of the method were examined by analyzing hair samples spiked with low (0.5 ng/mg of hair), medium (2.5 ng/mg of hair) and high (10 ng/mg of hair) concentrations of MA and AP (n=19). The seven aliquots of each sample were analyzed

on the first day, followed by triplicates for four consecutive days. For LOD and LOQ determination, hair samples spiked with MA and AP at concentrations below 0.20 ng/mg of hair (0.10, 0.15 and 0.20 ng/mg of hair) were evaluated, and the analyte concentrations were calculated by a calibration curve (n=10).

Analysis of hair samples from YABA abusers

The validated method was utilized for the analysis of hair from subjects in the previous drug abuse intervention project. The result of MA and AP hair analysis was compared to that reported by a previous protocol of analysis using the HS-SPME GC-MS technique.

Statistical analysis

The statistical analysis was carried out using SPSS for Windows Evaluation Version 17.0. The result of extraction and derivatization compared two variables using the One-Way ANOVA, to evaluate statistical difference. Statistical significance was evaluated at the p value= 0.05 level. Cohen's kappa was used to compare the degree of consensus between the results from the current and previous protocol.

RESULTS

Validation of method

The calibration curves showed linearity in the range from 0.20 to 10.0 ng/mg for MA and AP in hair (Table 1). The correlation coefficients of the calibration curves for MA and AP were 0.9992 and 0.9971, respectively.

The intra-day and inter-day coefficients of variation (C.V.) for 0.5, 2.5 and 10.0 ng of MA and AP per one mg of hair were 1.76 to 4.00% and 6.37 to 14.93%, respectively (Table 2). The relative recovery (R.R.) of the MA and AP from the spiked hair was, 96.16 to 109.55% and 93.82 to 110.36%, respectively. The accuracy and precision of this method was within a 15% range as indicated by the % R.R. and % C.V. , respectively, which is acceptable criteria following the US FDA guideline.

To identify the LOD and LOQ, three low concentrations, of MA and AP were used in this experiment. The MA was identified in all three low concentrations, but only 0.15 and 0.20 ng/mg of hair showed acceptable accuracy and precision (% R.R. and % C.V. less than 20%).[20] Therefore, the concentration of 0.15 ng of MA/mg of hair was selected as the LOQ and 0.10 ng/mg of hair as the LOD (Table 3). Concerning the AP,

Table 1. Linearity of MA and AP hair analysis (n=15 of each concentration)

Analyte	Linear range[a] (ng/mg of hair)	Regression equation[b]	Correlation coefficient (r^2)
MA	0.2-10	y = 0.4696(±0.0134)x - 0.043(±0.0178)	0.9992(±0.0005)
AP	0.2-10	y = 0.2145(±0.0185)x - 0.027(±0.0196)	0.9971(±0.0017)

[a] Linear range at concentration of 0.2, 0.5, 1, 2.5, 5 and 10 ng/mg of hair.

[b] x is the amount of analytes (ng/mg of hair) and y is the peak area ratio.

the result indicated that only 0.2 ng/mg of hair showed a %R.R. and %C.V. of less than 20%. Therefore, this concentration of AP was used as the LOQ. The AP at 0.15 ng/mg of hair could be identified, but the accuracy and precision were over 20%, so this concentration was indicated as the LOD for AP analysis. The summary of method verification of MA and AP hair analysis is shown in Table 4.

Analysis of hair samples from YABA abusers

Forty-five hair samples were collected from 45 subjects, who participated in the previous drug abuse intervention project, and analyzed for MA and AP using this current protocol. These subjects admitted using YABA at least three times during the past three months. A chromatogram example of MA and AP in the hair from one subject is shown in Figure 1. MA was detected in

