CHAPTER 4

DISCUSSION AND CONCLUSION

4.1 Discussion

Hair analysis of AM or MA is an alternative method for diagnosing YABA abuse. Its window of drug detection is longer than that in urine and blood analysis [16]. However, the amount of substance presented in hair is quite low compared to urine [52]. Therefore, an analytical technique of hair analysis must be sensitive.

Many analytical techniques have been reported [21]. Using GC-MS is specific and sensitive enough to detect and quantify substances in hair. Before analyzing by GC-MS, a specimen is subjected to extraction and derivatization to increase sensitivity. Recently, Nishida et al. [53] reported using HS-SPME for a specimen preparation before analysis by GC-MS. HS-SPME has many advantages such as no other extraction processes required and derivatization may not be needed for some substances [21]. Since HS-SPME-GC-MS for AM and MA hair analysis was introduced to this author's institute for the first time, and in Thailand to the best of her knowledge, this technique needed to be verified.

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A GC-MS condition in this protocol can clearly identify AM, MA and their derivatives, with different RT and mass spectra. Ketamine, another potential club drug, was also identified by this GC-MS condition.

To optimize the HS-SPME condition, types of SPME fibers were tested. In many studies, PDMS was used, since it can detect many low molecular weights or volatile compounds [54]. It is, therefore, suitable to analyze many drug abuses in one

specimen [21, 39]. PDMS/DVB fiber is more specific to volatile polar compounds such as amines and nitroaromatic compounds [55]. The extraction efficiency of both fibers was compared in this study. The result demonstrated that to investigate only amine substance, PDMS/DVB has more advantages than PDMS. This result supported those reported by Chia et al. [50]. The universal SPME fiber, PDMS, may not be appropriate if an analysis is mainly focused on AM or MA.

SPME fiber can be exposed to analytical substance either by direct immersion to the specimen or by headspace [41]. If the compound is easily volatile, headspace exposure is preferred. The headspace technique was suitable for AM and MA extraction in hair samples [21, 39, 54]. To determine a proper headspace volume, total sample volume was determined. The results showed that a 2 ml total sample volume in a 10 ml vial yielded proper responsibility and reproducibility.

Adding salt can increase the ionic strength of the sample solution by making the target compounds less soluble and more volatile [21]. Several kinds of salts have been used by other authors, such as sodium sulfate, potassium carbonate and sodium chloride [56]. From the study of Raikos et al., 2003 [57], the recovery of AM in the presence of K_2CO_3 (1g/ml, complete saturation) was higher than that of NaCl (0.7g/ml NaCl plus 100µl of 1 M NaOH). Concentrations of K_2CO_3 were investigated in this study and 1 M K_2CO_3 had good reproducibility (Table 3.4 and Figure 3.14).

The temperature in SPME condition was set at 90 °C to increase AM and MA volatility [21]. This study's preliminary result also showed that a better AM and MA response was yielded at 90 °C, and a 3-30 min incubation time did not give a significant MA response. A 5 min incubation time was selected, since it yielded a better response than a 3 min one and did not make the analytical cycle longer.

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MA was then extracted from an SPME fiber. The longer the extraction time, the more MA detected. There was a significant difference of MA detection from 5, 10, 20 or 30 min extraction. However, 20 or 30 min extraction took a long analytical cycle time, and is not suitable for daily laboratory work. Therefore, 10 min extraction time was used in this study.

Desorption at 250°C for 1, 3, 5 and 10 min did not show any difference in MA detection. A desorption time that is too short may cause a carry over effect to the next sample [56]. A 5 min desorption time was selected, since it showed a good response. From these experiments, the SPME condition took 20 min per cycle, which was faster than that reported by other studies [58].

Any remaining substance in a fiber may be eluded in the next sample analysis, thus causing a false positive. 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. Therefore, if the previous specimen shows more than 20 ng/mg of hair or higher in hair sample analysis, awareness of the carry over effect is necessary.

Although several methods for hair extraction, such as alkaline digestion [34], enzymatic digestion [28] and methanol sonication [30], were reported, this study used acid extraction [21]. By using this method, AM and MA were easily eluted from hairs into acid solution with good response. It also could simultaneously analyte AM-like and other commonly used drugs [21, 58].

