Investigation of Pencil Leads Fiber Efficiency for SPME of Trace Amount of Methamphetamine from Human Saliva Prior to GC–MS Analysis

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Abstract

The efficiency of pencil lead fiber was investigated for effective head-space solid-phase microextraction (HS-SPME) of methamphetamine (MAMP) from aqueous standard solutions without chemical derivatization prior to gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) analyses. Most effective experimental parameters such as extraction temperature and time, sample pH, and salting out were studied and optimized. At the optimum conditions, the efficiency of this fiber was compared with polyacrylate (PA) commercial fiber, which is most selective for volatile and semi-volatile compounds. The results obtained prove the suitability of modified pencil-lead fiber for sampling of the studied compound from aqueous solutions. Under optimum conditions, the calibration plot was linear in the range of 40–8000 ng/mL (r = 0.998), and the detection limit was 27 ng/mL (n = 3). The proposed method was successfully applied for HS-SPME of MAMP from 200 µL human saliva, which has been spiked with trace amounts of MAMP (160 ng/mL) followed by GC–MS monitoring.

Introduction

Amphetamines are a major class of central nervous system stimulants. Abuse of amphetamines and derivatives has increased dramatically in recent years. They are drugs of abuse as well as doping agents in sports. The analysis of amphetamines becomes of increased interest in toxicology, occupational medicine, and law enforcement. Methamphetamine (MAMP) and 3,4-methylenedioxymethamphatamine (MDMA, ecstasy) are the most important derivatives of amphetamine (AMP) (Figure 1).

The identification and quantification of amphetamines have been described using a variety of techniques such as immunoassay (1), liquid chromatography (LC) (2–4), gas chromatography–mass spectrometry (GC–MS) (1,5–7), and capillary electrophoresis (8). Among these methods, GC–MS has been the most widely used analytical method because of its sensitivity and selectivity.

These drugs are frequently monitored in very complex matrices such as biological samples. Thus, appropriately preparing samples is a prerequisite for chromatographic analysis. Solid-phase microextraction (SPME) is a powerful, simple, rapid extraction method for the separation and sample introduction into GC injector for determination of wide variety of volatile and semi-volatile drugs such as amphetamines. The sample molecules are adsorbed onto the fiber and subsequently thermally desorbed into the GC injection port for analysis. Because this method of preparation does not involve destruction of the sample itself, this is an ideal technique for forensics. The extraction can also serve to concentrate the sample molecules, thus providing a lower limit of detection for the analyte(s) of interest. Unlike other popular sample preparation techniques (e.g. solid-phase extraction, liquid–liquid extraction), SPME is considered to be "environmentally friendly" because of the lack of organic solvents required. Headspace (HS)-SPME minimizes interactions between the sample and the fiber and has been proven useful for these analyses. Fused-silica fibers coated with polydimethylsiloxane (PDMS), polyacrylate (PA), Carbowax, and Carboxen are widely used in this area. Most of these fibers are fragile and very expensive for routine analyses. Several new fibers based on metal wires coated with inorganic salts (9–11) and organic compounds (12–14), modified pencil lead (15), and fibers based on molecularly imprinted polymer (16,17) have been developed and used as SPME fibers. Within these, modified pencil lead fiber has demonstrated some drastic ability in the

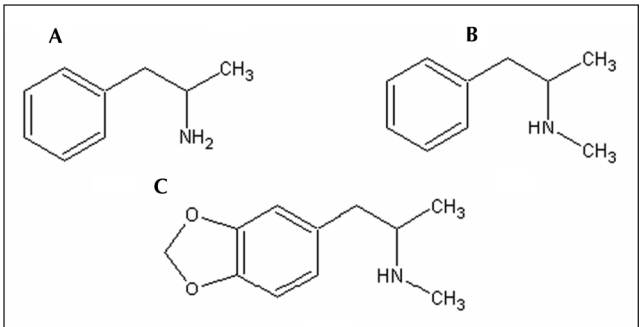

Figure 1. Structures of amphetamine (A), methamphetamine (B), and 3,4-methylenedioxymethamphatamine (C).

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wide range of applications for direct and headspace extraction of semi-volatile and volatile compounds from aqueous samples (18,19) and seems to be a useful and highly suitable fiber in this work.

