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*Full Paper*

# Selective fiber used for headspace solid-phase microextraction of abused drugs in human urine

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**Abstract:** A sensitive and selective fiber for simultaneous analysis of three drugs of abuse (amphetamine, methamphetamine and ephedrine) in urine samples was explored using headspace solid phase microextraction and gas chromatography with flame ionization detection. Several parameters affecting extraction, viz. extraction temperature, extraction time, pH of solution, and salt addition were investigated. Among five commercially available fibers, divinylbenzene/carboxen/ polydimethylsiloxane was the most sensitive and selective fiber at the extraction temperature of 80 °C for 20 minutes and solution pH of 9.5-10.0, with added NaCl and desorption temperature of 220 °C for 2 minutes. Under these optimal conditions, the proposed solid phase microextraction method provided good linearity in the ranges of 0.1-10 µg/ml for amphetamine and methamphetamine and 0.5-20 µg/ml for ephedrine. The detection limits for amphetamine, methamphetamine and ephedrine were 9, 3 and 30 ng/ml, respectively. The recoveries of the three drugs in urine samples exceeded 85%.

**Keywords:** amphetamine, methamphetamine, ephedrine, solid phase microextraction.

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## Introduction

Amphetamine (AMP), methamphetamine (MET) and ephedrine (EPH) are closely related in chemical structure (Figure 1). They are a class of central nervous system stimulants, usually without producing hallucination [1]. AMP and MET are generally manufactured in clandestine laboratories. They are significantly abused drugs in Thailand over the past 10 years. Called “ya ba” (crazy medicine) in Thai, AMP and MET are thought to come largely from small mobile production units across the border of the country. Synthesized MET is mainly produced from EPH. The varieties of illicit samples have been found to contain MET or MET mixed with EPH [2]. Since 2001, the most commonly abused drugs in Thailand have been amphetamine-type stimulants. Although Thailand has laws prohibiting the sale and use of amphetamines, the use of amphetamines has increased [3].

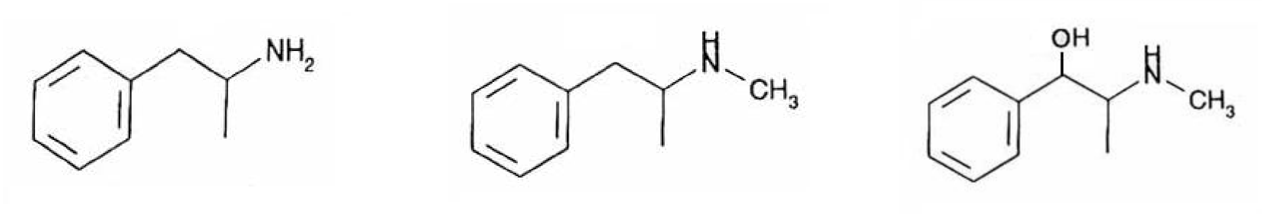
The determination of abused drugs usually begins with the extraction of target compounds from the sample. Generally, liquid-liquid extraction (LLE) is widely used for extracting abused drugs. The method requires an excessive amount of organic solvent and it is time consuming. Solid phase extraction (SPE) is an alternative to LLE with the advantages of being cheaper and faster, however it requires a large variety of adsorbents and toxic solvents still have to be used as in LLE [4-7].

Solid phase microextraction (SPME) is a sample preparation technique which has been widely applied to extract AMP and MET from biological samples such as urine [8-12], hair [13-14], blood [15] and saliva [16]. The method does not require any organic solvent as the analytes are directly adsorbed onto a fiber usually made of fused silica. The fiber works as a cross-linked or stationary phase coated onto the fiber surface. The additional advantages are that SPME requires small sample volume and possesses extraction simplicity.

SPME-GC-MS analysis has been employed for synthetic drugs in hair samples [13] with detection limits of 1.29 and 0.37 ng/mg for AMP and MET, respectively. Solid-phase dynamic extraction (SPDE) using a stainless steel needle coated with a 50 μm film of polydimethylsiloxane (PDMS) and 10% of activated carbon was used for determining AMP and synthetic drugs in hair samples [14]. Limits of detection were found to be 0.04 ng/mg for AMP and 0.05 ng/mg for MET. Namera et al. used polydimethylsiloxane (PDMS) fiber for the extraction of AMP and MET in whole blood [15] with a detection limit of 10 ng/ml for AMP and MET.

