

## Preparation of ionic liquid based solid-phase microextraction fiber and its application to forensic determination of methamphetamine and amphetamine in human urine<sup>☆</sup>

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### ABSTRACT

A new solid-phase microextraction (SPME) procedure using an ionic liquid (IL) has been developed. Reusable IL-based SPME fiber was prepared for the first time by fixing IL through cross-linkage of IL impregnated silicone elastomer on the surface of a fused silica fiber. 1-Ethoxyethyl-3-methylimidazolium bis(trifluoromethane) sulfonylimide ([EeMim][NTf<sub>2</sub>]) ionic liquid was employed as a demonstration and the prepared fiber was applied to the forensic headspace determination of methamphetamine (MAP) and amphetamine (AP) in human urine samples. Important extraction parameters including the concentration of salt and base in sample matrix, extraction temperature and extraction time were investigated and optimized. Combined with gas chromatography/mass spectrometry (GC/MS) working in selected ion monitoring (SIM) mode, the new method showed good linearity in the range of 20–1500 µg L<sup>-1</sup>, good repeatability (RSD < 7.5% for MAP, and < 11.5% for AP, n = 6), and low detection limits (0.1 µg L<sup>-1</sup> for MAP and 0.5 µg L<sup>-1</sup> for AP). Feasibility of the method was evaluated by analyzing human urine samples. Although IL-based SPME is still at the beginning of its development stage, the results obtained by this work showed that it is a promising simple, fast and sensitive sample preparation method.

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### 1. Introduction

Room temperature ionic liquids (ILs) are salts that are composed of cations and anions that do not fit simply into a rigid matrix, often involving one being bulky while the other is relatively small. While known for quite some time, ILs have become of particular interest to chemists (and others) during the past decade owing to their potential utility for a variety of “green” processes. Because of the wide selection of both cations and anions, the possible combination of ILs can be as much as 10<sup>18</sup> [1]. The term “designer solvents” has been applied to them owing to the possibility of providing continuous property modification with changes in structure, generally of the cationic portion, and the combination of cations and anions. In particular, ILs are gaining popularity because their physical and chemical properties, such as hydrophobicity, viscosity, thermal stability, selectivity and vapor pressure, can be tuned by controlling the nature and functionality of the cation or anion. Applications of

ILs have been seen frequently in various fields including separation science in recent years [2–7].

Solid-phase microextraction (SPME) is a simple solvent free sample preparation technique integrating extraction, concentration, and sample cleanup in one step, and can be easily coupled with various analytical instruments. The key part of the SPME device is an extraction fiber, usually made of a fused silica fiber or metal wire with an extraction coating, which determines the extraction efficiency [8]. ILs are very attractive to be used as SPME coating because they can be custom-designed according to target analytical system, and thus improve the selectivity and sensitivity of the extraction. Although IL-based SPME methods have been reported by Liu et al. [9] and Hsieh et al. [10], fiber preparation in their work was tedious and time-consuming, limiting the practical use of the technique. In Liu's work, 100 µL of dichloromethane was added to 900 µL of IL (1-octyl-3-methylimidazolium hexafluorophosphate [C<sub>8</sub>Mim][PF<sub>6</sub>]) to prepare a coating solution. A clean stainless steel or fused silica fiber was dipped vertically into the coating solution and held for 1 min, and then removed from the coating solution and kept in air for another 1 min to evaporate dichloromethane. The dipping and evaporating process were repeated several times in order to get a thicker coating [9]. Hsieh et al. described a procedure to develop Nafion membrane supported

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IL SPME fibers for analyzing ultra trace PAHs in water samples, in which Nafion, a cation exchange polymer, was coated on the surface of a bare fused silica tip, then ILs were absorbed on the fiber surface through the electrostatic interaction between IL and the Nafion membrane [10]. Both techniques require washing the fiber clean by organic solvent after each use and re-coating it before the next extraction. Additionally, ILs are present as a liquid state on the fiber, causing inconvenience in fiber handling. Furthermore, the precision of analysis suffered because the reproducibility of the coating thickness for each extraction is difficult to control. We made efforts to prepare [EeMim][NTf<sub>2</sub>] IL-based fibers using the above-mentioned two methods, however, the difficulties encountered during the experiments and the unsatisfactory results convinced us that in order to prepare a reliable and reusable IL-based fiber the IL has to be physically supported or immobilized on the surface of the extraction fiber. Very recently, Zhao et al. reported that SPME fiber's thermal stability, reproducibility and life-time could be improved by using polymeric ionic liquids [11]. Three homologous polymeric imidazolium-based ionic liquids, poly(ViHim<sup>+</sup>NTf<sub>2</sub><sup>-</sup>), poly(ViDDim<sup>+</sup>NTf<sub>2</sub><sup>-</sup>), and poly(ViHDDim<sup>+</sup>NTf<sub>2</sub><sup>-</sup>) were synthesized by free radical polymerization of 1-vinyl-3-hexylimidazolium chloride, 1-vinyl-3-dodecylimidazolium bromide, and 1-vinyl-3-hexadecylimidazolium bromide, respectively. The halogen anions were subsequently exchanged with the bis[(trifluoromethyl)sulfonyl]imide anion (NTf<sub>2</sub><sup>-</sup>) in an effort to increase the thermal stability of the ionic liquid [11].