The present paper describes a headspace SPME using pencil lead fiber and GC and/or GC–MS assay for the detection of MAMP in saliva. Because saliva is a blood filtrate, salivary drug concentration should reflect plasmatic concentration (20).

Materials and Methods

Chemicals

Methamphetamine hydrochloride (MAMP.HCl), amphetamine sulphate [$(AMP)_2SO_4$], and ecstasy (MDMA) were from local Criminal Investigation Labs (Tehran, Iran), and other analytical-grade chemicals were obtained from Merck (Darmstadt, Germany). Helium as carrier gas (99.999%) was purchased from Arad Gas Gostar Gas Co. (Air Product, Middle East Dubai, United Arab Emirates).

Standard aqueous solution with a concentration of 80 µg/mL of MAMP was prepared by dissolving 2 mg of analyte in doubly-distilled water and was adjusted to 25 mL. This solution was used to prepare aqueous buffered solution containing various amounts of MAMP. For this purpose, an appropriate volume of standard was adjusted to 25 mL by phosphate buffer (0.05 mol/L) with pH 7, 9.2, and 11 separately. The solutions were stored at 4°C.

Blank saliva sample was collected on the day of analysis from subject with no history of having received abused drugs. The test specimen, 200 µL saliva spiked with 160 ng/mL of the methamphetamine.

GC and GC–MS apparatus

Monitoring of the analytes was performed by 3800 CP GC coupled with Saturn 2000 MS, both from Varian (Palo Alto, CA). The chromatographic column used was a Chrompack CP-Sil8-CB (30 m × 0.25 mm i.d.) (Palo Alto, CA). For optimization of extraction condition, a Shimadzu GC model GC-15A (Kyoto, Japan) equipped with a FID, and a split/splitless injector was used. The column used for the separations was a 30 m × 0.25 mm i.d. capillary column coated with a 0.25-µm film of SPB-50 Supelco (Dorset, UK).

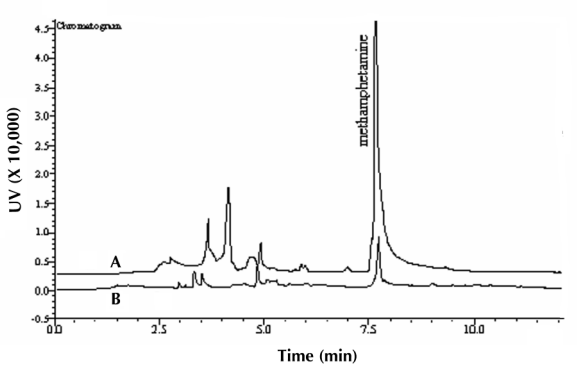

Figure 2. Extraction efficiency of MAMP by different types of fibers: modified pencil lead (A) and PA fiber (B). The volume of aqueous sample was 1 mL (phosphate buffer, 0.05 mol/L) containing 100 ng/mL of analyte. HS-SPME was performed for 50 min at 80°C. MAMP was desorbed by split/splitless injection at an injector temperature of 270°C. The split valve was opened after 1 min, and the split ration was 1:10.

In this study, the column temperature was programmed at 15°C/min from 90°C to 210°C, which was maintained for 2 min, and then at 30°C/min to 250°C, which was held for 0.5 min in both GC and GC–MS. The carrier gas velocity was 25 cm/s, and make up-gas flow was 30 mL/min. The injector was held at 270°C. Splitter was opened after 1 min, and split ratio was 1/10. All ions were detected within the pre-selected mass range (i.e., *m/z* of 40 to 200).

SPME apparatus

A HB-type of pencil lead (diameter 0.35 mm, length 60 mm) from Rotring (Hamburg, Germany), prepared as described previously (9), was mounted in the homemade SPME device, and the exposed fiber was trimmed to 25 mm. A SPME manual sampling holder, and a polyacrylate (PA) fiber with 85-µm film thickness was purchased from Supelco (Dorset, UK). Extraction of the analyte was performed in a 4-mL sample vial sealed with a silicone-rubber septum cap (Supelco) and contained a teflon stirring bar. Samples were agitated during SPME by a magnetic stirrer (Gerhardt, Konigswinter, Germany) operated at 600 rpm.

Fiber conditioning

The ability of two types of SPME fibers, (A) pencil-lead and (B) PA fiber, which is selective and recommended fiber for the SPME of polar semi-volatile compounds, was investigated. PA fiber was initially conditioned at 270°C under helium flow into GC injector port. Various types of commercial pencil-leads were modified by heating for 60 min in the presence of water vapor in the furnace at ~ 600°C. Before using, they were conditioned inside GC injection port for 15 min at 270ºC under helium flow.