Several groups have also reported the analysis of amphetamine-like drugs and their derivatives in urine samples using various fibers, for example, the PDMS fiber [8, 9, 11, 17], the carboxen polydimethylsiloxane (CAR/PDMS) and polydimethylsiloxane divinylbenzene (PDMS/DVB) fibers [17], the heptakis (2,6-di-*O*-methyl)-β-cyclodextrin blended with hydroxyl-terminated silicone oil (DM-β-CD/OH-TSO) fiber [18]. Yashiki et al. [9] reported that the minimum detectable levels of both AMP and MET were 100 ng/ml whereas the limit of detection values of those compounds was 30 ng/ml as reported by Raikos et al. [11]. The detection limits obtained using the PDMS fiber derivatized with heptafluorobutyric anhydride were 0.05 and 0.02 ng/ml for AMP and MET, respectively [17]. Employing the DM-β-CD/OH-TSO fiber, the limits of detection were 0.60 and 0.33 ng/ml for MET and EPH, respectively [18].

As stated earlier, many methods for the determination of AMP and MET in urine samples using the PDMS fiber either with or without derivatization have been developed. However, few methods on extraction of EPH using commercial SPME fiber were reported. Recently, EPH mixed with MET has been used extensively in the production of the so called “ya ba” stimulant drugs. In this study, the selectivity of SPME fiber for simultaneous analysis of MET, AMP and EPH was investigated using five commercially available SPME fibers. Variables affecting the SPME process, viz. extraction temperature, extraction time, pH of solution and salt addition were evaluated and optimized.



Amphetamine (AMP) Methamphetamine (MET) Ephedrine (EPH)

**Figure 1.** Chemical structures of abuse drugs used in this study.

## Materials and Methods

### *Materials*

Amphetamine sulfate, methamphetamine hydrochloride, ephedrine hydrochloride and phentermine hydrochloride (used as internal standard) were obtained from Medical Science Department (Bangkok, Thailand). All chemicals were of analytical grade with purity above 99 %.

Five different fibers: PDMS with coating thickness 100 μm, polyacrylate (PA) 85 μm, carboxen/polydimethylsiloxane (CAR/PDMS) 75 μm (Stable Flex), carbowax/divinylbenzene (CW/DVB) 65 μm (Stable Flex), and DVB/CAR/PDMS 50/30 μm (Stable Flex) were purchased from Supelco (Bellefonte, PA, USA). All fibers were conditioned according to the supplier's instructions.

### *Gas chromatography*

The gas chromatograph used was a Hewlett Packard HP-5890 Series II equipped with a flame ionization detector. The column was HP-5MS (30m x 0.25 mm I.D., 0.25 μm film thickness). The oven temperature was: initial 55 °C, held for 0.5 min, programmed to 230 °C at 20 °C/min, and then held for 2 minutes. The carrier gas was nitrogen maintained at a flow rate of 1.2 ml/min. The temperature of injector and detector were set at 220 °C and 260 °C, respectively. A split/splitless injector was used in the splitless mode.

### *SPME procedure*

The parameters that affect the SPME process such as fiber type, extraction temperature, extraction time, pH of solution and salt addition were evaluated and optimized. Spiked aqueous solutions used for extraction efficiency were adjusted to pH 9.5 with 1.0 M sodium hydroxide.

First of all, the selection of the fiber type was investigated. Each spiked aqueous solution (2 ml; 1 μg/ml of each analyte) and 0.6 g sodium chloride, adjusted to pH 9.5 with 1.0 M sodium hydroxide was placed in a 10-ml headspace vial. The vial was sealed with a septum and aluminum cap. Then it was immersed in a thermostatic water bath at 80 °C and stirred at speed of 300 rpm. The fiber was then exposed to the headspace over the solution for 20 minutes and thermally desorbed in the GC injection port for 2 minutes.