Other available IL-based microextraction methods often employed a single IL drop to couple with GC or HPLC analysis. When combined with GC, extra attention must be given to prevent IL entering into GC column due to the low-volatility of ILs. Recently, a removable interface enabling the introduction of analytes into a GC/MS system while preventing ionic liquid from entering column was designed [12]; however, the setup is complicated. Generally, it is more convenient to use ILs as extraction solvents for drop based liquid phase microextraction (LPME) combined with LC analysis due to its unique properties of low-volatility, adequate viscosity and immiscibility with water. It can be used for direct immersion and headspace LPME. Compared with solvents commonly used in LPME, such as octanol, a larger IL drop can be suspended on the syringe tip for longer time [4]. IL-based LPME had been applied to bioanalysis [13,14] and determination of environmental pollutants such as PAHs [4], formaldehyde [15], pesticides [16], chlorobenzenes [17], chlorinated anilines [18], and phenols [19].

In order to make IL-based microextraction more compatible with GC, in this work, we developed a procedure to prepare reusable IL-based SPME fiber by fixing IL through cross-linkage of IL impregnated silicone elastomer on a bare fused silica fiber. 1-Ethoxyethyl-3-methylimidazolium bis(trifluoromethane)sulfonylimide ([EeMim][NTf<sub>2</sub>]) ionic liquid was used as demonstration because of its polar functional structure and good thermal stability (see details below). This newly developed fiber looks like a commercially available SPME fiber and can be used in the same way as the conventional fiber. Methamphetamine (MAP), an illicit drug, and amphetamine (AP), its major metabolite, were employed as model compounds with respect to optimization of the extraction parameters. The feasibility of the new method was demonstrated by analyzing human urine samples. The results indicated that the coating technique developed by this work provided a practical approach to prepare reusable IL-based SPME fibers.

## 2. Experimental

### 2.1. Reagents and materials

MAP and AP were purchased from Sigma (St. Louis, MO, USA). L- $\alpha$ -Methyl benzylamine (L- $\alpha$ -MBA) (Acros, Geel, Belgium)

was used as an internal standard (IS). The stock solutions of MAP at 0.935 mg mL<sup>-1</sup>, AP at 0.452 mg mL<sup>-1</sup> and L- $\alpha$ -MBA at 1.512 mg mL<sup>-1</sup> were prepared in ultrapure water (Millipore, Bedford, MA, USA). A 10  $\mu$ g mL<sup>-1</sup> secondary stock solution was prepared freshly daily, and all working solutions were prepared by spiking the secondary stock solution. NaOH and NaCl were supplied by Fisher (Fair Lawn, NJ, USA). 1-Methylimidazole, 2-bromoethyl ethyl ether, acetonitrile, and bis(trifluoromethane)sulfonylimide lithium salt were purchased from Aldrich (St. Louis). Gelest filler-free 2-part silicone elastomer was obtained from Gelest (Morrisville, PA, USA). Bare fused silica SPME fibers and a manual SPME device were obtained from Supelco (Bellefonte, PA, USA). 10 mL headspace sample vials (23 mm  $\times$  46 mm, National Scientific, Rockwood, TN, USA) and 20 mm crimp seal caps with septa were used for all extractions.