HS-SPME Procedure

Extractions were performed from headspace of 1 mL aqueous sample (phosphate buffer, 0.05 mol/L) at pH 11 that was poured

Table I. Comparison of Ability of Different Types of Pencil Lead Modified Under Various Conditions

Modification methods	GC Response			
	Staedler HB 0.35	Staedler HB 0.3	Rotring HB 0.35	Sanford HB 0.35
a: Heated at 600ºC with water vapor for 60 min	ND*	ND	175365 ± 11395 (n = 3)	ND
b: Floated infused NaOH at 400ºC with water vapor for 30 min	ND	ND	ND	ND
c: Immersed in Carbon glue and then heated at 600ºC with water vapor for 60 min	ND	ND	ND	ND
d: No preparation	ND	ND	ND	ND

* ND = not detected.

into a vial containing a teflon stirring bar, and 5% NaCl was added. The stirring speed was adjusted at 600 rpm. SPME was accomplished by 25 mm of fiber for 50 min at 80°C in a water bath. After extraction, the fiber was immediately inserted into the GC and/or GC–MS injection port for thermal desorption of the analytes at 270°C for 1 min.

In the case of real sample, 200 µL of spiked saliva has been transferred into a vial and adjusted to 1 mL with buffer and the experiment was continued as described for aqueous standards samples.

Results and Discussion

Pencil lead modification and conditioning

The main goal in this process is the increasing of adsorption efficiency of pencil lead fibers. For this purpose pencil leads were heating for a long time (60 min) at high temperature (600°C) in the presence of water vapor (method A) (16). Treatment with fused NaOH was limited to 400°C for 30 min because pencil leads are destroyed if this temperature or time is exceeded (method B). For studying of carbonization probability, fibers were immersed in carbon glue and then heated at 600°C with water vapor for 60 min (method C). The ability of these fibers for extraction of amphetamines from headspace of aqueous samples was investigated. As illustrated in Table I, pencil-lead type HB modified by heating at ~ 600°C in the presence of water vapor (method A) adsorbed the studied compound. Therefore, pencil lead modified by heating with water vapor can be an attractive and precious fiber for the extraction of MAMP.

Extraction capacity of the pencil leads fiber

The ability of two types of fibers: (A) modified pencil lead and (B) PA fiber for the extraction of MAMP was investigated and compared with each other. HS-SPME procedure was carried out using each of selected fiber at the same experimental conditions followed by GC analyses. Obtained chromatograms were shown in Figure 2. Comparing these chromatograms and MAMP peak area reveals clearly that in the optimum conditions, the extraction efficiency with pencil lead fiber is higher than commercial PA fiber. Because of easy preparation, inexpensiveness, high suitability of pencil lead fiber in comparison with the commercial fibers, it can be a preferable SPME fiber for the extraction and sampling of MAMP.

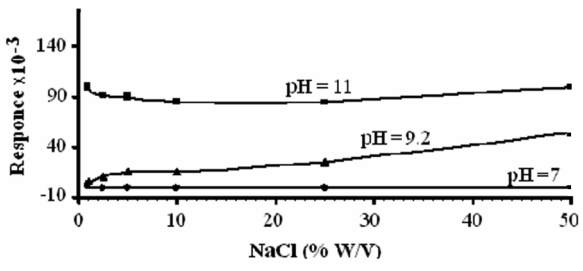

Figure 3. Effect of pH and amount of salt amount on the extraction efficiency of MAMP: pH 7 (A), pH 9.2 (B), and pH 11 (C). The volume of aqueous sample was 1 mL containing 100 ng/mL of analyte. HS-SPME was performed for 50 min at 80°C.