The effect of extraction temperature was investigated by varying in the range of 50-90 °C. The extraction time was evaluated from 5 to 30 minutes. Then the pH of the aqueous solutions was varied from 8.5 to 11.0. Finally, the influence of salt types on the extraction was evaluated employing aqueous solutions containing sodium chloride or sodium sulfate. All experiments were carried out in the spiked aqueous solutions. For recovery test, urine samples were spiked with appropriate concentrations of the three abused drugs and 0.1 µg/ml of phentermine hydrochloride was also added as internal standard. To avoid carry-over, each fiber was kept in the GC injection port for another 10 min before the next run. It was also confirmed that no carry-over effect was observed after 10 minutes.

## Results and Discussion

### *Selection of fiber*

In order to achieve maximum efficiency of extraction of the three target drugs from the spiked aqueous solution, five different fibers, viz. PDMS, PA, CAR/PDMS, CW/DVB and DVB/CAR/PDMS, were initially evaluated in this study. The result of the evaluation showed that all three drugs (Figure 1) could be sufficiently extracted by the fibers, except for the CAR/PDMS fiber (Figure 2). This may be rationalized by the fact that the CAR/PDMS is semi-polar fiber while the PDMS fiber is nonpolar, whereas the PA, CW/DVB and DVB/CAR/PDMS fibers are polar. As indicated in Figure 2, all fibers exhibited somewhat lower efficiency for extracting EPH. This is because the existence of the β-hydroxyl group of EPH makes it more soluble in water [19]. Also, small and broad peaks for EPH were observed. Among the five fibers studied, the DVB/CAR/PDMS provided high extraction efficiency, particularly for AMP and MET as observed in Figure 2. On the other hand, the CAR/PDMS is least sensitive to the three drugs studied. The DVB/CAR/PDMS was therefore selected for further method development and applications

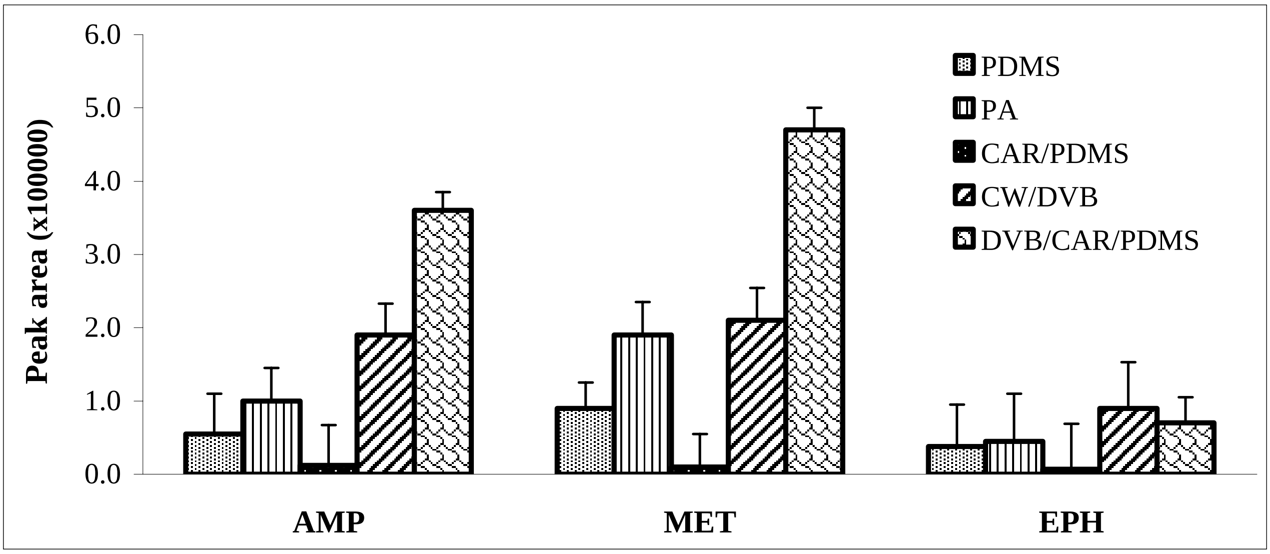


**Figure 2.** Effect of fiber coating type on extraction amount and percent relative standard deviation (%RSD) of three abused drugs (n = 3). Spiked water samples containing 1 µg/ml of each compound and 0.6 g sodium chloride, adjusted to pH 9.5 with 1.0 M sodium hydroxide were extracted at 80 °C for 20 min.