### 2.2. Preparation of ionic liquid

Preparation of 1-ethoxyethyl-3-methylimidazolium bromide: 1-methylimidazole (0.122 mol) was dissolved in acetonitrile (40 mL). To this 2-bromoethyl ethyl ether (0.122 mol) was added dropwise. The reaction mixture was refluxed overnight. The solvent was evaporated under reduced pressure, leaving behind an orange viscous liquid. The liquid was re-dissolved in 2-bromoethyl ethyl ether (35 mL) and passed through a 600 mL glass frit containing a layer of charcoal and a layer of alumina. The filtrate was evaporated under reduced pressure, leaving behind a pale yellow liquid. The liquid (9.60 g, 33.5% yield) was dried under high vacuum. NMR ( $\delta$ , DMSO-D<sub>6</sub>)—<sup>1</sup>H: 1.05 (t, *J*=6.8 and 7.2 Hz, 3H); 3.41–3.46 (q, *J*=6.8 and 7.2 Hz, 2H); 3.72 (t, *J*=4.8 and 5.2 Hz, 2H); 3.88 (s, 3H); 4.39 (t, *J*=4.8 and 5.2 Hz, 2H); 7.78–7.82 (m, 2H); 9.29 (s, 1H). <sup>13</sup>C: 13.7, 34.5, 47.5, 64.1, 66.2, 121.2, 122.0, 135.4.

Preparation of 1-ethoxyethyl-3-methylimidazolium bis(trifluoromethane)sulfonylimide ([EeMim][NTf<sub>2</sub>]): 1-ethoxyethyl-3-methylimidazolium bromide (0.041 mol) was dissolved in distilled water (40 mL). In a separate flask, a charge equivalent of bis(trifluoromethane)sulfonylimide lithium salt (0.041 mole) was dissolved in distilled water (20 mL). Both solutions were then combined. The reaction mixture was stirred overnight. The solvent was carefully decanted and the resulting liquid washed with water until there was no detectable halide present. The resulting liquid (9.7 g, 57% yield) was dried under high vacuum. NMR ( $\delta$ , DMSO-D<sub>6</sub>)—<sup>1</sup>H: 1.09 (t, 3H); 3.43–3.48 (q, 2H); 3.71 (t, 2H); 3.87 (s, 3H); 4.34 (t, 2H); 7.67–7.72 (m, 2H); 9.07 (s, 1H). <sup>13</sup>C: 14.7, 35.7, 48.8, 65.5, 67.4, 114.6, 117.8, 121.0, 122.6, 123.4, 124.2, 136.7. The synthesis and structure of [EeMim][NTf<sub>2</sub>] is shown in Fig. 1. Table 1 summarizes the physicochemical properties of [EeMim][NTf<sub>2</sub>]. Previously prepared and commonly used ILs, often based on alkyl modified methylimidazolium base structure, depend on differences in chain length and thereby relative hydrophobicity to provide selectivity. Once the modifying chain has been significantly lengthened, the variability in the characteristics of the species decreases. Introduction of polar functional groups provide additional means of specific interaction and thereby introduce additional means for gaining selectivity. It was anticipated that the polar ether linkage in [EeMim][NTf<sub>2</sub>] would provide a particular interaction with polar amine group in MAP and AP to enhance extraction.

### 2.3. Preparation of immobilized IL-based SPME fiber

The two parts (A and B) of the silicone elastomer were thoroughly mixed in a 1:1 ratio (w/w) in a 2 mL GC sample vial using a microspatula. The vial containing the mixture was then placed in an 80 °C water bath (IsoTemp 205, Fisher) for 4 min. Twenty percent (w/w) of ionic liquid was then thoroughly mixed with the elastomer using a microspatula and returned to the 80 °C water bath.

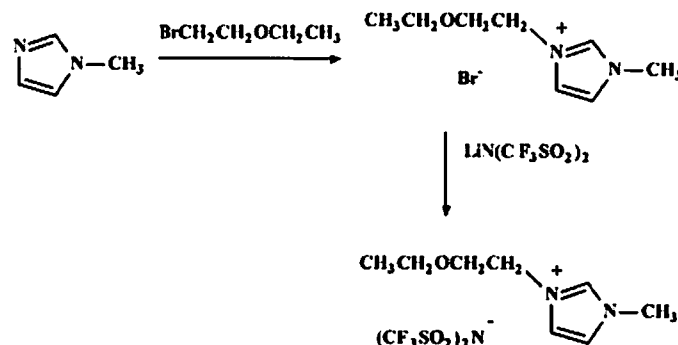


Fig. 1. The synthesis and chemical structure of [EeMim][NTf<sub>2</sub>] ionic liquid.

The elastomer-IL solution then remained in the water bath for about 1 h until the viscosity was thick enough to coat the bare fused silica SPME fiber. The consistency of the coating was controlled by viscosity of the mixture through adjusting the time spent in the water bath. A piece of silica fiber was repeatedly dipped into the coating solution and removed to test the viscosity. Prior to being coated, the bare SPME silica fiber was washed using acetone, followed by a 10 min conditioning in the 220 °C GC injection port. The pre-treated silica fiber was vertically dipped into the viscous elastomer-IL mixture and immediately slowly removed from the mixture. The fiber was immediately placed in a 200 °C oven for 5 min to achieve complete cross-linking of the polymer. After being attached to a manual SPME device, the fiber was conditioned in the GC injection port (220 °C) for approximately 10 min. The fiber was also reconditioned at the beginning of every day for approximately 10 min prior to use.