Table 2. Accuracy and precision of MA and AP hair analysis

Analyte	Expected conc. (ng/mg of hair)	Measured conc. (ng/mg of hair) (± SD)		Precision C.V. (%)[a]		Accuracy R.R. (%)[b]	
		Intra-day (n = 7)	Inter-day (n = 12)	Intra-day (n = 7)	Inter-day (n = 12)	Intra-day (n = 7)	Inter-day (n = 12)
MA	0.5	0.55 (±0.01)	0.55 (±0.02)	2.45	4.00	109.55	109.32
	2.5	2.40 (±0.05)	2.42 (±0.04)	2.19	1.76	96.16	96.89
	10	9.83 (±0.26)	10.01 (±0.21)	2.63	2.07	98.33	100.09
AP	0.5	0.55 (±0.04)	0.50 (±0.06)	6.79	12.52	110.36	100.68
	2.5	2.62 (±0.32)	2.35 (±0.15)	12.12	6.37	104.87	93.82
	10	9.65 (±0.92)	10.04 (±1.50)	9.58	14.93	96.50	100.42

[a] C.V., coefficient of variation; [b] R.R., relative recovery.

Table 3. LOD[a] and LOQ[b] of MA and AP

Analyte	Expected conc. (ng/mg)	Measured conc. (ng/mg) (± SD)	C.V. (%)	R.R. (%)	LOD and LOQ
	0.20	0.19 (±0.01)	3.78	92.58	
MA	0.15	0.13 (±0.01)	4.68	88.77	LOQ
	0.10	0.07 (±0.004)	5.72	71.25	LOD
AP	0.20	0.23 (±0.03)	14.13	117.35	LOQ
	0.15	0.23 (±0.04)	17.11	150.50	LOD
	0.10	Not detect	-	-	

Ten replicates for each concentration point: 0.10, 0.15 and 0.20 ng/mg of hair in spiked hair samples were calculated by calibration curve range 0.2-5 ng/mg of hair.

[a] LOD = limit of detection; identified by %R.R., %C.V. and relative abundance (mass/ratio).

[b] LOQ = limit of quantitation; identified by %R.R., %C.V. and relative abundance (mass/ratio).

Table 4. Summary of method verification

Analyte	Calibration curve (ng/mg)	Correlation coefficient (r^2)	Accuracy (% R.R.)	Precision (% C.V.)	LOD (ng/mg)	LOQ (ng/mg)
MA	0.2-10	0.9992	96.16-109.55	1.76-4.00	0.10	0.15
AP	0.2-10	0.9971	93.82-110.36	6.37-14.93	0.15	0.20

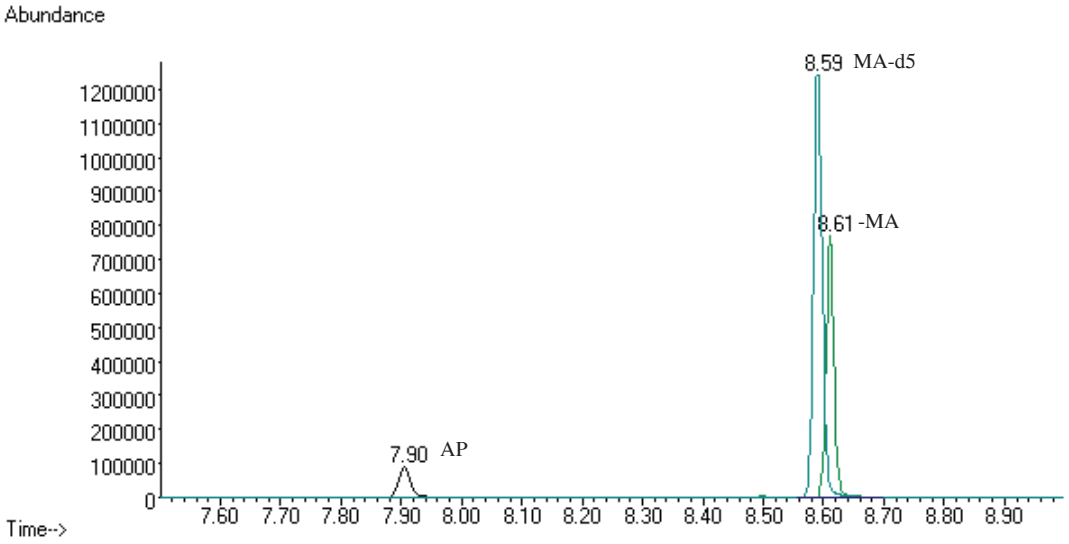

Figure 1. Extracted ion chromatograms of the derivatives from a YABA abuser

46.67% of cases, with a concentration range from 0.20 to 20.06 ng/mg of hair. AP was detected in 33.33% of cases, with a concentration range from 0.22 to 2.76 ng/mg of hair (Table 5).