*Effect of extraction temperature and extraction time*

For the SPME method, temperature is an important factor in the extraction efficiency as it affects the kinetics of the reaction and diffusion of the analytes [20]. The effect of temperature on extraction yield, as represented by the peak area, was investigated by extracting a spiked aqueous solution in a thermostatic water bath for 15 minutes. The extraction temperature was increased in 5-10°C steps from 50 to 90°C. The extraction yield of AMP and MET increased with temperature until it reached a maximum at 80 °C, whereas the extraction yield of EPH continued to increase with temperature until 90 °C (Figure 3). This is because EPH is more soluble in water and can form more hydrogen bonding, which makes it more difficult to volatilize. The peak areas of AMP and MET obtained at 80 °C were increased by 2 and 3 times respectively, as compared to those obtained at 50°C. In order to avoid water boiling, the temperature selected for the extraction of the three drugs was 80°C.

Extraction time was also found to affect the extraction efficiency of the three drugs as shown in Figure 4. At extraction time from 5 to 20 minutes, peak areas were increased 61%, 74% and 67% for AMP, MET and EPH, respectively. Increasing extraction time beyond this period provided lower amounts of analytes extracted. Thus, the results at 80°C indicated that extraction time of 20 minutes was suitable for determining AMP, MET and EPH in aqueous solution.