The bare fused silica fiber and prepared IL-based fiber were observed (Fig. 2) and their diameters were measured by an Olympus BX41 microscope (Olympus America Inc., Center Valley, PA, USA). The coating thickness was determined by the difference of these two measurements. The typical coating thickness prepared in this work averaged about 50 μm.

#### 2.4. Extraction procedure

Solid NaCl was weighed and transferred to a 10 mL headspace sample vial. After adding a micro-stirring bar (8 mm × 1.5 mm, Fisher, Pittsburgh, PA, USA), 4 mL NaOH was added to the vial and spiked with MAP, AP, and IS secondary stock solution to obtain the desired concentration. Then the vial was quickly sealed, slightly mixed, and pre-heated in a temperature controlled water bath for 30 min. Pre-heating, which usually was performed in parallel when a previous sample was analyzed, was performed to help establish equilibrium before analysis.

For extraction, the pre-heated sample was placed on top of a magnetic-hot plate (Super-Nuova, Barnstead, Chicago, IL, USA). The sample vial was housed in a custom made Styrofoam block that fit the vial snugly around the sides, and had an open bottom for the vial to be in contact with the hotplate (Fig. 3). This Styrofoam block insulated the sample vial from the surrounding air. The SPME device was fixed with a clamp to the stand that was attached to the hotplate

after its needle pierced the septum of the sample vial. The fiber was then carefully exposed to the headspace above the solution. Sample solution was continuously heated at the same temperature as that of the water bath and was stirred at the rate of 1200 RPM during extraction. After extraction, the fiber was withdrawn back into the needle of the SPME device, and the entire device was removed from the vial and directly transferred to GC for subsequent analysis.

#### 2.5. GC/MS analysis

The analysis of MAP and AP was performed on a HP5890 Series II GC coupled with a HP5971A MSD (Hewlett-Packard, CA, USA). The GC was fitted with a DB-1MS column (30 m × 0.250 mm × 0.25 μm film, J&W Scientific, Santa Clara, CA, USA). Helium with a flow rate of 1 mL min<sup>-1</sup> was used as a carrier gas. The injector temperature was 220 °C, and the detector temperature was 275 °C. The temperature of the column oven started at 60 °C and held for 2 min, then ramped to 140 °C at 20 °C min<sup>-1</sup>, to 190 °C at 10 °C min<sup>-1</sup>, to 250 °C at 20 °C min<sup>-1</sup>, and held for 1 min. The ions used by selected ion monitoring (SIM) were *m/z* 91 and 120 for AP, *m/z* 91 and 134 for MAP, and *m/z* 79 and 106 for the IS.

A Varian CP-3800 GC-FID equipped with a DB-5 column (30 m × 0.320 mm × 1.0 μm film, J&W Scientific) was used during optimization of the extraction parameters. All GC settings were same as those for the GC/MS.

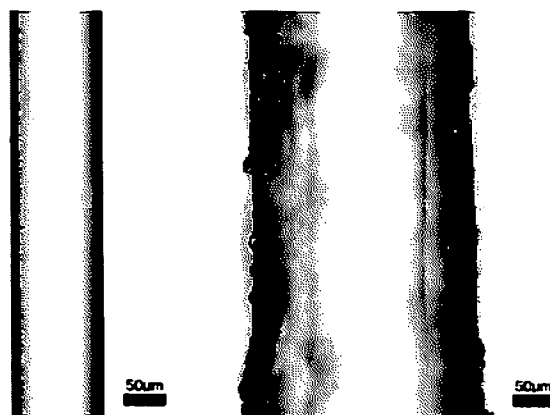


Fig. 2. Microphotograph of a bared silica fiber (left) and an IL-based fiber (right) viewed using transmitted light at a total magnification of 200.

Table 1  
Physicochemical properties of [EeMim][NTf<sub>2</sub>] IL.