Optimization of sample pH and salt amount

The efficiency of extraction of MAMP from aqueous solution containing different amounts of sodium chloride was investigated at various pH. Due to the NaCl presence in biological fluids such as human saliva and its concentration level, which is usually more than 0.7% (w/v), salting out process was investigated by adding 1 to 50% (w/v) NaCl into standard solutions. Plots of peak area versus NaCl amount (%) in standard aqueous solutions adjusted at pH 7, 9.2, and 11, are presented in Figure 3. The results obtained show that the presence of NaCl at the studied amounts, particularly at pH 11, have not considerable effect on the HS-SPME efficiency of MAMP. On the other hand, these results reveal also that the extraction efficiency increases with pH. This is in good concordance with theoretical aspects because due to the pKa value of MAMP (9.2), at pH 11, it is in non-ionic form and can be extracted easily. Therefore, for further analysis, NaCl concentration was buffered by addition of 5% (w/v) NaCl into standard or real samples, and pH was adjusted at 11 by phosphate buffer.

Optimization of extraction time and temperature

HS-SPME relies on the equilibrium between the concentration of analytes in the sample solution and gas phase and also between their concentration in the gas phase and that on the fiber. Exposure time and temperature are, therefore, important factors affecting the extraction efficiency. For this reason, extraction of MAMP was performed at different temperatures for periods from 10 to 60 min. Isothermal extraction-time profiles

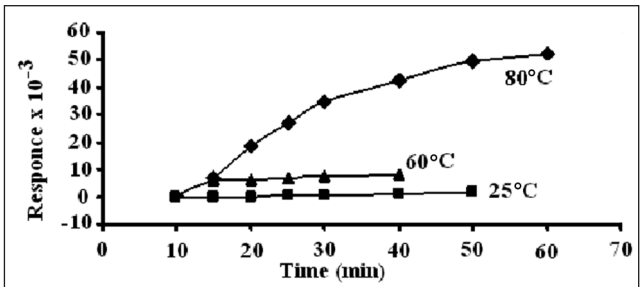

Figure 4. Effect of extraction time and temperature on the extraction efficiency of MAMP: 25°C (A), 60°C (B), and 80°C (C). The aqueous sample containing 100 ng/mL of analyte at pH 11.

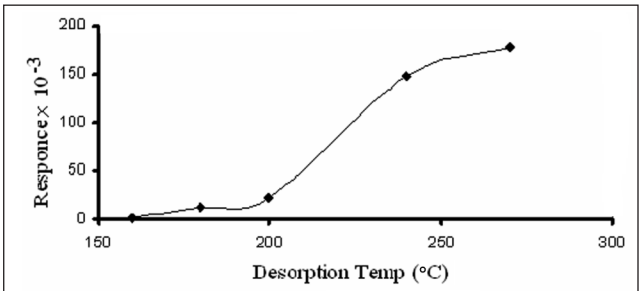

Figure 5. Results from optimization of desorption temperature. The aqueous sample containing 100 ng/mL of analyte. HS-SPME was performed for 50 min at 80°C.

were constructed by plotting the mass adsorbed, measured as chromatographic peak area, against extraction time. As shown in Figure 4, the extraction efficiency increases considerably by increasing of temperature and exposure time and reaches a plateau after 50 min exposure of the fiber in headspace of solution at 80°C. Further SPME procedures were performed therefore at 80°C for 50 min.

Optimization of stirring speed

Considering the equilibrium distribution of analyte in SPME, stirring speed of the sample solution could also be an affecting parameter. The extraction efficiency of the studied compound was measured with various stirring speeds. The results obtained showed that magnetic stirring of the samples at 600 rpm improved the performance of the extraction and was used throughout.

Optimization of desorption temperature

Higher desorption temperature can reduce desorption time and carryover. On the other hand, thermal degradation of the fibers and injector septum or analyte limits the elevation of desorption temperature. High thermal stability of pencil lead fibers allows us to increase desorption temperature and, therefore, we can improve the efficiency of thermal desorption. In this way, complete desorption of thermal stable compounds such as MAMP can be achieved. Plots of extraction recovery against desorption temperature are presented in Figure 5. The results obtained showed that extraction recovery of MAMP increased as desorption temperature was increased. In order to prevent an eventual thermal degradation of injector septum or other compounds present, injector port temperature was adjusted at 270°C.

Table II. Quantitative Characteristic of SPME-GC Method for the Analysis of Methamphetamine

Compound	Calibration Graph Equation*	LOD (ng/mL)	LDR (ng/mL)	RSD%	*R*
MAMP	$y = -428.5 + 39.7x$	27	40-8000	6.5	0.998

* y = response, x = analyte concentration (ng/mL).