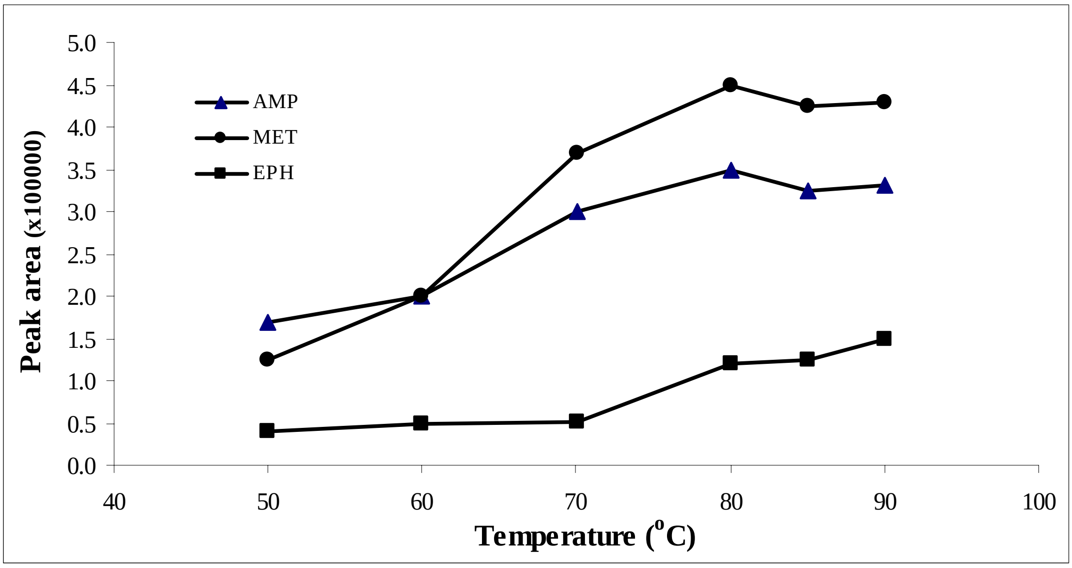


**Figure 3.** Temperature profiles for the extraction of three drugs using DVB/CAR/PDMS fiber.

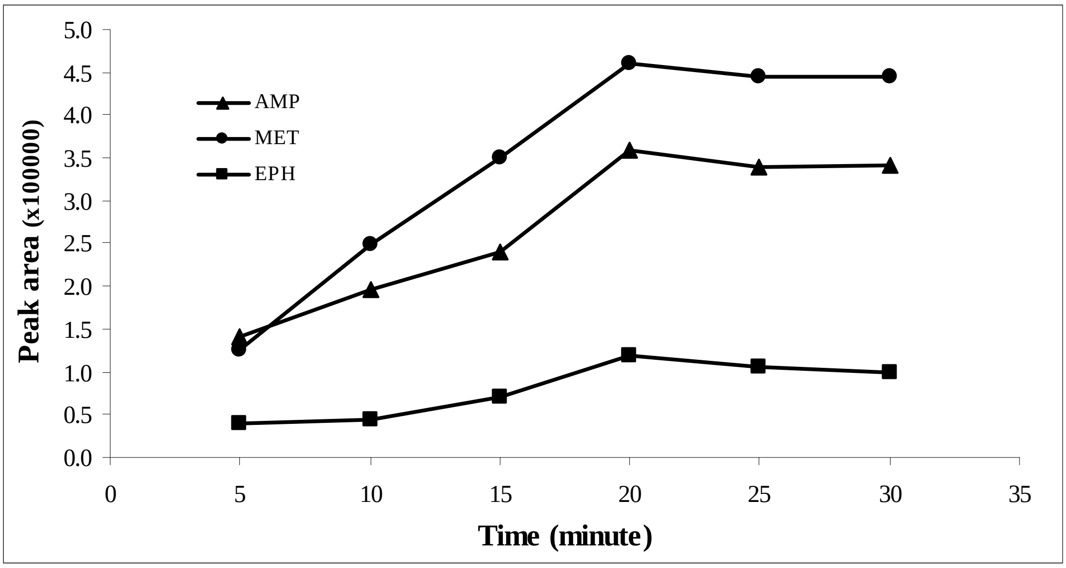


**Figure 4.** Extraction time profiles for the extraction of three drugs using DVB/CAR/PDMS fiber at 80 °C.

*Effect of solution pH*

It has been reported that the determination of AMP, MET and their derivatives is satisfactory in alkaline solution [8, 11, 12, 21]. In this work, the pH of solution was varied from 8.5 to 11.0 for SPME of AMP, MET and EPH. As shown in Figure 5, the extracted amount of the three drugs (the peak area) increased with increasing pH to a maximum at 9.5 (for AMP and MET) and 10 (for EPH). This may be explained by the fact that at a high pH, the acid-base equilibria of the three drugs, all being a weakly basic compound, significantly shift toward the neutral forms [22, 23], which have a higher affinity for the fiber, thereby increasing the amounts extracted. At still higher pH, however, the extraction efficiency began to decrease. This might be due to the formation of the new ionized forms of the three drugs starting to occur, thereby decreasing the neutral forms available for adsorption onto the fiber.