Molecular mass	423 g mol <sup>-1</sup>
Density [32]	14622 g cm <sup>-3</sup>
Viscosity [32]	5612 mPa s
Conductivity [32]	3316 mS cm <sup>-1</sup>

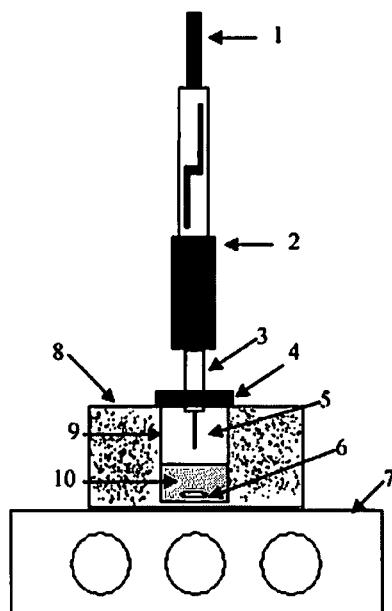


Fig. 3. Schematic of SPME extraction setup. (1) SPME plunger; (2) SPME device; (3) septum piercing needle; (4) septum; (5) SPME fiber; (6) stirring bar; (7) hot/stir plate; (8) Styrofoam block; (9) sample vial; (10) sample.

## 2.6. Urine sample collection

All urine samples were donated by drug-free volunteers. Institutional Review Board (IRB) protocols for research involving human subjects were followed in entire investigation to protect human subjects. Samples were collected in Nalgene HDPE sample bottles (Nalgene, Rochester, NY, USA), and kept frozen at  $-20^{\circ}\text{C}$ . Before the samples were used each day, they were thawed at room temperature and filtered with disposable syringe filters ( $0.2\ \mu\text{m}$  pore size, Whatman, Florham Park, NJ, USA).

## 3. Results and discussion

### 3.1. Preparation of IL-based SPME fiber

We successfully prepared IL-based SPME fibers by cross-linking IL impregnated silicone elastomer on the surface of a bare silica fiber. IL was trapped and supported by the silicone cross-linkage. The coating was stable and we never observed it was peeled-off from the fused silica fiber at both room and elevated temperature ( $220^{\circ}\text{C}$ ). This fiber looks like a commercially available SPME fiber and is used in the same way as the commercial fiber. The average thickness of IL fiber coating was determined to be about  $50\ \mu\text{m}$  and the volume of the coating was therefore calculated to be approximately  $2.5 \times 10^{-10}\ \text{m}^3$ . Upon analysis, the fibers exposed in GC injector and analytes were thermally desorbed by carrier gas. The [EeMim][NTf<sub>2</sub>] IL-based fiber coating was thermally stable under GC operation condition. The thermal stability of [NTf<sub>2</sub>] ILs has been verified by differential scanning calorimetry (DSC) test in our previous study [20]. Fundamentally, the bistriflimides exhibit decomposition only over  $300^{\circ}\text{C}$ , higher than the other anionic forms of ILs. In addition, Fig. 4(a) shows a chromatogram of a blank [EeMim][NTf<sub>2</sub>] IL-based SPME fiber, which was desorbed at  $220^{\circ}\text{C}$  for 6 min, and no fiber bleeding was observed. For a  $1.0\ \mu\text{g mL}^{-1}$  MAP and AP solution, no carryover was observed when the fiber was desorbed at  $220^{\circ}\text{C}$  for 6 min, a desorption duration that was com-

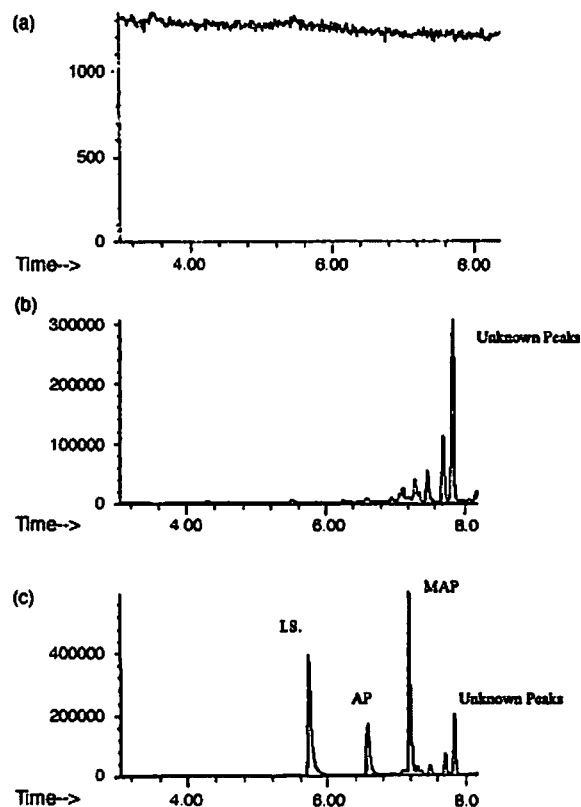


Fig. 4. The chromatogram of (a) a blank [EeMim][NTf<sub>2</sub>] based SPME fiber to show the thermal stability of the fiber, (b) a blank urine sample and (c) a urine sample spiked with  $200\ \mu\text{g L}^{-1}$  of MAP and AP at optimum extraction condition. A smaller scale was used to show the details in (a) and (b).

monly used in SPME analysis. The [EeMim][NTf<sub>2</sub>] IL-based SPME fiber was durable as well. More than 100 extractions were performed using one single fiber, and no changes in its efficiency were observed.