DISCUSSION

The analysis of drugs in biological fluids is important in diagnosing drug abuse. Although blood and urine are specimens of choice, hair is increasing its role in drug abuse monitoring. Drugs can be detected in hair for a longer period of time after use than blood and urine.[21] However, the level of substances in hair is quite low. Therefore, the analysis method has to be sensitive enough to detect low amounts of drugs. A recommended method for MA and AP hair analysis is GC-MS. An extraction with SPME was applied for MA and AP hair analysis, since it is less complicated and not time-consuming.

Table 5. Hair analysis of MA and AP from 45 YABA abusers

Hair sample	MA	AP
No. of positive (%)	21 (46.67)	15 (33.33)
Range (ng/mg of hair)	0.20-20.06	0.22-2.76

From our previous MA and AP hair analysis protocol, we used SPME coupled with a GC-MS technique to detect MA in hair of YABA abusers. However, AP could not be detected using that protocol.[16] The LOQ from previous methods for MA detection complied with the Société Française de Toxicologie Analytique (SFTA) guideline.[17] Nevertheless, the sensitivity of AP hair analysis did not fit the SFTA guidelines. To improve the sensitivity of MA and AP hair analysis, we conducted specimen derivatization. This process transformed MA and AP into more stable and volatile compounds. These derivatizing reagents can reduce chemical polarity by replacing active hydrogens with an alkyl or acyl group of esters, ethers, alkyl amines or acyl halide.[22] The appropriate reagents for the derivatization of amine compounds such as MA and AP are acylation reagents, for instance, HFBA,[23] TFAA,[12] HFBCl[15] and N-Methyl-bis(trifluoroacetamide) (MBTFA),[24] and silylation reagents like N-(t-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA).[25] Most of these derivatizing reagents are hydrolytically unstable and they must be protected from moisture.[26] Furthemore, uncontrolled formation of unexpected minor derivatives can be produced if the conditions of the reaction are not well established.[22] Meanwhile, HFBCl can be used for derivatization in aqueous solution and more stable for analysis.[15] However, using only HFBCl is expensive and sample preparation has some difficulties, because it drips rapidly and evaporates quickly. The mixture of HFBCl and HFBA could overcome these problems. Therefore, HFBCl combined with HFBA (8:2 v/v) in aqueous solution was used in this study as dervatizing reagent, and the sample was analyzed for MA and AP derivatives.

In this study, we have established an automated GC-MS method for the analysis of MA and AP in human hair using an in-matrix derivatization and SPME technique. A good linear range was observed from 0.2 to 10 ng/mg of hair for both MA and AP. The correlation coefficients were greater than 0.99 for both MA and AP. Accuracy of both intra-day and inter-day was less than 110.36%, as expressed in the relative recovery. Precision of both intra-day and inter-day was less than 14.93%, as expressed in the coefficient of variation. The methods of accuracy and precision were within US FDA guidelines. The LOQ in this method was 0.15 and 0.2 ng/mg of hair for MA and AP, respectively. This sensitivity complied with the SoHT guideline in that the LOQ for hair analysis should be lower than 0.2 ng/mg of hair. This validation method is suitable for use in the intended field.