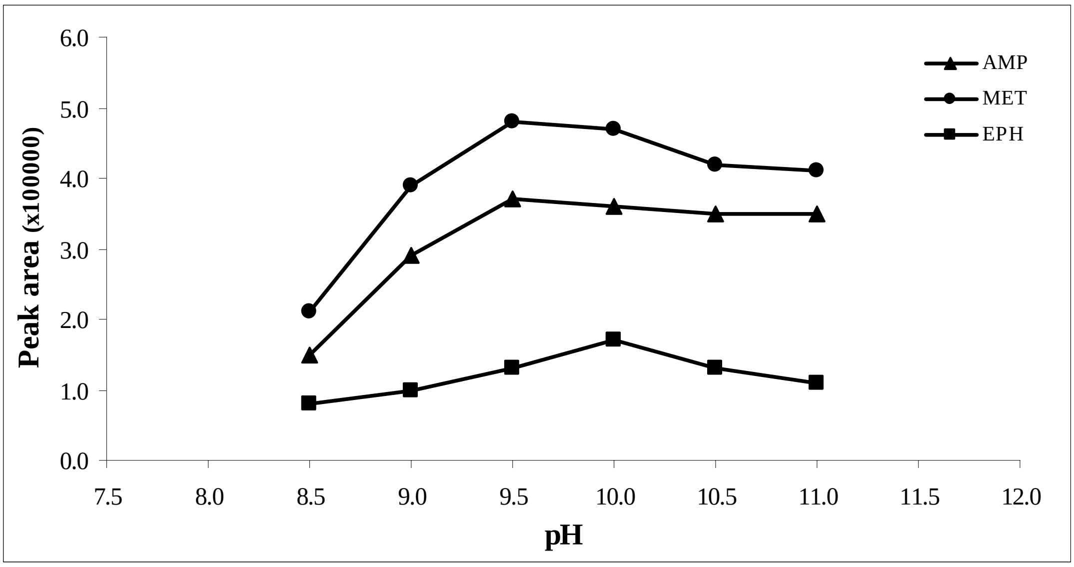


**Figure 5.** Effect of solution pH on the extraction of the three drugs using DVB/CAR/PDMS fiber at 80°C.

### *Effect of salt addition*

In order to enhance the extraction efficiency, two types of salt (sodium chloride and sodium sulfate) added at the amount of 0.4 g in the aqueous solution (2 ml) containing 1 μg/ml of each drug were investigated. This effect was studied in solutions of pH 10.0 (adjusted with 1.0 M sodium hydroxide). As demonstrated in Figure 6, the extracted amounts of the three drugs increased significantly with the addition of each salt (5 times for AMP, MET and 7 times for EPH compared to the absence of salt). This may be explained by the fact that the addition of salt to the aqueous solution causes a decrease in solubility of the drugs in the aqueous phase, consequently increasing adsorption of the drug onto the fiber surface [22, 24]. The results obtained for AMP and MET are in good agreement with other reports [8, 22, 24]. In addition, the extraction efficiency obtained using sodium chloride and sodium sulfate was similar. Sodium chloride was therefore selected for further investigation.