### 3.2. Optimization of extraction parameters

The newly developed fiber was applied to determination of MAP and AP in urine samples. Both MAP and AP are basic drugs and volatile at elevated temperatures [21]. The sample matrix was therefore adjusted to alkaline pH with NaOH so that the target compounds were in their neutral form and easily released into the headspace [22]. The salting-out effect can also enhance the extraction efficiency by adding NaCl to sample solution [22]. The factors affecting extraction, including NaCl concentration, NaOH concentration, extraction temperature and extraction time were investigated and optimized by using a  $50\ \mu\text{m}$  [EeMim][NTf<sub>2</sub>] IL-based SPME fiber.

The effect of NaCl on extraction was investigated. The NaCl concentration was varied from 0% (w/v) to 35% (w/v). For a 4 mL 4 M NaOH sample solution containing  $1.0\ \text{mg L}^{-1}$  MAP and AP and extracted at  $50^{\circ}\text{C}$  for 10 min, addition of 35% (w/v) NaCl increased the peak area of MAP and AP for 3.0 times and 3.9 times, respectively, in comparison with no salt addition (Fig. 5). As a result, 35% (w/v) NaCl concentration was used in following extractions.

The NaOH concentration was optimized by varying its concentration from 0.5 to 4 M. The optimum value was found to be 4 M, which agrees with our previous study [22]. Because both MAP

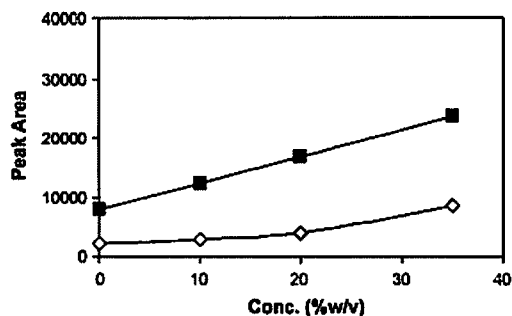


Fig. 5. The effect of NaCl concentration. The solid square is MAP, and the open diamond is AP.

and AP are basic drugs ( $pK_a = 10.1$  for both drugs) [23], they were presented in solution as undissociated forms in the investigated concentration range. However, compared with a concentration of 0.5 M, the extraction of MAP and AP was improved approximately 3.5 times for each analyte with the increase of NaOH concentration to 4 M (Fig. 6), which was because NaOH further increased the ionic strength of sample solution so that it additionally acted as a salting-out reagent [22].

Temperature affects two equilibria, i.e., the distribution of analytes between sample matrix and headspace, and the distribution between headspace and fiber coating, in an opposite way [24]. Increasing temperature can enhance mass transfer of analytes from the sample solution to headspace; however, it also decreases the amount of analytes that can be retained by the fiber coating [8]. Extraction temperatures ranging from room temperature (24 °C) to 80 °C were investigated. For a 4 mL 4 M NaOH sample solution containing 1.0 mg L<sup>-1</sup> MAP and AP and 35% (w/v) NaCl and extracted for 10 min, the peak area was increased from room temperature to 50 °C (about two times for both analytes), and then decreased (Fig. 7). Therefore, 50 °C was chosen in following experiments.

The kinetics of extraction was investigated. As shown in Fig. 8, the extraction efficiency increases quickly over the beginning 20 min and reaches equilibrium after 20 min. Therefore, 20 min was used for all further extractions. Such results are similar to what have been obtained by previous studies using a 100 μm PDMS fiber to extract MAP and AP, in which the extraction time ranged from 15 to 30 min [25–28].