This validated method was applied to analyze 45 hair samples from subjects who admitted using YABA at least three times during the past three months. MA was detected in 46.67% of cases with a concentration range from 0.20 to 20.06 ng/mg of hair. AP was detected in 33.33% of cases, with a concentration range from 0.22 to 2.76 ng/mg of hair. A comparison between results from the current protocol (SPME GC-MS with sample derivatization) and the previous one (SPME GC-MS without sample derivatization) is shown in Table 6. With sample derivatization, more cases of MA and AP were detected. In 15 samples, we detected AP only when using this recent method. To investigate the agreement between both protocols, the kappa statistic was used. The calculation is based on the difference between previous results and this current one. A kappa value of 0.819 was considered ac-

Table 6. Comparison of the results from current protocol with previous protocol

Previous reports	Current results			
	MA[a]		AP[b]	
	Positive	Negative	Positive	Negative
Positive	17	0	0	0
Negative	4	24	15	30

[a] Kappa = 0.819; very good of agreement; [b] Kappa = 0; poor of agreement

ceptable for MA analysis in both methods. This means sample derivatization does not affect MA hair analysis significantly. On the other hand, both protocols showed a poor degree of agreement when analyzing of AP in hairs. Without sample derivatization, AP could not be detected,[16] but the recent technique could detect AP in more than 33% of the subjects. In terms of proving YABA abuse, both MA and AP should be identified as a proper ratio in a particular subject.[27]

In conclusion, we reported a method to verify MA and AP hair analysis. The protocol starts with cleaning hair with distilled water and acetone, followed by sodium hydroxide extraction, and then derivatization with HFBCl combined with HFBA (8:2 v/v). The sample is then subjected to HS-SPME for extraction and analyzed by a GC-MS. This validated method is sensitive and complies with international hair analysis guidelines. The protocol is not so complicated and the automated SPME make easier and suitable for routine forensic and clinical analysis of MA and AP in hair.

ACKNOWLEDGEMENT

The authors would like to express their sincere appreciation to the Faculty of Medicine for financially supporting this project. We also would like to extend our thanks to Assistant Professor Dr. Apinun Aramrat for his generosity in allowing us to use the leftover hair specimens from his research project.

REFERENCES

1. Office of Narcotic Control Board. Situation trend in 2009. [online]. Available from: http://www.nccd.go.th/upload/content/situationtrendin2009(published).pdf (9 February 2007).
2. Adam P. Drug characterization of methamphetamine pills as a scientific tool to help identify drug production and trafficking network. Ph.D. Thesis, Chiang Mai University, 2005.
3. Wu AHB. Mechanism of interferences for gas chromatography/mass spectrometry analysis of urine for drugs of abuse. Ann Clin Lab Sci 1995;25:319-29.
4. Moeller MR, Steinmeyer S, Kraemer T. Determination of drugs of abuse in blood. J Chromatogr B 1998;713(1):91-109.
5. Wenning R. Potential problems with the interpretation of hair analysis results. Forensic Sci Int 2000;107:5-12.
6. Moeller MR, Fey P, Wening R, Sachs H. Hair analysis as evidence in forensic cases. J Forensic Sci Int 1993;63:185-206.
7. Zhao H, Brenneisen R, Scholer A, et al. Profiles of urine samples taken from ecstasy users at rave parties. J Anal Toxicol 2001;25:258-269.
8. Baumgartner AM, Jones PF, Baumgartner WA, Black CT. Radioimmunoassay of hair for determining opiates abuse histories. J Nuclear Med 1979;20:748-52.