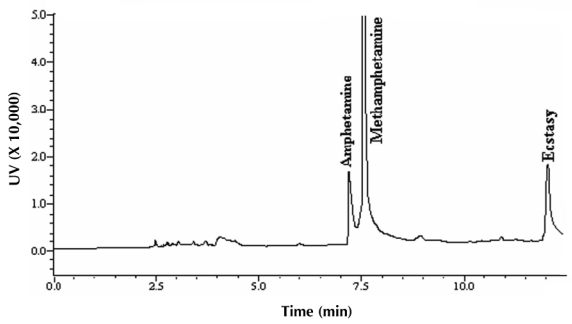

Figure 6. Typical GC chromatogram obtained from 1 mL standard aqueous solution containing AMP, MAMP, and MDMA (100 ng from each compound) at pH 11. HS-SPME was performed for 50 min at 80°C. MAMP was desorbed by split/splitless injection at an injector temperature of 270°C.

Analytical approach

The method's discrimination capacity was evaluated analyzing by HS-SPME–GC a representative mixture of amphetamines under the optimal conditions. Chromatogram presented in Figure 6 show a good ability of the proposed method for discrimination of AMP, MAMP, and MDMA in mixture samples. On the other hand, due to the low sensitivity of AMP and MDMA in direct GC analysis, these compounds were normally analyzed after derivatization. In this work quantitative analyses were limited to MAMP.

Table II shows some analytical characteristic parameters for three replicated analysis including limit of detection, dynamic range, correlation coefficient of calibration graphs, and relative standard deviation obtained by the proposed fiber.

Limit of detection

The limit of detections (LOD) obtained for the studied was 27 ng/mL, which is in the range of admissible concentrations. The obtained values prove the excellent ability of the proposed fiber comparing commercial PA fiber for the extraction of studied compound. These results are remarkable, considering rapid and simple extraction from aqueous solutions without any practical difficulty.

Linearity

The obtained values of correlation coefficient (r) are 0.998. The quantitative analysis of MAMP can be performed in a relatively wide concentration range: 40–8000 ng/mL by the external standard method.

Precision

The precision of the method for three replicates analysis of model aqueous solutions for the solution containing 160 ng/mL of MAMP, the relative standard deviations was below than 10%. Taking into account the very low concentration level of the analyte and easy extraction from aqueous sample, the precision is acceptable.

Application

To evaluate the reliability of the proposed pencil lead fiber for

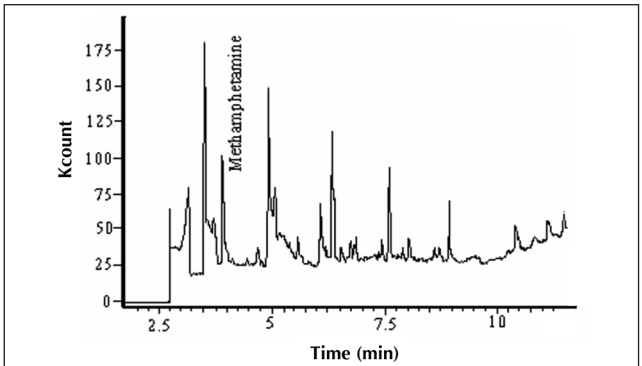

Figure 7. Typical TIC GC–MS chromatogram obtained from human saliva. Solution was adjusted at pH 11 and taken on day from subject with no history of having received abused drugs and spiked with 160 ng of MAMP; HS-SPME was performed for 50 min at 80°C; MAMP was desorbed by split/spliyless injection at an injector temperature of 270°C. Mass spectrum of MAMP (B).

HS-SPME of MAMP from real biological fluids, a human saliva sample delivered from volunteer with no history of having received abused drugs was spiked and analyzed. Two hundred microliters of sample was pipetted into 4-mL sealed vial, and volume adjusted to 1 mL with phosphate buffer at pH 11. HS-SPME procedure was performed using optimized conditions. Subsequent recognition and confirmation was performed by GC–MS. Results in Figure 7 show the presence of MAMP in the studied sample. GC quantitative analysis reveals that in the optimum conditions, more than 93% of the added MAMP can be recovered from spiked human saliva and analyzed.

Conclusion

These experiments have conclusively demonstrated that HS-SPME using modified pencil lead fiber can be an effective method for the selective extraction of metamphetamine from biological fluids prior to GC and/or GC–MS analysis. The preconcentration afforded by this method allows limits of detection at the parts-per-billion levels for sampling from samples matrix.

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