### 3.3. The effect of silicone on extraction

The contribution of the silicone to extraction was investigated because it was a major component in fiber coating. The extraction of a blank fiber, which was coated with silicone only, and an IL-based

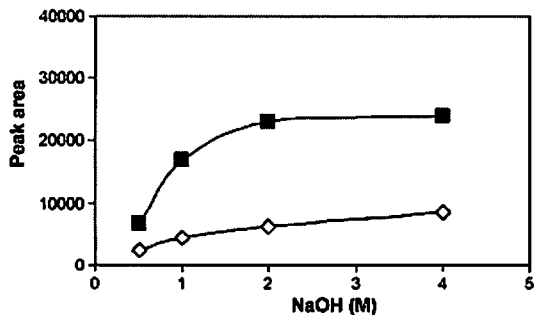


Fig. 6. The effect of NaOH concentration. The solid square is MAP, and the open diamond is AP.

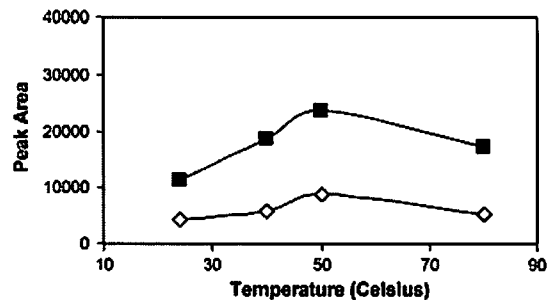


Fig. 7. The effect of extraction temperature. The solid square is MAP, and the open diamond is AP.

fiber containing 20% of [EeMim][NTf<sub>2</sub>] IL was compared. Because the coating thickness was slightly different for these two fibers, extraction efficiency expressed by peak area per unit coating volume ( $10^{-10} \text{ m}^3$ ) was used and shown in Fig. 9. Although the content of [EeMim][NTf<sub>2</sub>] IL in the prepared fiber was only 20%, the extraction has been increased 4.2 times for MAP and 7.3 times for AP for a 4 mL NaOH sample solution spiked with 1.0 mg L<sup>-1</sup> of MAP, AP and IS under optimum extraction conditions. Such results further demonstrated the selectivity exhibited by IL.

### 3.4. [EeMim][NTf<sub>2</sub>] IL-based fiber versus commercial PDMS fibers

IL-based SPME fiber was compared with a 7 μm and a 100 μm commercial polydimethyl siloxane (PDMS) fiber. PDMS fiber has demonstrated its effectiveness to extract MAP and AP from biological samples by previous studies [27–30]. For a sample solution with 100 μg L<sup>-1</sup> of MAP, AP and IS and extracted at the optimum condition, the peak areas obtained by a 50 μm IL-based fiber were larger than those from a 7 μm thickness PDMS fiber, but smaller than those from a 100 μm thickness PDMS fiber (Table 2). It was somewhat expected because 100 μm PDMS has a much thicker coating.

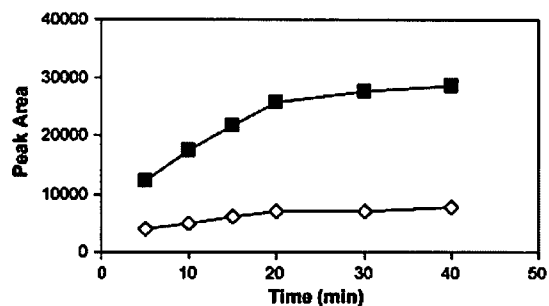


Fig. 8. Kinetics of SPME by using a 50 μm IL-based SPME fiber. Extraction conditions: 4 mL NaOH sample solution with 35% (w/v) NaCl, 1.0 mg L<sup>-1</sup> of MAP, AP and IS. Sample was pre-heated at 50 °C for 30 min, followed by SPME extraction at 50 °C. The solid square is MAP, and the open diamond is AP.

Table 2

Comparison of peak area obtained by a 50 μm [EeMim][NTf<sub>2</sub>] IL-based, a 100 μm PDMS and a 7 μm PDMS fiber (n = 3).

	IS	AP	MAP	AMP/IS	MP/IS
50 μm 20% IL	469715	298241	1320909	0.63	2.81
100 μm PDMS	7844058	2181648	4318406	0.28	0.55
7 μm PDMS	<LT. <sup>a</sup>	<LT.	71789	n/a	n/a

<sup>a</sup> Integration threshold.