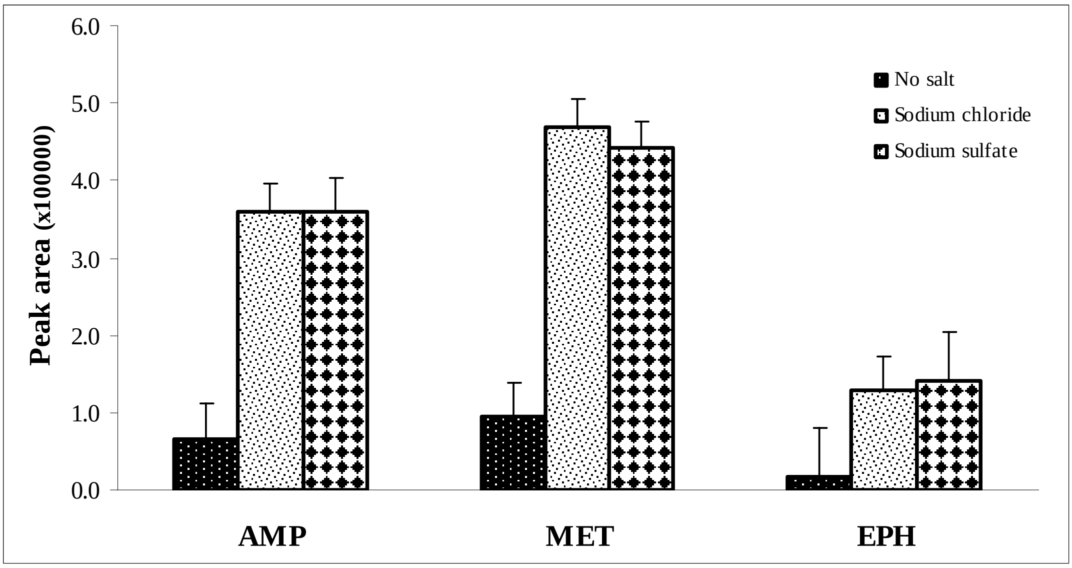


**Figure 6.** Effect of salt type on the extraction of the three drugs using DVB/CAR/PDMS fiber at 80 °C.

### *Characteristics of the developed HS-SPME method*

An example of the chromatograms of the abused drug standards obtained by HS-SPME method under SPME optimum conditions is depicted in Figure 7. Calibration data obtained using phentermine hydrochloride as internal standard, limits of detection (LOD) and limits of quantitation (LOQ) are summarized in Table 1. Excellent linearity was obtained over the entire concentration ranges with correlation coefficient ($r^2$) greater than 0.999. The LOD calculated from low concentration value calibration curves were 9 ng/ml for AMP, 3 ng/ml for MET and 30 ng/ml for EPH with a signal-to-noise ratio of 3 (S/N = 3). The LOD of AMP and MET obtained in this work are lower than those presented by other groups employing a commercial PDMS fiber [9, 11]. Although the LOD values of MET and EPH are higher than those reported by Zhou and Zeng [18], a simple commercial fiber instead of a sophisticated fiber was used in this study. In addition, the LOD and LOQ obtained in this study are lower than the concentration (e.g., 10 µg/ml for EPH) which is regarded as positive for illegal use of amphetamine drugs. The extraction time of SPME method was less than that reported by Zhou and Zeng [18].

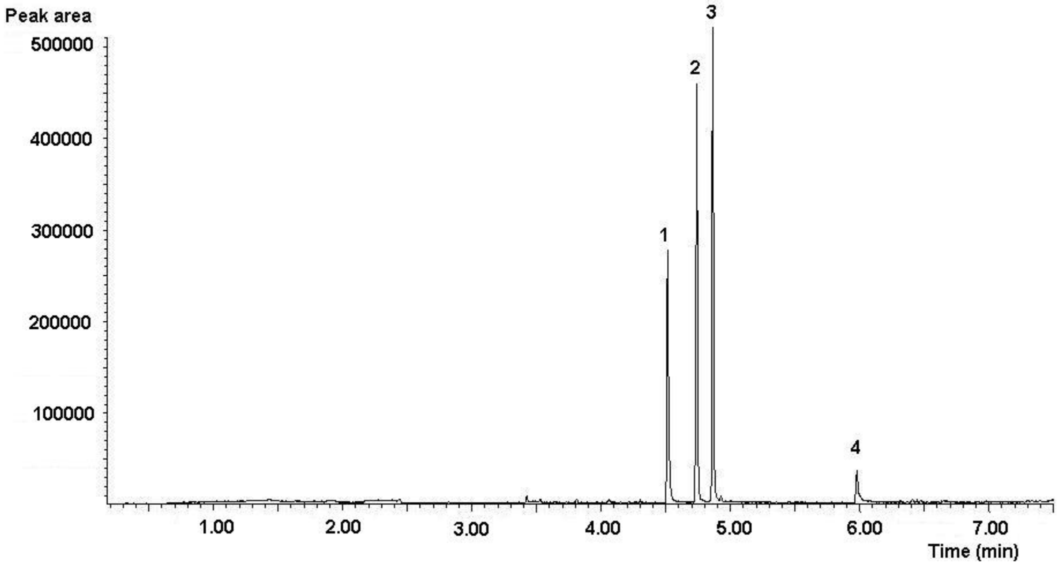


**Figure 7.** Chromatogram of standard mixture of the three abused drugs obtained using the DVB/CAR/PDMS fiber under SPME optimum conditions. Peak assignment: (1) amphetamine, (2) phentermine, (3) methamphetamine and (4) ephedrine.

**Table 1.** Linearity, detection limit and quantitation limit of the developed method

| Compound | Range of linearity (µg/ml) | Correlation coefficient ($r^2$) | Limit of detection (ng/ml) | Limit of quantitation (ng/ml) |
|---|---|---|---|---|
| AMP | 0.1-10 | 0.9993 | 9 | 30 |
| MET | 0.1-10 | 0.9999 | 3 | 10 |
| EPH | 0.5-20 | 0.9990 | 30 | 100 |

The accuracy and precision values of the three standards spiked at two concentration levels in blank urine from different sources were investigated to determine the effect of different matrices of urine samples. The results demonstrated that the developed HS-SPME-GC method provides good recovery in the range of 86-98%, with the standard deviation (SD) ranging from 1.1 to 3.1, as shown in Table 2. The intra-day and inter-day RSD values at two different concentrations ranged from 1.8-4.9% and 3.8-6.8%, respectively (Table 2).