After establishing the optimized HS-SPME-GC-MS conditions for determination of AM and MA, the suitable conditions are summarized in Table 3.10. The limit of

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detection and limit of quantitation, linearity, accuracy and precision were investigated by spike standards into drug-free hair samples.

A good linear calibration curve was observed over the 2.5–10 ng/mg of hair for AM and 0.5-10 ng/mg of hair for MA (Table 3.11-3.12). The correlation coefficients were greater than 0.98 and 0.99 for AM and MA, respectively.

Accuracy as expressed in the relative recovery in this experiment was 93.50-111.22%. Precision, as expressed in the coefficient of variation, was 3.14-13.09%. The accuracy and precision were ± 15%, by following the FDA guideline [48].

The limit of quantitation in this method was 2.5 and 0.5 ng/mg of hair for AM and MA, respectively. The LOQ for MA complied with the SFTA guideline in that LOQ for hair analysis should be lower than 0.5 ng/mg of hair [59]. Yet, this was not the case for AM hair analysis. The sensitivity of MA hair analysis in this study was still less than that reported by others. Miki A. reported an LOQ for MA analysis of 0.2 ng/mg of hair from 10 mg of hair [60]. Compared to the report by Gentili et al [21], their LOQ was higher than that in this study. However, 10 mg of hair was used in their experiment, while 20 mg of hair was used in this study.

The hair analysis method verified in this study was tested with real hair samples from both YABA abusers and drug-free subjects. It can detect MA in about 57-67% of YABA abusers, but no MA was detected in negative control subjects. The SIM for MA in negative and positive hairs was clearly demonstrated (Figure 3.17, 3.18). MA concentration measured in hair section 1, 2 and 3 of abusers was 3.03 (±4.85), 4.47 (±7.40) and 5.21 (±11.43) ng/mg, respectively. Section 1 of hair was closest to the scalp and represented the most recent month, while section 3 represented a period from about 3 months previously. From this result, it was

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observed that MA concentration in the hair of abusers decreased along with time. Although these subjects admitted they had used YABA at least 3 times during the past 3 months, hair analysis could not detect MA in all of them. This may be due to the amount and frequency of drug used. Further investigations were made by this study into any correlation between the time of last use and detection of MA in hair. In the group that had used YABA within the last 30 days, 82.35, 84.21 and 76% were MA positive in hair section 1, 2 and 3, respectively. The presence of positive findings was higher than in the groups that used YABA for more than one month. A limitation of this study was the rather low number of subjects in this group (Table 3.19). The average number of YABA tablets used from self-reports was compared to MA detection in hairs. Subjects with a history of using more than 4 YABA tablets per month had a higher tendency to be MA hair positive. This assumption relied on the validity of the self-reports. In animal studies, in which the drug dosage delivered is controlled, a correlation between dosages and drug concentrations in hair was demonstrated [38, 61]. However, the results from this study showed no significant correlation between MA concentration in hair and the amount of YABA used. Only the concentration of MA in hair section 3 showed some degree of correlation with the number of YABA tablets used.

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4.2 Conclusion

An HS-SPME-GC-MS technique for hair analysis of AM and MA has been verified in this study. This analytical method was quite simple, with no-derivatization, and rapid and good specificity. The sensitivity of this protocol depended on the amount of hair tested. At least 20 mg of hair was required to meet the recommended LOQ. The accuracy and precision were within the FDA guideline [48]. The linear calibration curve was 2.5-10 ng/mg of hair for AM and 0.5-10 ng/mg of hair for MA. This method clearly separated other amphetamine derivatives and ketamine from AM and MA.

By using this laboratory protocol, MA was detected in the hair of YABA abusers. Although it could not be detected in all subjects, the percentage of hair positives was about 56-66%, depending on hair section. There was no strong correlation between the number of YABA tablets used and the MA concentration detected in hair. Further studies are needed to improve the sensitivity of this analytical technique, with less amount of hair needed.

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