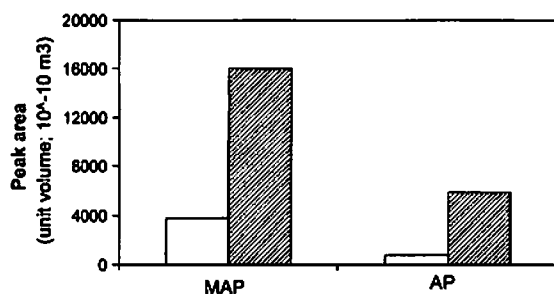


Fig. 9. Comparison of extraction efficiency between a blank silicone elastomer fiber and an IL-based fiber. Extraction conditions: 4 mL NaOH sample solution with 35% (w/v) NaCl, 1.0 mg L<sup>-1</sup> of MAP, AP and IS. Sample was pre-heated at 50 °C for 30 min, followed by SPME extraction at 50 °C for 20 min. The blank bar represents silicone elastomer blank fiber, and the filled bar is IL-based fiber containing 20% [EeMim][NTf<sub>2</sub>] ionic liquid.

### 3.5. Quantitative analysis

The linearity, limits of detection (LOD), and precision (%RSD) were investigated at optimum conditions for the established IL-based SPME procedure. Standard solutions were prepared in ultrapure water. Both MAP and AP exhibited good linearity in the investigated concentration range of 20–1500 µg L<sup>-1</sup> with a coefficient of determination (*R*<sup>2</sup>) of 0.9901 and 0.9895, respectively. The error in the slope of the calibration curve was 4.6% for AP and 4.5% for MAP. The LODs (*S*/*N*>3) were 0.1 µg L<sup>-1</sup> for MAP and 0.5 µg L<sup>-1</sup> for AP. The precision was measured by repeatedly extracting standard solutions with analyte concentrations of 100, 200, and 500 µg L<sup>-1</sup>. The relative standard deviations (RSDs, *n*=6) ranged from 3.9 to 11.3% for AP, and 4.0 to 7.5% for MAP.

### 3.6. Urine sample analysis

The IL-SPME fiber was tested for its capability to extract MAP and AP from urine samples. All urine samples were found MAP and AP free. In order to investigate the relative recovery of the method, urine samples were spiked with 100, 200, 500 and 1000 µg L<sup>-1</sup> of MAP and AP. Such concentration levels were chosen because the urinary cutoff concentration of abuse set by the U.S. Substance Abuse and Mental Health Services Administration (SAMHSA) is 1000 ng mL<sup>-1</sup> of total amphetamines by initial test, and 500 ng mL<sup>-1</sup> of AP, or 500 ng mL<sup>-1</sup> of MAP with at least 200 ng mL<sup>-1</sup> AP by confirmatory test [31]. All urine samples were adjusted to 4 M NaOH through 1:1 dilution with 8 M NaOH. Acceptable relative recoveries and repeatability was obtained (Table 3). The chromatograms of the extraction of a blank urine sample and a urine sample spiked with 200 µg L<sup>-1</sup> of MAP and AP are shown in Fig. 4(b) and (c).

### 3.7. Perspective of the work

The significance of this work is to develop a concept to prepare reusable IL-based SPME fibers by fixing ILs through cross-linkage

Table 3  
Results of determination of AP and MAP in spiked human urine samples.

Spiked conc. (µg L <sup>-1</sup> )	% Relative recovery (% RSD, <i>n</i> =3)	
	AP	MAP
100	89.0 (1.9)	113.8 (4.7)
200	102.6 (1.2)	112.2 (5.0)
500	94.0 (0.5)	108.9 (0.4)
1000	91.9 (1.3)	94.4 (1.3)

of IL impregnated silicone elastomer on the surface of a fused silica fiber, and develop a procedure to make a prototype fiber in laboratory using [EeMim][NTf<sub>2</sub>] IL as a demonstration and apply it to forensic analysis of MAP and AP in urine samples. The developed fiber preparation technique is suitable for other room temperature ILs. Although IL-based SPME is still relatively new and in its early development stage, analytical results demonstrated that IL-based SPME fiber is a very promising technique that we foresee can be widely used in various fields such as environmental, biological, pharmaceutical, forensic, and food analysis. IL-based SPME will be more specific than conventional SPME because the fiber properties can be fine-tuned according to the characteristics of target analysis system by choosing or designing molecular structure of the IL. This feature provides more flexibility and versatility in terms of choosing fiber coating material. Specifically, the volatility of [NTf<sub>2</sub>] type of ionic liquids, such as what we used in this study, is low and they are stable at significantly high temperature, providing an acceptable stability of the fiber and prolonging the life-time of the fiber [20].

## 4. Conclusion

The present study demonstrated the development and application of an [EeMim][NTf<sub>2</sub>] IL-based SPME method to the determination of MAP and AP in human urine samples. This method provided a practical way to prepare reusable IL-based SPME fiber. Wide linear range, good sensitivity, and reproducibility have been achieved under optimum extraction conditions.

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