**Table 2.** Recovery and relative standard deviation of intra-day and inter-day analysis of the three drugs.

| Concentration (μg/ml) | Compound | Recovery (%) (mean ± SD) | RSD (%) | |
|---|---|---|---|---|
| | | | Intra-day (n =5) | Inter-day (n = 5) |
| 0.5 | AMP | 89.2 ± 2.1 | 3.7 | 4.9 |
| | MET | 91.7 ± 1.5 | 2.2 | 4.2 |
| 1.0 | AMP | 94.6 ± 1.8 | 1.8 | 4.5 |
| | MET | 98.5 ± 1.1 | 1.3 | 3.8 |
| | EPH | 85.8 ± 3.1 | 4.9 | 6.8 |
| 2.0 | EPH | 91.3 ± 2.3 | 3.3 | 5.1 |

*Application to real samples*

The developed HS-SPME-GC method was applied to the analysis of AMP, MET and EPH in urine samples collected from three suspected persons. An example of the chromatograms of the abused drugs in urine samples is shown in Figure 8. All resulting chromatograms were obtained without endogenous interferences. The amounts of AMP and MET in all urine samples were found in the range of 23-128 ng/ml, whereas EPH was not present (Table 3).

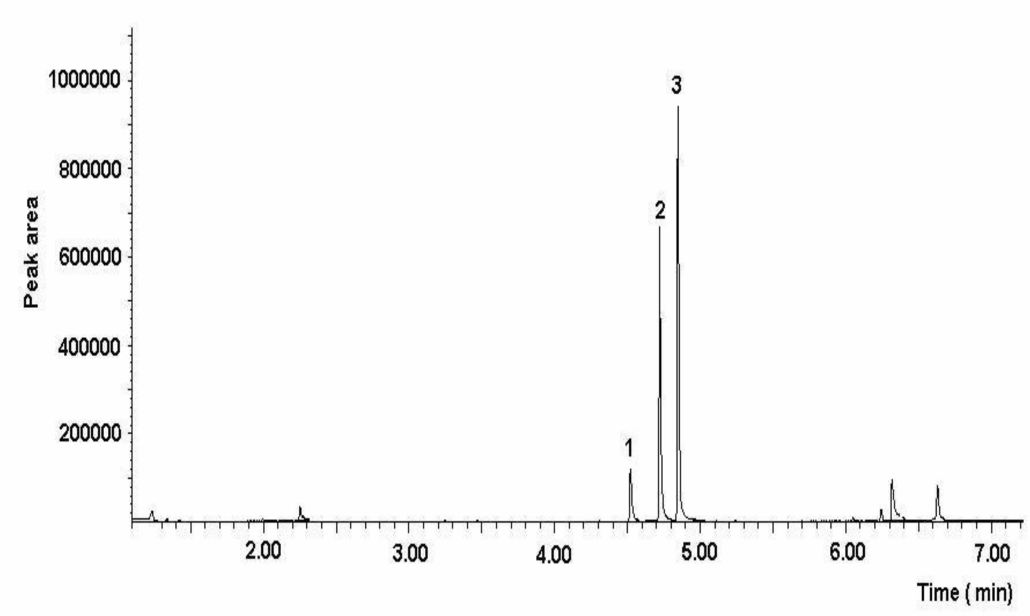


**Figure 8.** Chromatogram of abused drugs present in a urine sample using DVB/CAR/PDMS fiber under SPME optimum conditions. Peak assignment: 1 = amphetamine, 2 = phentermine, 3 = methamphetamine.

**Table 3**. Concentrations of the three drugs in urine samples

| Sample | Amount±SD (ng/ml) | | |
|---|---|---|---|
| No. | AMP | MET | EPH |
| 1 | 28.3±1.2 | 127.7±1.1 | ND |
| 2 | 22.9±2.3 | 91.3±1.9 | ND |
| 3 | 34.9±1.1 | 103.2±1.3 | ND |

ND: not detected.

## Conclusion

Headspace SPME coupled with GC-FID is a rapid and simple method for extraction and quantitative analysis of AMP, MET and EPH in human urine. In this study, the DVB/CAR/PDMS fiber was found to give higher extraction efficiency than other commercially available fibers, especially PDMS. Under the proposed method, the results were obtained with low limits of detection, good precision, linearity dynamic ranges, and interference minimization. In view of the simplicity, sensitivity and selectivity, the present method is recommendable for doping control.

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