9. Gentili S, Cornetta M, Macchia T. Rapid screening procedure based on headspace solid-phase microextraction and gas chromatography–mass spectrometry for the detection of many recreational drugs in hair. J Chromatogr B 2004;801:289-96.
10. Knapp DR. Handbook of analytical derivatization reactions. John Wiley and Sons;New York:1979.
11. Skender L, Karacic V, Brcic I, Bagaric A. Quantitative determination of amphetamines, cocaine, and opiates in human hair by gas chromatography/mass spectrometry. Forensic Sci Int 2002;125:120-6.
12. Lee S, Park Y, Yang W, et al. Development of a reference material using methamphetamine abusers' hair samples for the determination of methamphetamine and amphetamine in hair. J Chromatogr B 2008;865:33-9.
13. Arthur CL, Pawliszyn J. Solid phase microextraction with thermal desorption using fused silica optical fibers. Anal Chem 1990;62:2145.
14. Kataoka H, Saito K. Recent advances in SPME techniques in biomedical analysis. J Pharm Bio Anal 2011;54:926–50.
15. Liu J, Hara K, Kashimura S, Kachiwagi M, Kageura M. New method of derivatization and headspace solid-phase microextraction for gas chromatographic-mass spectrometric analysis of amphetamines in hair. J Chromatogr B 2001; 758:95-101.
16. Pengwong M. Development of hair analysis for amphetamine and methamphetamine in YABA abusers using automated headspace solid-phase microextraction and gas chromatography-mass spectrometry technique. M.S. Thesis, Chiang Mai University, 2008.
17. Pragst F, Balikova MA. State of the art in hair analysis for detection of drug and alcohol abuse. J Clin Chim Acta 2006;370:17-49.
18. The proposed SAMHSA (Substance Abuse and Mental Health Services Administration) levels are from: Proposed Revisions to Mandatory Guidelines for Federal Workplace Drug Testing Programs. Federal Register 2004;69:19673-732.
19. Society of Hair Testing. Recommendation for hair testing in forensic cases. Forensic Sci Int 2004;145:83-4.
20. United States Food and Drug Administration. Guidance for Industry: Analytical Procedures and Methods Validation Chemistry, Manufacturing, and Controls Documentation Food and Drug Administration (FDA), 2005. [online]. Available from: http://www.fda.gov/cber/gdlns /methval.pdf [10 April 2008].
21. Kronstand R, Grundin R, Jonsson J. Incidence of opiates, amphetamines and cocaine in hair and blood in fetal case of heroin overdose. Forensic Sci Int 1998;92:29-38.
22. Segura J, Ventura R, Jurado C. Derivatization procedures for gas chromatographic-mass spectrometric determination of xenobiotics in biological samples, with special attention to drugs of abuse and doping agents. J Chromatogr B 1998; 713:61-90.
23. Wu YH, Lin KL, Chen SC, Chang YZ. Integration of GC/EI-MS and GC/NCI-MS for simultaneous quantitative determination of opiates, amphetamines, MDMA, ketamine, and metabolites in human hair. J Chromatogr B 2008;870:192-202.
24. Cordero R, Paterson S. Simultaneous quantification of opiates, amphetamines, cocaine and metabolites and diazepam and metabolite in a single hair sample using GC-MS. J Chromatogr B 2007;850:423-31.
25. Melgar R, Kelly RC. A novel GC/MS derivatization method for amphetamines. J Anal Toxicol 1993;17:399-402.
26. Pierce 2003-2004 Applications Handbook & Catalog. GC Derivatization. [online]. Available from: http://www.msg.ku.edu/mass/GC%20Derivatization.pdf (28 November 2010).
27. Lee S, Han E, Park Y, Choi H, Chung H. Distribution of methamphetamine and amphetamine in drug abusers' head hair. Forensic Sci Int 2009;190:16-8.

การทดสอบความถูกต้องของวิธีวิเคราะห์เมทแอมเฟตามีนและแอมเฟตามีน ในเส้นผม และประยุกต์ใช้ในผู้ที่เสพยาบ้า

พงศ์พิชาญ หอเจริญ, วท.ม., อนงพันธ์ จันทร์กุย, วท.ม., พงษ์รักษ์ ศรีบัณฑิตมงคล, พ.บ., Ph.D.

ภาควิชานิติเวชศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่

บทคัดย่อ

วัตถุประสงค์ เพื่อทดสอบความถูกต้องของวิธีวิเคราะห์การตรวจเมทแอมเฟตามีน (MA) และแอมเฟตามีน (AP) ในเส้นผม โดยใช้การเตรียมอนุพันธ์ร่วมกับเทคนิค automated HS-SPME GC-MS จากนั้นประยุกต์ใช้วิธีการนี้ในการตรวจเส้นผมของผู้ที่เสพยาบ้า

วิธีการศึกษา ตัวอย่างเส้นผมได้รับจากผู้เสพยาบ้าที่เข้าร่วมในโครงการ "การลดพฤติกรรมเสี่ยงต่อการติดเชื้อเอชไอวี และโรคติดต่อทางเพศสัมพันธ์ในเยาวชนที่เกี่ยวข้องกับยาเสพติดในภาคเหนือของประเทศไทย: ระยะที่ 2 การแทรกแซง" ส่วนเส้นผมกลุ่มควบคุมเก็บจากอาสาสมัครที่ไม่มีประวัติการใช้สารเสพติด ทำการล้างเส้นผมด้วยน้ำกลั่นและอะซีโตน นำเส้นผม 20 มิลลิกรัม สกัดภายใต้สภาวะด่าง และทำการเตรียมอนุพันธ์ด้วย heptafluorobutyric chloride (HFBCl) ร่วมกับ heptafluorobutyric anhydride (HFBA) ในสัดส่วน 8:2 (v/v) หลังจากการทดสอบความถูกต้องของวิธีวิเคราะห์ ได้นำวิธีการนี้ไปทำการตรวจ MA และ AP ในผู้ที่เสพยาบ้าจำนวน 45 ราย

ผลการศึกษา กราฟมาตรฐานอยู่ในช่วงความเป็นเส้นตรง 0.2-10 นาโนกรัมต่อมิลลิกรัมเส้นผม ซึ่งแสดงค่าสัมประสิทธ์สหสัมพันธ์ (r^2) > 0.99 ความเที่ยงของการตรวจหา MA และ AP มีค่าไม่เกินร้อยละ 15 ส่วนความถูกต้องมีค่าไม่เกินร้อยละ 110.36 ค่าต่ำสุดที่ตรวจได้ (LOD) ของ MA และ AP เท่ากับ 0.10 และ 0.15 นาโนกรัมต่อมิลลิกรัมเส้นผม และค่าต่ำสุดที่วัดปริมาณได้ (LOQ) ของ MA มีค่าเท่ากับ 0.15 และของ AP เท่ากับ 0.20 นาโนกรัมต่อมิลลิกรัมเส้นผม เมื่อตรวจเส้นผมผู้ที่เสพยาบ้า ตรวจพบ MA คิดเป็นร้อยล่ะ 46.67 ของผู้เสพทั้งหมด และมีช่วงของความเข้มข้นเท่ากับ 0.20-20.06 นาโนกรัมต่อมิลลิกรัมเส้นผม ตรวจพบ AP คิดเป็นร้อยละ 33.33 ของผู้เสพทั้งหมด มีความเข้มข้นเท่ากับ 0.22-2.76 นาโนกรัมต่อมิลลิกรัมเส้นผม เมื่อเปรียบเทียบกับวิธีการตรวจวิเคราะห์เส้นผมโดยปราศจากการเตรียมอนุพันธ์ (derivatization) ที่รายงานก่อนหน้านี้ พบว่าการตรวจ ทั้ง 2 วิธี ให้ผลการตรวจ MA ที่สอดคล้องกัน แต่สำหรับ AP วิธีที่รายงานในการศึกษานี้ได้ผลดีกว่า

สรุป วิธีการตรวจวิเคราะห์สารในเส้นผมด้วยการเตรียมอนุพันธ์และวิเคราะห์ด้วย SPME GC-MS นี้มีความไวในการตรวจหา MA และ AP สูงและเป็นไปตามแนวทางการวิเคราะห์เส้นผมที่นานาชาติยอมรับ การเตรียมตัวอย่างที่ไม่ซับซ้อนมากสามารถนำมาใช้ตรวจในผู้เสพยาบ้าได้ **เชียงใหม่เวชสาร 2554;50(2):31-41.**

คำสำคัญ: เมทแอมเฟตามีน แอมเฟตามีน การวิเคราะห์เส้นผม การเตรียมอนุพันธุ์ โซลิด-เฟสไมโครเอ็กซแทรกชัน